Biomaghreb

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TPHA
Haemagglutination Test for the
Serodiagnostic of Syphilis

IN VITRO DIAGNOSTIC USE

IVD

REF	49018	100 Tests
REF	49025	300 Tests

CLINICAL SIGNIFICANCE

Syphilis is a venereal disease caused by T. pallidum infection. T. pallidum transmission occurs by direct contact with a productive lesion. The incubation period is about 20 days and the disease progress trough 3 different stages with different symptomatology. The anti-T. pallidum antibodies appears in the first stage and may persist in the 85-90% of treated patients after they have been treated and cured

PRINCIPLE

Avian erythrocytes sensitized with treponema palladium antigen will agglutinate in the presence of serum anti treopnema antibodies to give characteristic patterns micro titration plate. No specific reactions are defined by erythrocytes control (without sensitization).

REAGENT COMPOSITION (100 Tests)

Test Cells	Stabilized avian erythocyte suspension sensitized with T. pallidum antigen 1 vial of 7,5 ml
Control cells	Stabilized avian cells suspension not sensitized 1 vial of 7,5 ml
Diluant Buffer	1 vial of 20 ml
Positive control serum	Pre-diluted at 1/20 1 vial of 1 ml
Negative control serum	Pre-diluted at 1/20 1 vial of 1 ml
V.U. Well microtitration plates	3 plates (100 Tests)

*Serum :

Fresh serum, the serum can be stored at 2 - 8°C for 5 days, for longer periods, the serums should be frozen (-20° C).

*Plasma :

EDTA plasma samples can be used for screening in blood banks.

SAFETY CAUTIONS

Biomaghreb reagents are intended for use by qualified personnel for in vitro use (do not pipette by mouth).

- Refer to the current SDS available on request or at www.biomaghreb.com;
- Verify the integrity of the reagents before use; and
- Disposal of waste: comply with the legislation in force.

For safety reasons, treat any specimen or reagent of biological origin as potentially infectious.

Respect the legislation in force.

PRESERVATION AND STABILITY

At 2-8°C until the expiry date indicated on the label.

PROCEDURE

QUALITATIVE TEST :

- Well n° 1: add 10 µl of test serum to 190 µl of buffer to obtain a 1:20 dilution.
- Mix thoroughly: dispense 25 µl of this dilution into wells 2 and 3.
- Well n° 2 : add 75 µl of control cells
- Well n° 3: add 75 µl of test cells.
- Mix, cover the plate, wait 45 minutes at room temperature, away from any source of heat and vibration.

Caution: Positive and negative control sera, which must be introduced in each series, are pre-diluted to 1/20: make only cups 2 and 3.

QUANTITATIVE TEST

Well	A	B	C	D	E	F	G	H
Buffer	25	25	25	25	25	25	25	25
1/20 serum from cup 1 (µl)	25 ↳	25 ↳	25 ↳	25 ↳	25 ↳	25 ↳	25 ↳	25 ↳
Test Cells(µl)	75	75	75	75	75	75	75	75
Dilution 1/N	80	160	320	640	1280	2560	5120	10240

* Homogenize, cover the plate, and wait at least 45 minutes at room temperature away from all sources of heat and vibration.

INTERPRETATION

- Read the results after 45 minutes.
- Results are stable for 24 hours.
- The images below correspond to reactions performed in U microplates.

4+ : Homogeneous veil of cells covering the entire well, sometimes with folded edges.

3+ : Homogeneous veil of cells partially covering the well bottom

2+: Veil of cells surrounded by a red circle of cells

1+ : Veil of cells surrounded by a thicker red circle of cells

± Cell button with a small central opening

-: Cell button with, or without, a very small central opening

Positive : from (4+) to (1+)

Doubtful : ±

Négative : -

For the interpretation of the results, it is imperative to compare them with the agglutinates of the control sera.

Agglutination in well 2 of the screening test indicates the presence of non-specific agglutination. In this case treat the serum as follows:

For 100 µl of serum, add 400 µl of control cells, shake, and wait 1 hour at laboratory temperature, centrifuge for 5 min at 1000 rpm; repeat the test with the supernatant, bearing in mind that the serum is diluted 1/5 by this treatment (redilute to 1/4 to obtain the equivalent of well 1).

The negative control must give a negative result.

The positive control should give a positive result in the qualitative test and react positively up to the labelled titre ± double dilution in the quantitative test.

QUALITATIVE TEST :

A positive result indicates the presence of T pallidum antibodies resulting from past or present infection.

A negative result indicates the absence of anti-T pallidum antibodies (see method limitations). A questionable result in the qualitative test may correspond to low antibody levels in early stages of syphilis or residual antibody levels in treated syphilis. In this case, an additional specimen may be tested to demonstrate a possible increase in antibody titer.

QUANTITATIVE TEST :

The approximate titre will correspond to the last dilution giving a reaction.

LIMITS

* Although serum is the standard specimen for all syphilis tests, EDTA plasma samples can be used for screening in blood banks. However, some reactions may be false positive. In this case, serum should be re-tested on all initially positive or questionable tests.

* Specific antibodies can persist for a long time, even after successful treatment of the disease. In order to estimate the response to treatment, the use of a reagin re-test (RPR re-test) is recommended

* TPHA may cross-react with other forms of treponemal infections and give false positive reactions with specimens from patients with infectious mononucleosis, leprosy, drug addiction or autoimmune diseases

Occasionally, in some cases of early primary syphilis, specific antibodies may not be detected by the TPHA technique.

Manufacturer	Use by	In Vitro Diagnostic	Temperature Limitation	Catalogue number	See insert	Keep away from light	Sufficient for < n > essais	Batch number