TPHA kit (Syphilis)

Qualitative and semiquantitative determination
by agglutination on a microplate
of antigens to Syphilis

96 tests **REF 6009**

PRINCIPLE

Syphilis or Lue is a venereal infectious disease, whose etiological agent is Treponema Pallidum. The infection is transmitted through wounds on skin. Treponema antigenic structure is not completely known, but three different kinds of antigens can be distinguished: lipoid antigen, proteic antigen and polysaccharide antigen.
The diagnosis can be performed directly on Treponema Pallidum (TPHA method) or indirectly (serum diagnosis), highlighting specific antibodies anti-Treponema (reagins).
TPHA is a specific treponemic test which uses the species-specific polysaccharide antigen. It is a passive hemoagglutination test, which uses avian red blood cells sensitized with an extract of Treponema Pallidum. The red blood cells react and agglutinate with the specific antibodies eventually present in the serum.

REAGENTS

Kits components: **REF 6009**

REAGENT 1 (liquid) **2 x 4 ml**
Test erythrocytes: avian erythrocytes sensitized with Treponema Pallidum extract cultured in rabbit testicles, ready to use.

REAGENT 2 (liquid) **1 x 10 ml**
Control erythrocytes: avian erythrocytes non-reactive for Treponema Pallidum, ready to use.

REAGENT 3 (liquid) **1 x 20 ml**
Diluent: saline solution, ready to use

REAGENT 4 (liquid) **1 x 0,5 ml**
Positive control, ready to use.

REAGENT 5 (liquid) **1 x 1 ml**
Negative control, ready to use.

WARNING: the reagents contain sodium azide (< 0.1%) as preservative. Handle with caution and avoid ingestion and contact with skin.

MICROPLATE U-bottom **1 x 96 wells**

STABILITY: sealed and stored at 2-8°C, reagents are stable up to the expiration date on the label.

SAMPLE

Non hemolized serum.
STABILITY: 1 day at 2-8°C, 1 month at -20°C.

PREPARATION OF THE REAGENTS

Let the reagents reach room temperature before use. Shake Reagent 1 and Reagent 2 gently but thoroughly immediately before use.

MANUAL PROCEDURE OF THE QUALITATIVE TEST

Pour the reagents in the proper microplate wells, as in the following table, using only the first three wells. Before adding the Reagents 1 and 2, prepare the whole curve as per the table. Incubate the plate at room temperature on a perfectly flat surface for at least 50 minutes, covered and away from light.

Well	1	2	3
Diluent (µl)	190	---	---
Sample (µl)	10	25 from well 1	25 from well 1

Once sample dilutions are done, add the Reagents 1 and 2, as per the following table:

Well	1	2	3
Reagent 2 (µl)	---	75	---
Reagent 1 (µl)	---	---	75
Corresponding titer	---	Control	1:80

The positive control is prediluted 1:20 and it is ready to use. It is to be used directly by adding 75 µl of Reagent 1 to 25 µl of control.
The first 1:20 sample dilution can be performed on a separate test tube, to avoid wasting a well.

READING AND RESULTS

The result is negative if on the bottom of the well there is a very defined button of non-hemoagglutinated erythrocytes (if there is a small hole in the middle of the bottom, the result is still negative).
The result is positive if a light layer of agglutinated erythrocytes or an agglutination ring is visible.
If the erythrocytes deposit on the bottom of the well and form a very defined ring, the result is not reliable.
On the bottom of well 2 (control) the erythrocytes deposited must be non-agglutinated. Otherwise (when positive), the serum is not suitable for the test as it contains specific antibodies and the test is to be considered not valid.
In this case follow the below listed procedure for the serum and repeat the test:
- adsorb 25 µl of sample with 0,5 ml of Reagent 2 and incubate at R.T. for 30 minutes
- centrifuge for 5 minutes at 1000 rpm
- collect 25 µl of supernatant and add 75 µl of Reagent 2
- repeat the test.

QUANTITATIVE TEST PROCEDURE

Prepare the dilution curve for the sera as per the table, then add Reagent 1 and 2.

Well	1	2	3	4	5	6	7	8
Diluent (µl)	190	---	---	25	25	25	25	25
Serum (µl)	10	25 from 1	25 from 1	25 from 1	25 from 4	25 from 5	25 from 6	25 from 7 eliminate 25 µl
Before adding the Reagents 1 and 2, prepare all dilutions								
Reagent 2 (µl)	---	75	---	---	---	---	---	---
Reagent 1 (µl)	---	---	75	75	75	75	75	75
Title	---	Control	1:80	1:160	1:320	1:640	1:1280	1:2560

Incubate the same way as for the qualitative method.
The positive control is prediluted 1:20 and is ready to use. It is to be used instead of the step for well 1 stated in the table.

RESULTS

The sample titer is defined as the highest dilution showing reactive results. Follow the same indications given for of the qualitative method.

NOTES

1. Longer reaction times may give false positive results.
2. Always compare the results with the controls.
3. The titer of the positive control is approx. 1:1280.
4. False positive values can be found in leprosy, L.E.S. and viral pneumonia cases.
5. Microplates for reading are available.
6. All reagents have been deactivated and tested for HIV I and II, HBsAg and HCV antibodies. However, they should be treated as potentially infectious.

REFERENCES

1. Tomizawa, T., Kasamatsu, S., (1966). Japan J. Med. Sci. Biol., 19,305-308.
2. Garner, M.F. Bachouse, J.L., Daskalapouls, G. Walsh, J.L. (1972). Brit. J. Vener. Dis., 48, 470-473.
3. Sequeira, PJ.L., Eldridge, A. E. (1973). Brit. J. Venr. Dis.,49, 242-248.

KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	read instructions for use

IVD CE Ed. 02 - Mar 2015 MS

MANUFACTURER

FAR
Via Fermi, 12 - 37026 Pescantina - VERONA - ITALY
phone +39 045 6700870
website http://www.fardiag.com
e-mail: order@fardiag.com e-mail: fardiag@fardiag.com