Atlas Medical

TPHA TEST KIT

A passive particle agglutination assay for the qualitative and semi-quantitative detection of IgG and IgM antibodies to *Treponema pallidum*

IVD For *in vitro* diagnostic and professional use only

2°C 8°C Store at 2° to 8° C CE

INTENDED USE

TPHA test kit is designed for the detection of antibodies to *Treponema pallidum* (IgG and IgM antibodies) in human serum or plasma based on the principle of passive particle agglutination.

INTRODUCTION

Syphilis is a venereal disease caused by the spirochaete microorganism *Treponema pallidum*. As this organism cannot be cultured on artificial media the diagnosis of syphilis depends on the correlation of clinical data with the specific antibody demonstrated by serological tests. Serological screening tests for syphilis using cardiolipin and lecithin as antigens are simple to perform but biological false positive (BFP) reactions occur frequently because the tests use non-treponemal antigens.

The TPI and FTA-ABS tests utilize pathogenic *Treponema pallidum* as the antigen but these tests present some difficulties for routine serodiagnosis. The TPI test requires living pathogenic *T.Pallidum* and the FTA-ABS test requires a fluorescence microscope. Both tests require a high level of expertise.

TPHA test kit has been shown to be a convenient and specific test for the diagnosis of treponemal infection, having specificity similar to that of the TPI test and sensitivity comparable to that of the FTAABS test. It requires minimum laboratory equipment and is very simple to perform.

PRINCIPLE OF THE TEST

Atlas TPHA uses preserved avian erythrocytes coated with extracted antigens of T.pallidum (Nichols strain). Specific antibodies present in a sample of plasma or serum bind to these antigens when the sample is incubated with the particles. This causes the particles to agglutinate, then settle to form a characteristic pattern in the test well.

Non-specific reactions are eliminated by the use of absorbents. The assay can be run and interpreted manually or with an auto-analyzer using an agglutination interpretation program.

MATERIALS PROVIDED

- Test cells; avian erythrocytes coated with antigens of *T. Pallidum.*
- Control cells; avian erythrocytes.
- Sample Diluent; Saline solution containing absorbents.
- Positive control; Rabbit antiserum, titer 1/1280, **Pre-diluted.**
- Negative control; Normal Rabbit Serum, **Pre-diluted.**
- Package Insert.

MATERIALS NEEDED BUT NOT PROVIDED

- Micropipettes capable of delivering: 10, 25, 75 and 190µl.
- U-Well microtitration plates.

PACKAGING CONTENTS

REF **8.00.19.0.0200 (2x20 ml Diluent, 2x8.5 ml Control Cell, 2x8.5 ml Test Cells, 1x1 ml Positive Control, 1x1 ml Negative Control)**

REF **8.00.19.0.0100 (20ml Diluent, 8.5ml Control Cell, 8.5ml Test Cells, 1ml Positive Control, 1ml Negative Control)**

PRECAUTIONS

- For i*n vitro* diagnostic and professional use.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- If spillage of reagent occur clean with disinfectant (disinfectant used could be irritable so handle with care).
- The test is for well-trained professional health user not for lay user.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Reagents and controls contain 0.1% sodium azide as a preservative which is toxic and can be absorbed through the skin when drained, the drains should be thoroughly washed with water.
- The reagent is considered toxic, avoid drinking, ingestion and contact with skin or mucus membrane.

REAGENTS HANDELING

- All the reagents must be allowed to equilibrate to room temperature before use.
- Do not freeze any of the reagents.

REAGENTS STORAGE

- Store bottles upright at **2–8°C**.
- **Do not freeze**
- Do not use after the expiry date.

SAMPLE PREPARATION AND HANDLING

- Use fresh serum or plasma samples free of cells and microbial contamination.
- Samples may be stored at 2-8°C for up to 7 days prior to testing.
- Samples can be frozen at -20°C or lower, these should be thawed and mixed prior to testing.

INTERFERING SUBSTANCES AND LIMITATION OF THE TEST

- Atlas TPHA test kit can be used for serum and plasma samples.
- No interfering substances have been identified.
- Atlas TPHA test kit can cross react with other treponemal infections such as *T.pertenue* and *T.carateum* so positive results should be confirmed by another method.
- In early primary syphilis, occasionally, specific antibodies may not be detected.

PROCEDURES

Bring all reagents and samples to room temperature before use.

Kit controls must be run with each assay.

Ensure Test and Control Cells are thoroughly re-suspended.

QUALITATIVE METHOD

Each sample requires 3 wells of a microtitration plate.

1. Add 190 µl of diluent to Well 1.
2. Add 10 µl sample to Well 1. (Sample dilution 1:20).
3. Using a micropipette, mix contents of Well 1 and transfer 25 µl to Wells 2 & 3.
4. Ensure that the Test and Control Cells are thoroughly suspended. Add 75 µl of control cells to Well 2. Add 75µl of Test Cells to Well 3.
5. Tap the plate gently to mix the contents thoroughly.
6. Incubate 45-60 minutes at 15-30° C. **Caution! Keep the plate away from heat, direct sunlight and any source of vibration.**
7. Read results. Results are stable if the plate is covered and the above precautions are observed.

NOTE

Kit controls must be run in parallel and are diluted and ready for use.

SEMI-QUANTITATIVE TEST

9 wells are needed for each sample.

Sample Dilution (to 1 in 20)

1. Add 190µL of sample diluent to a well.
2. Add 10µL of sample to the same well. Mix thoroughly.

Note: Kit controls are pre-diluted (i.e. diluted 1 in 20)

Titration

1. Leave the first well empty, add 25µL of diluent all other wells in the sequence.

2. Transfer 25µL from step 1 to the first well.
3. Transfer 25µL from step 1 to the second well and mix, then serially dilute along the well sequence, discard the excess 25µL from the final well.

Test

Re-suspend the Test and Control Cells thoroughly

4. Add 75µL of Test Cells to each well.

(Final sample dilution is 1 in 80 – 1 in 10,240)

5. Mix wells thoroughly.
6. Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes. Caution! Keep the plate away from heat, direct sunlight and any source of vibration.
7. Read results. Results are stable if the plate is covered and the above precautions are observed.

The titer of the sample is the reciprocal of the final positive sample dilution.

INTERPRETATION AND ASSAY VALIDATION

Assay Control

The Kit Controls must be give the correct result; Negative is Negative and Positive is Positive. When the Kit Positive is titrated the expected end point is 640 – 2560.

Positive **Equivocal Negative**

A sample where the Test Cell well is non-reactive should be considered as **negative for *T.pallidum***. Reactivity less than equivocal is considered negative.

A sample where the Test Cell well is reactive indicates antibodies to *T.pallidum* resulting from a syphilis infection. The sample should be repeated in duplicate. Where 2 or more wells are positive the sample should be considered as **positive *for T.pallidum***.

A repeatable equivocal sample should be considered positive.

Where a sample is reactive in both Test and Control Cells, if the agglutination is greater in the Test Cells, then the sample is considered positive and should be repeated as above.

Where a sample has greater or equal agglutination in the Control Cells then the sample should be absorbed using the following procedure.

Absorption of Non-specific Reactions

1. Add 10µL of sample to 190µL of re-suspended Control Cells, mix thoroughly and leave for 30 minutes.
2. Centrifuge to deposit the cells at a minimum of 1500g for 3 minutes.
3. Add 25µL of supernatant from step 2 to each of 2 wells.
4. Ensure Test and Control Cells are re-suspended.

   Add 75µL of Test Cells to the first well.
   Add 75µL of Control Cells to the second well.
5. Mix wells thoroughly and Incubate at 15-30°C on a vibration-free surface for 45 - 60 minutes
6. Read and interpret patterns as above.

PERFORMANCE CHARACTERISTICS

Specificity

A study on 300 donor serum showed 100% specificity. (95% confidence limits 98.8 – 100%).
A study on 300 donor EDTA plasma showed 100% specificity. (95% confidence limits 98.8– 100%).

Sensitivity

A study on 100 syphilis positive samples showed 100% sensitivity. (95% confidence limits 96.6 – 100%).

Analytical sensitivity

Atlas TPHA has an expected sensitivity of between 0.1 and 0.025 IU/ml against the 1st IS for human syphilitic plasma IgG and IgM NIBSC code: 05/132

REFRENCES

1. Rathlev T. - Haemagglutination tests utilizing antigens from pathogenic and apathogenic Treponema pallidum WHO/VDT/RES 1965 ; 77 : 65.
2. Tomizawa T, Kasamatsu S. - Haemagglutination tests for diagnosis of syphilis. A preliminary report. Japan. J. Med. Sci. Biol. 19, 305-308, 1966.
3. Rathlev T. - Haemagglutination test utilizing pathogenic Treponema pallidum for the serodiagnosis of syphilis. Br J Vener Dis 1967 ; 43 : 181-5
4. Tomizawa T. Kasamatsu S. Yamaya S. - Usefulness of the haemagglutination test using Treponema pallidum antigen (TPHA) for the serodiagnosis of syphilis. Jap J Med Sci Biol 1969 ; 22 : 341-50.
5. Sequeira P,J,L. Eldridge A,E. - Treponemal Haemagglutination test. Br J Vener Dis 1973 ; 49 : 242-8.
6. Larsen S.A., Hambie E.A., et coll., Specificity, sensitivity and reproducibility among the fluorescent treponemal antibody absorption test, the microhemagglutination assay for Treponema pallidum antibodies, and the hemagglutination treponemal test for syphilis. J. Clin. Microbiol., 1981; 14 : 441 – 445.
7. Wasley G.D. & Wong H.H.Y. Syphilis Serology Priciples and Practice. Oxford Medical Publications 104 - 105

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REF	Catalogue Number		Temperature limit
IVD	In Vitro diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry

***: Indication of the introduced modifications.**

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