**1. INTENDED USE**

Treponeema pallidum haemagglutination test (TPHA) for the serodiagnosis of Syphilis.

Syphilis is a sexually transmitted disease. The causative organism is *Treponeema pallidum*, a spirochaete which cannot be grown on culture media or in tissue culture. Diagnosis of infection is normally performed by the detection of antibodies specific for *Treponeema pallidum* in the patient’s blood or CSF.

Detection of the antibody becomes possible after 3–4 weeks following infection. Detectable levels may remain for long periods after treatment. Two groups of antibodies are formed in response to infection:

- **1.1. Antibodies reactive with non-treponemal antigens** (reagin antibodies)
  - Reagin antibodies are normally found in the active disease. They are detected by the VDRL/Carbon Antigen and RPR tests (Oxoid VDRL Carbon Antigen Test (DR525M)). Reagin antibodies usually appear after successful treatment.

- **1.2. Antibodies reactive with the specific antigens of T. pallidum**
  - Specific antibodies persist long after the infection has been successfully treated.

The TPHA test will detect these antibodies. The Oxoid TPHA test is a sensitive passive haemagglutination test specifically for the detection of antibodies to *T. pallidum*.

**2. PROCEDURE**

- **Tanned fowl erythrocytes** are coated with specific antigen and suspended in a diluent. When diluted positive samples are mixed with the test cell suspension, antibody to the sensitising antigen causes agglutination of the cells. The cells form a characteristic pattern of cells in the bottom of a microtitration plate.

**3. COMPONENTS OF THE KIT**

**DRS31 Test Cell Suspension**
- 2 bottles each containing 8-5 ml of uncoated formalised tanned fowl erythrocytes. The dropper bottle will dispense 75 μl drops. Each kit contains sufficient suspension for 200 tests.

**DRS32 Control Cell Suspension**
- 2 bottles each containing 8-5 ml of uncoated formalised tanned fowl erythrocytes. The dropper bottle will dispense 75 μl drops. Each kit contains sufficient suspension for 200 tests.

**DRS33 Diluent Buffer**
- 2 bottles each containing 20 ml of buffer.

**DRS34 Positive Control Serum**
- 1 bottle containing 2 ml of pre-diluted (1/20) serum, positive for antibodies to *T. pallidum*. The serum should cause agglutination in the screening test and will remain positive to a serum dilution of 1/2560 plus or minus one doubling dilution in the quantitative test.

**DRS35 Negative Control Serum**
- 1 bottle containing 2 ml of pre-diluted (1/20) serum, negative for antibodies to *T. pallidum*.

The human sera used in the manufacture of the controls have been shown to be negative for HBsAg (Hepatitis B surface antigen), Hepatitis C and HIV 1 and 2 antibodies by FDA approved tests.

**4. PRECAUTIONS**

**In vitro**

- This product is for in vitro diagnostic use only.
- Do not freeze.

**Oxoid TPHA test reagents contain 0.095% sodium azide as a preservative.**

**5. STORAGE**

- Store kit at room temperature. Bring reagents to room temperature before use.

**6. CONTROL PROCEDURES**

- The positive and negative control sera provided should be used daily to check the correct working of the test and control cell suspensions before routine tests are performed.

The positive control serum should cause the cells in the test cell suspension to agglutinate to form the characteristic pattern of cells on the bottom of the microtitre well within 60 minutes at room temperature.

Agglutination should not occur with the negative control serum within the 60 minutes. A compact button of cells forms on the base of the wells.

**7. IMPORTANT PROCEDURE NOT**

- Do not allow the reagents to become contaminated by allowing the dropper tip to touch the samples in the microtitre wells.

Ensure that the caps are securely fitted after use to prevent contamination and drying out of the reagents. After use return the kit to the refrigerator ensuring that the bottles are stored in an upright position. The control cells should not show agglutination in the tests. If the control cells show agglutination then anti-cell antibody is present. The test should be repeated after first carrying out the following absorption step on the test serum.

- **Dilute the serum 1 in 4 with the control cells (100 μl + 300 μl).**
- **Allow to stand at room temperature for 45–60 minutes.**
- If centrifuging the sample (1000 rpm/5 minutes) the supernatant should be diluted 1 in 5 (100 μl + 400 μl) in Diluent Buffer. Test this dilution directly (without further dilution) using the Test and Control cell suspensions. It is recommended that a FTA-ABS® test is used as confirmation.

**Kit controls are pre-diluted and should be added directly into individual wells in well 3 and 4 (no diluent required).**

**8. SPECIMEN COLLECTION AND PREPARATION OF TEST MATERIAL**

Obtain a sample of venous blood from the patient and allow clot to form and react. Centrifuge clotted sample and collect clear serum. Do not use specimens which are haemolysed. Serum samples must be clear and particle free. Serum samples must be clear and particle free.

**9. QUALITATIVE ASSAY**

Each test requires 4 wells of a microtitration plate.

**Note:** Kit controls are prediluted and should be added directly into individual wells in row 3 and 4 (no diluent required).

**Method**

1. **Dispense Diluent into the microtitration plate as follows:**
   - In well 1 – 25 μl
   - In well 2 – 100 μl
   - In wells 3 and 4 – 25 μl
2. **Dispense 25 μl of each sample into well 1.**
3. **Dilute samples as follows:**
   - Using a microtitration diluter (or a multichannel pipette set to 25 μl) mix well 1 and transfer 25 μl to well 2. Mix and transfer 25 μl to well 3. Mix and discard 25 μl from well 3.
   - Transfer 25 μl from well 2 to well 4, mix and discard 25 μl from well 4.
4. **Shake the Test and Control cell bottles to fully resuspend before use.**
5. **Add one drop (75 μl) of Test cells to well 4 and one drop (75 μl) of Control cells to well 3.**
6. **Set to 25 μl**

**10. READING AND INTERPRETATION OF RESULTS**

Agglutinated cells form an even layer over the bottom of the well. Non-agglutinated cells form a compact button at the centre of the well. Weakly agglutinated cells form a characteristic ring pattern.

**Agglutination of the Test cells but not the Control cells indicates the presence of specific antibody to *T. pallidum* Absence of agglutination indicates that any antibody if present is below the limit of detection of the system. Do not use the Control cell pattern as an indication of a negative result since they give a more compact button in cells. The test is invalid if there is agglutination of the Control cells.

**11. QUANTITATIVE ASSAY**

**Note:** Kit controls are prediluted and 25 μl should be added directly into individual wells in rows 1, 2 and 3 with doubling dilutions commencing from row 3 (no diluent is required in row 1 or row 2).

- It is intended to routinely quantitate positive results, the screening procedure may be modified by omitting the Control cells and preparing only one final dilution. Most samples will be negative or genuinely positive, and the Control cells may be used in the quantitative procedure below.

1. **Dispense diluent into a microtitration plate as follows:**
   - For each sample and control, dispense 25 μl (1 drop) into each well in one column of the plate.
2. **From the original screening plate transfer 25 μl (1 drop) from well 2 to well 1 of the quantitative plate.**
3. **Prepare doubling dilutions from well 2 to well 8.**
4. **Using the appropriate reagents, add 1 drop (75 μl) of Control cells to wells 1 and 1 drop (75 μl) of Test cells to wells 2 and 8.**

**Method**

1. **Dispense Diluent into the microtitration plate as follows:**
   - In well 1 – 25 μl
   - In well 2 – 100 μl
   - In wells 3 and 4 – 25 μl
2. **Dispense 25 μl of each sample into well 1.**
3. **Dilute samples as follows:**
   - Using a microtitration diluter (or a multichannel pipette set to 25 μl) mix well 1 and transfer 25 μl to well 2. Mix and transfer 25 μl to well 3. Mix and discard 25 μl from well 3.
   - Transfer 25 μl from well 2 to well 4, mix and discard 25 μl from well 4.
4. **Shake the Test and Control cell bottles to fully resuspend before use.**
5. **Add one drop (75 μl) of Test cells to well 4 and one drop (75 μl) of Control cells to well 3.**
6. **Set to 25 μl**

**12. READING AND INTERPRETATION OF RESULTS**

The titre is the highest dilution showing agglutination. The Reactive Control serum should produce a titre within one doubling dilution of 1/2560. The starting dilution for the quantitative procedure is 1/80.

**13. PERFORMANCE CHARACTERISTICS**

The performance of the Oxoid TPHA kit has been validated by an independent European centre. The samples were tested using the Oxoid kit and another commercially available TPHA test. Results were confirmed by FITAABS Test.

A total of 252 samples were tested. The results are shown in Table 1.

**Table 1: Reactivity of Oxoid TPHA test with Clinical Samples:**

<table>
<thead>
<tr>
<th>No. of positives</th>
<th>No. of negatives</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid TPHA</td>
<td>163</td>
<td>83</td>
<td>98.7%</td>
</tr>
<tr>
<td>Commercially available TPHA</td>
<td>161</td>
<td>79</td>
<td>97.5%</td>
</tr>
<tr>
<td>FITAABS</td>
<td>165</td>
<td>87</td>
<td>100%</td>
</tr>
</tbody>
</table>
14. LIMITATIONS

Vinyl microtitration plates are not suitable for use with this test. The use of samples other than serum or CSF 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 treponemas i.e. *T. pertenue* and *T. carateum*.

It is recommended that all positive results be confirmed with the FTA-ABS procedure as samples from some patients with connective tissue disorders, lepromatous leprosy or infectious mononucleosis have been reported to give false positive reactions in this type of test. Antibody activity as detected by tests such as Oxoid TPHA may persist even after successful treatment of the disease. Thus a positive result can indicate a past or present infection.

A fresh infection can be differentiated from a past infection by quantitatively testing sequential samples from the patient. A rising antibody titre is indicative of an acute infection. An FTA-ABS test which can differentiate between IgM (acute) and IgG antibodies should be used for confirmation.

In order to assess the clinical response to treatment it is advisable to use a quantitative reagin test. Oxoid VDRL Carbon Antigen test is available for this purpose.

In early primary syphilis, tests such as Oxoid TPHA test may give a negative result with some samples. In these cases an FTA-ABS test should be performed.

15. REFERENCES


For technical assistance please contact your local distributor.