BIOLABO
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TPHA

Haemagglutination Assay for qualitative and semi-quantitative determination of antibodies to *Treponema pallidum* in human serum or plasma.

REF 4500100 : 100 tests	**R1**	20 mL	**R2**	7.5 mL	**R3**	7.5 mL	**R4**	1.0 mL	**R5**	1.0 mL
REF 4500200 : 200 tests	**R1**	2 x 20 mL	**R2**	2 x 7.5 mL	**R3**	2 x 7.5 mL	**R4**	1.0 mL	**R5**	1.0 mL

TECHNICAL SUPPORT AND ORDERS
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CE

IVD IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1) (2)

Syphilis is a veneral disease caused by the spirochaete micro-organism *T.pallidum*. As the organism cannot be cultured on artificial media the diagnosis of syphilis depends on the correlation of clinical data with the detection of specific antibody 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 (*Treponema Pallidum* Immobilization) and FTA-ABS (Fluorescent Treponema Assay) tests utilise 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.

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

PRINCIPLE

TPHA reagents are used to detect human serum/plasma antibodies to *T.pallidum* by means of an indirect haemagglutination (IHA) method.
Preserved avian erythrocytes are coated with antigenic components of pathogenic *T.pallidum* (Nichol's strain). These Test Cells agglutinate in the presence of specific antibodies to *T.pallidum,* and show characteristic patterns in microtitration plates.
Any non-specific reactions occurring are detected using the Control Cells, which are avian erythrocytes not coated with *T.pallidum* antigens.
Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes, included in the Test cells suspension.
Test results are obtained in 45-60 minutes and the cell agglutination patterns are both easily read and long lasting.

REAGENTS

Vial R1 **Diluent Buffer**

Specimen diluent

Vial R2 **Test cells Suspension**

Preserved avian erythrocytes sensitised with *T. pallidum* antigen.

Vial R3 **Control cells Suspension**

Preserved avian erythrocytes.

Vial R4 **Positive Control Serum (prediluted 1/20)**

Human serum containing antibodies against *T. pallidum*.
Ready for use. This will give an equivalent titer of 1/640 to 1/2560 with the quantitative test.

Vial R5 **Negative Control Serum (prediluted 1/20)**

Human serum free of antibodies against *T. pallidum*.

REAGENTS PREPARATION

Reagents are ready for use.

MATERIAL REQUIRED BUT NOT PROVIDED

1. Accurate pipettes for delivering 10, 25, 75, 190 µL.
2. U well micro titration plates.

STABILITY AND STORAGE

Store at 2-8°C away from light.

- When free from contamination, stored in the original vial and used as described in this technical data sheet, reagents are stable until expiry date stated on the label of the kit and components.
- Reagents should be stored all times in an upright position.
- Discard any reagent if contaminated or do not demonstrate correct activity with controls.
- Do not use reagents after the expiry date.
- The reagents in each kit have been standardised to produce the proper reaction and reagents should not be interchanged with those from other batches.

SAFETY CAUTIONS

BIOLABO reagents are designated for professional, in vitro diagnostic use.

- Use adequate protections (overall, gloves, glasses).
- Do not pipette by mouth.
- Do not use damaged or contaminated components.
- In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water.
- Reagents contain sodium azide (concentration < 0.1%) which may react with copper and lead plumbing. Flush with plenty of water when disposing.
- Controls contain human serum. Human serum used have been tested and found to be negative for HIV, HCV and HbsAg. Because no known test method can offer complete assurance that infectious agents are absent, this material should be handled as potentially infectious.
- For further information, Material Safety Data Sheet is available upon request.
- Waste disposal : Respect legislation in force in the country. Non disposable apparatus must be sterilised after use by an appropriate method. Disposable apparatus must be treated as biohazardous waste and autoclaved or incinerated. Spillages of potentially infectious material should be absorbed and disposed of as above. The site of spillage must be sterilised with disinfectant or 70% alcohol.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

SPECIMEN COLLECTION AND HANDLING

Serum: 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.

Plasma: Obtain a sample of venous blood from the patient and add to plasma collection vial. Centrifuge specimen and collect clear plasma. Fresh plasma samples are required.

Do not use haemolysed, contaminated or lipaemic serum or plasma for testing as this will adversly affect the results.

Specimen may be stored at 2°C to 8°C for up to 7 days prior to testing.

If longer storage is required, store at –20°C (once only).
Thawed specimens must be mixed prior to testing.

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation | REF Catalogue number | See insert | LOT Batch number | Store away from light | sufficient for | → dilute with

INTERFERENCES

Syphilis antibodies detected in the TPHA test persist after successful treatment. Therefore a positive test may indicate past or present infection.

In common with other serological tests TPHA cannot distinguish between syphilis and other pathogenic treponemal infections, eg. Yaws.
Clinical evidence should always be considered.

Although the TPHA test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders.

For confirmation the FTA-ABS test should be used, since it allows a differentiation between IgG and the early IgM antibodies. The FTA-ABS test is also very useful in very early syphilis where the haemagglutination test may be negative.
For therapeutic control it is advisable to use a quantitative test such as VDRL or RPR test.

QUALITY CONTROL

Positive and Negative control included in this kit.
External quality control program.

It is recommended to control in the following cases :

- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.

If control is is not correct, apply following actions :

1. Repeat the test with the same control.
2. If control is still not correct, try again with a new vial of control(s).
3. If control is still not correct, try again with a new vial of reagent.
4. If control is still not correct, please contact BIOLABO technical support or your local Agent.

PERFORMANCES CHARACTERISTICS

Specificity : Two independent studies on 2900 donor sera each showed 100 % consensus with existing test methods. The initial reactive rate was 0.1%, and the repeat reactive rate was 0%.
An independent study on 200 antenatal sera showed 100% specificity (95% confidence 98.04 – 100%).
Sensitivity :In-house studies on 110 known positive specimens gave 100% positive results. (95% confidence 98.04 – 100%). This included 2 specimens negative by other commercially available TPHA tests but positive by FTA and specific IgM EIA tests.

MANUAL PROCEDURE

QUALITATIVE METHOD

Each sample requires 3 wells of a microtitration plate.

1. Allow each component to reach room temperature before use.
2. Add 190 µL of diluent to Well 1.
3. Add 10 µL serum to Well 1.
4. Using a micropipette, mix contents of Well 1 and transfer 25 µL to Wells 2 and 3.
5. Shake gently and re-suspend Test and Control Cells.
6. Add 75 µL of control cells to Well 2.
7. Add 75 µL of Test Cells to Well 3.
8. Shake the plate gently to mix the contents thoroughly.
9. Cover the plate and protect to direct sunlight, heat and any source of vibration.
10. Incubate 45-60 minutes at room temperature.
11. Read results. Results are stable for 24 hours if the plate is covered and the above precautions are observed.
12. At the end of the test discard any used plate (see § Safety Caution).

SEMI-QUANTITATIVE METHOD

Each sample requires 8 Wells of a microtitration plate. Labelled wells A to H.

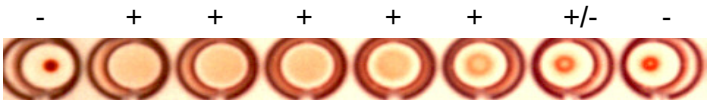
1. Add 25 µL of diluent (vial R1) to wells B to H inclusive.
2. Transfer 25 µL of this 1/20 serum dilution from screening test to Wells A and B. Mix well the contents of well B.
3. Take 25 µL of diluted serum from Well B and serially dilute from Wells B to H inclusive in 25 µL aliquots, discarding 25 µL of diluted serum from Well H.
4. Ensure the Test Cells are thoroughly resuspended. Add 75 µL of Test cells to wells A to H inclusive. This will give a dilution of serum of 1/80 in Well A to 1/10240 Well H.
5. Shake the plate gently to mix the contents thoroughly.
6. Cover the plate and protect to direct sunlight, heat and any source of vibration.
7. Incubate for 45-60 minutes at room temperature.
8. Read results. Results are stable for 24 hours if the plate is covered and the above precautions are observed.
9. At the end of the test discard any used plate (see § Safety Caution).

INTERPRETATIONS OF RESULTS

Qualitative method :

RESULTS	TEST CELLS	CONTROL CELLS
Strong Positive	Full cell pattern covering the bottom of the well.	No agglutination tight button
Weak Positive	Cell pattern covers approx. 1/3 of well bottom	No agglutination tight button
Indeterminate	Cell pattern shows a distinctly open centre	No agglutination tight button
Negative	Cells settled to a compact bottom, typically with a small clear centre.	No agglutination tight button
Non-specific *	Positive reaction	Positive reaction

Quantitative method :



The titer is the highest diution showing agglutination.

Any specimen giving less agglutination than "+/-"is Negative.
Any specimen giving greater agglutination than "+/-"should be noted as provisionally positive, and the test procedure repeated as above, but in duplicate, adding the Control Cells provided to one set of wells, and Test Cells to the other.
If the agglutination with Test Cells is greater than with Control Cells, the specimen is positive for anti-treponemal antibody, and should be subjected to further tests for confirmation.

If the agglutination with Control Cells is greater or equal to that with Test Cells, the procedure below for absorption of non-specific reactions should be applied.

* Non-specific adsorption « Procedure » :

1. Add 100 µL of test serum to a small tube then add 400 µL of Control Cells. Mix well and let stand for 1 hour.
2. Centrifuge for 15 minutes at 1000 rpm and test the supernatant by the qualitative method.

Note: The sample is now at 1/5, this should be taken into account when preparing the dilutions. If the result is repeatedly non-specific the sample should be tested by another method eg. Reagin or FTA-ABS.

Interpretations of results :
Strong positive reactions may show some folding at the edge of the cell mat. When the Test well is positive, the Control well should be observed.
The Control cells should settle to a compact button. They should not be used as a comparison for Non-Reactive serum patterns since the Control Cells will give a more compact pattern than the Test Cells.
Agglutination in the Control well indicates the presence of non-specific agglutinins in the sample, the test should be reported as **INVALID**. A serum that gives this result may be absorbed using the Control Cells as detailed under Non-specific absorption.
A doubtful reaction with Test Cells should be reported as **INDETERMINATE.** This result may indicate a low level of antibody in early primary syphilis or yaws. This sample should be first retested in the qualitative test then a further sample should be tested at a later date to determine whether or not there is a rising titre. It is also advisable to perform a reagin test and/or another confirmation test (FTA-ABS) to complete the profile of the test serum.

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