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.

7. *T. pallidum* is a spirochetal bacterium which causes the sexually transmitted disease Syphilis. Despite the absence of any infective stage in the early stages of the disease, resulting in chronic debilitating suffering caused by the disease, limited information about *T. pallidum* is available. 7. *T. pallidum* can 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 easily and in the late stages of the disease treatment can limit any permanent damage caused by this bacteria (1). Syphilis can also be acquired congenitally. If the fetus is infected early in pregnancy, spontaneous abortion occurs usually (1). This can stop either 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.

**INDICATIONS**

**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 simple one step wash, it is ideally suited for large numbers of samples.

For professional use only.

**PRINCIPLE OF THE TEST**

Purified antigen derived from *Treponema pallidum* is bound to the surface of microtiter 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. Addition of the Substrate, stabilized 3,3',5,5'-Tetramethyl Benzidine (TMAB), 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 H2SO4. 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 Serodiagnosis Tests for Treponematoses - Ref 3 – 1993.

**CONTENTS**

- Microtitre Plate
- Stop Solution: Sulphuric Acid
- Wash Buffer concentrate: Tris based buffer
- Control Solution: Clear solution, of human serum contained in resealable foil bag with a desiccant.
- Treponema pallidum HRP Conjugate: A purified antigen derived from *Treponema pallidum* in human sera. 1. 2. 3.
- EIA Data Recording Sheet provided.

**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 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 HRP-enzyme system.

Do not freeze Departmental Use only.

**LIMITATION OF USE**

The use of serum samples other than serum has not been validated in this test. No serological haemagglutination test can discriminate between antibody due to 1) *T. pallidum* infection and antibody due to infection with other pathogenic treponemes, i.e. 1) *T. pertenue* and 1) *T. carateum*.

The test is not intended for use in patients who are known or suspected to have an abnormal immune response to antigen or are immunocompromised. In such cases an interpretation of the test 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 assay.

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 test sera 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.

5. 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 the addition of the first 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 1 on top of the strip. Incubate for 30 minutes at 37°C.

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. Before the addition of the Stop Solution, wash 5 times including a 30 second soak after each wash.

8. Wash the strips sharply against absorbent paper or paper towel to remove all residual wash droplets. 1

9. Dispense 10µl of Substrate (REAGENT 7) into each well. Replace the lid on to the plate and return to the incubator and allow the reaction to develop in the dark at 37°C for 15 minutes.

10. 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.

11. Wash the strips sharply against absorbent paper or paper towel to remove all residual wash droplets. 1

12. Place the plate lid provided and place 1 on top of the strip. Incubate for 30 minutes at 37°C.

13. Wash the strips sharply against absorbent paper or paper towel to remove all residual wash droplets. 1

14. Dispense 25µl 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 presence of antibody to *Treponema pallidum* antibodies. Strike the plate on to the air to remove the absorbance of each well, at 450nm. IMMEDIATELY after stopping the reaction.

All reagents should, however, be treated as potential biotoxins in use and disposal. Final disposal must be in accordance with local legislation.

**REFERENCES**

1. WHO Reference Serum for S. 1980

2. 1

3. 1

4. 1

5. 1

6. 1

7. 1

8. 1

9. 1

10. 1

11. 1

12. 1

13. 1

14. 1

**MATERIAL REQUIRED BUT NOT PROVIDED**

Micropipettes: 100µl, 200µl, 1000µl and 5000µl

Dispensing pipette

Thermoshake

Microtitre plate reader

Graph paper

Biohazard container

**PRECAUTIONS**

**PATHOZYME SYPHILIS COMPETITION** contains materials of human origin which have been tested and confirmed negative for HIV, HCV, HBV and HMP by approved procedures at single donor level. Because no test can completely assure that products derived from human sera will not transmit infectious agents it is recommended that the reagents within this kit be handled with due caution during use and disposal. All reagents should, however, be treated as potential biotoxins in use, and for disposal. Do not ingest.

**PATHOZYME SYPHILIS COMPETITION** reagents do not contain dangerous substances as defined by UK Chemicals (Hazardous Information and Packaging for Supply) regulations.
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.

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.

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, cooled 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 dilute the test serum or controls.

Replace caps on all reagents immediately after use.

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.

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.

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 20°C to 25°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


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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