PATHOZYME® SYPHILIS COMPETITION Ref OD117/OD157

Competitive Enzyme-Immunoassay (EIA) for the detection of Total Antibodies to *Treponema pallidum* in human serum

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro use only.

INTRODUCTION

PATHOZYME SYPHILIS COMPETITION is a competitive enzyme-immunoassay (EIA) for the detection of total antibodies to *Treponema pallidum* in human sera.

T. pallidum is a spirochaete bacterium which causes the sexually transmitted disease Syphilis. Despite its worldwide importance as an infectious agent and the chronic debilitating suffering caused by the disease, limited information about *T. pallidum* is available. The organism cannot be cultured continuously *in-vitro*, no vaccine is available and the mechanisms of pathogenesis are not well understood (1, 2).

Infection with T. pallidum is systemic and complicated by periods of latency in the late stages of the disease. However, Syphilis can be cured relatively easily and in the late stages of the disease treatment can limit any permanent damage caused by this bacteria (1).

Syphilis can also be aquired congenitally. If the foetus is infected early in pregnancy spontaneous abortion usually occurs (1). This can be stopped by the diagnosis and treatment of Syphilis.

All the above features of this disease highlight the importance of serological techniques in the diagnosis of Syphilis. The **PATHOZYME SYPHILIS COMPETITION** kit is a highly sensitive and specific EIA for the primary screening of antibodies to *T. pallidum* as an aid to the diagnosis of Syphilis.

INTENDED USE

PATHOZYME SYPHILIS COMPETITION is an *in-vitro* diagnostic test for Screening for Syphilis.

The test detects primarily IgG and IgM and therefore has a high sensitivity to all disease stages (3). As this test requires no serum dilution or reagent preparation and has only a single wash step, it is ideally suited for automation to test large numbers of samples.

For professional use only.

PRINCIPLE OF THE TEST

Purified antigen derived from *Treponema pallidum* is bound to the surface of microtitration wells. Undiluted test sera are applied followed by anti-*T. pallidum* antibody conjugated to Horseradish Peroxidase (HRP).

Specific antibodies to *T. pallidum* in the test sera and the conjugate compete to bind to the antigen in the wells. Unbound material is then 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. This indicates the absence of human anti-*Treponema pallidum* antibody and is therefore a negative result. The enzyme 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) less than the cut off is considered positive. The intensity of the yellow colour produced after stopping the reaction is inversely proportional to the concentration of *T. pallidum* antibodies in the sample.

This test has been calibrated to the WHO Reference Serum for Serodiagnosic Tests for Treponemal Infections – Ref 3 – 1980

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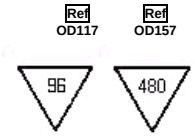

Microtitre Plate				**12 x 8**	**60 x 8**
Breakable wells coated with specific antigens contained in resealable foil bag with a desiccant.					
Control	**-**	**Reagent**	**2**	**0.5 ml**	**2.5 ml**
Negative Control. Clear solution, of human serum negative for antibodies to Treponema pallidum. Ready to use. (Blue)					
Control	**L**	**Reagent**	**3**	**0.5 ml**	**2.5 ml**
Low Positive Control. Clear solution, of human serum containing a low level of antibodies to Treponema pallidum . Ready to use. (green)					
Control	**H**	**Reagent**	**4**	**0.5 ml**	**2.5 ml**
High Positive Control. Clear solution, of human serum containing a high level of antibodies to Treponema pallidum . Ready to use. (Red)					
Washbuf	**20X**	**Reagent**	**5**	**50 ml**	**125ml x 2**
Wash Buffer concentrate: Tris based buffer containing detergents. (Colourless)					
Conj	**Reagent**	**6**		**15 ml**	**32.5 ml x 2**
Anti- Treponema pallidum HRP Conjugate: Anti-Treponema pallidum antibodies conjugated to Horseradish Peroxidase. Ready to use. (purple)					
Subs	**TMB**	**Reagent**	**7**	**15 ml**	**11 ml x 5**
Substrate Solution: 3,3', 5,5' Tetramethyl Benzidine in a citrate buffer. Ready to use. (Colourless)					
Soln	**Stop**	**H2SO4**	**0.2M**	**15 ml**	**32.5 ml x 2**
Reagent	**8**				
Stop Solution: Sulphuric Acid diluted in purified water. Ready to use. (Colourless)					
Instruction Leaflet and EIA Data Recording Sheet				**1 +1**	**1 +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 SYPHILIS COMPETITION 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 SYPHILIS COMPETITION 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 SYPHILIS COMPETITION 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 SYPHILIS COMPETITION 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.

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 (REAGENT 5) 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.

LIMITATION OF USE

The use of samples other than serum has not been validated in this test.

No serological haemagglutination test can discriminate between antibody due to T.pallidum infection and antibody due to infection with other pathogenic treponemes, i.e. T.pertenue and T.carateum.

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.

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 Standards 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 terst 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. DO NOT DILUTE THE TEST SERUM OR THE CONTROLS. Dispense 25µl of test serum or control serum (REAGENTS 2, 3 & 4) into the appropriate wells. The Control Serum should be added last to ensure accurate interpretation of the results. Immediately after all the test and control serum have been added dispense 100µl of anti-*Treponema pallidum* HRP conjugate (REAGENT 6) to each well containing test or control serum. The conjugate must be added not more than 30 minutes after the addition of the first serum. Gently shake for 5 seconds. Cover the plate with the plate lid provided and place it on top of some moist absorbent paper in a 37°C incubator for 90 minutes.
5. 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.
6. 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 5 times including a 30 second soak after each wash.
7. Strike the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
8. 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 including a 30 second soak after each wash..
9. Dispense 100µl of Substrate (REAGENT 7) into each well. Replace the lid onto the plate and return to the incubator and allow the reaction to develop in the dark at 37°C for 15 minutes.
10. Stop the reaction by adding 100µl of Stop Solution (REAGENT 8) to each well. This will produce a colour change from blue to yellow in wells containing enzyme which indicates the absence of anti-*Treponema pallidum* 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 serum, determine the average Optical Density (OD) value obtained in the wells.
Cut of level = (Average OD value of the duplicate Low Positive Controls (REAGENT 3)) x 1.2.
Equivocal Zone = OD values within, or equal to, 10% below Cut Off Level.
Assay Validation: The average OD of the Negative Control (Reagent 2) should be greater than 0.8 the Low Positive Control (Reagent 3) should greater than 0.35 and the High Positive Control (Reagent 4) should be less than 0.6 for the assay results to be valid.
Negative Result: A negative result should have an OD greater than the Cut Off value.
Suspected Positive: A low or suspected positive result should have an OD in the Equivocal Zone.
Positive Result: A positive result should have an OD less than the Equivocal Zone.
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.
At a European reference centre 120 samples were tested from patients with different forms of syphilis or untreated subjects. The control group of 345 samples comprised of possible cross reacting samples and known negative samples.
Sensitivity results: **PATHOZYME-SYPHILIS COMPETITION** 100%
Specificity results: **PATHOZYME-SYPHILIS COMPETITION** 100%
The co-efficient of variation of **PATHOZYME-SYPHILIS COMPETITION** is less than or equal to 10%.

REFERENCES

(1) **Cheesbrough, M** (1991). Spirochaetes, Chlamydiae, Rickettsiae, Mycoplasma and Bartonella. In: *Medical laboratory manual for tropical countries. Volume II: Microbiology.* Chap 45:312-318. Butterworth-Heinemann Ltd, Linacre House, Jordan Hill, Oxford.

(2) **Norris, S J & the T. PALLIDUM POLYPEPTIDE RESEARCH GROUP.** (1993). Polypeptides of *Treponema pallidum:* Progress toward understanding their structural, functional and immunologic roles. Microbiol. Rev.57(3). 750-779.

(3) **Independent Evaluation Performed By Bristol Supra Regionaltreponemal Serology UK.** April 1996.

QUICK REFERENCE TEST PROCEDURE

1. Do not dilute the test serum or controls.
2. Dispense 25µl of test serum or working strength Control Serum (REAGENTS 2, 3 & 4) to each well followed by 100µl of anti-*Treponema pallidum* HRP Conjugate (REAGENT 6). The conjugate must be added not more than 30 minutes after the addition of the first sera. Gently shake for 5 seconds.
3. Incubate for 90 minutes at 37°C.
4. Discard well contents and wash five times including a 30 second soak after each wash.
5. Dispense 100µl of Substrate solution (REAGENT 7) to each well.
6. Incubate in the dark for 15 minutes at 37°C.
7. Dispense 100µl of Stop Solution (REAGENT 8) to each well.
8. Read the OD with an EIA reader using a 450nm filter.
9. Interpret results as described in the interpretation of Results section.

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