SYPHILIS TPHA

Qualitative and semiquantitative determination, by passive hemagglutination, of anti-Treponema pallidum antibodies

TEST SUMMARY
Anti-Treponema Pallidum antibodies, contained in the serum or plasma, produce agglutination of erythrocytes coated with antigens of Treponema Pallidum.

SAMPLES
Serum or plasma citrate specimens should be free of blood cells. Stability 7 day at 2-8°C. Specimens needing longer storage should be frozen at -20°C. Frozen specimens should be thawed and well mixed before testing.

REAGENTS
Test cells
Chicken's tannate erythrocytes and coated with total extractive antigen of Treponema Pallidum cultivated in rabbit's testicles; conservative and stabilizer.

Control cells
Preserved chicken erythrocytes; conservative and stabilizer.

Diluent
Saline solution; conservative and stabilizer.

Positive control
Human base stabilized solution of anti-Treponema Pallidum antibodies with a titre that gives a clear agglutination.

Negative control
Proteicl solution not reactive with suspension.

REAGENTS PREPARATION AND STORAGE
Reagents are ready to be use.
Erythrocytes suspension must be resuspended with much care. Make the suspension homogeneous by sweet inversion.
Stability: The reagents are stable until expiration date on the label to 4°C.
Do not freeze.

MATERIAL REQUIRED BUT NOT SUPPLIED
Normal equipment of laboratory.
Microplate with U bottom, micropipette, centrifuge, test-tube for centrifuge.

PRECAUTION
All reagents contain 0.095% of sodium azide.
Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

QUALITATIVE PROCEDURE
Predispose dilutions of serum, into a microplate with "U" bottom, following scheme below, pipetting diluent and serum.
Using the same pipette (inspiring and discharging many times) mix with care contents of well before to transfer into the following well. Discharge 25 µl from last well of every series. Execute test using positive and negative control instead of sample.
The TPHA kit is intended for screening large numbers of specimens and contains only a small volume of Control Cells. It is intended that specimens are screened using only Test Cells in the first instance, and the Control Cells be used when repeating tests on specimens giving a positive result when first tested.

Reagents	Well 1	Well 2	Well 3
Diluent	40 µl	25 µl	25 µl
Samples	10 µl	25 µl from well 1	25 µl from well 2 (reject 25 µl)
Test cells	--	--	75 µl
Shake sweetly the microplate to obtain a mix of reagents into the wells. Incubation sheltered from the vibrations for 60 minutes.			

In case of positive result repeat the analysis using even the control cells as described below.

Reagents	Well 1	Well 2	Well 3
Diluent	40 µl	25 µl	25 µl
Samples	10 µl	25 µl from well 1	25 µl from well 2 (reject 25 µl)
Test cells	--	--	75 µl
Control cells	--	75 µl	--
Shake sweetly the microplate to obtain a mix of reagents into the wells. Incubation sheltered from the vibrations for 60 minutes.			

RESULTS INTERPRETATION
Positive (strong): Full cell pattern covering the bottom of the well.
Positive (weak): Cell pattern covers approx. 1/3 of the well bottom.
Negative: Cells settled to a compact button, typically with a small clear centre.
Indeterminate: Cells pattern shows a distinctly open centre.
Non specific: Positive reaction.

Intermediate results, for ex. a ring of hemagglutination with bottom in centre, indicate uncertain results. The well 2 (control well) has to give negative result to indicate sample fitness to be tested, in fact eventual agglutinates depend on a-specific agglutinines that invalidate the test, therefore they must be removed by incubation of 25 µl of sample with 500 µl of erythrocytes control for 30 minutes and following centrifugation.
Use the supernatant during the test.

SEMIQUANTITATIVE PROCEDURE
Follow the scheme of qualitative procedure continuing dilutions until 10° well.

Reagents	Well 1	Well 2	Well 3	…	Well 10
Diluent	40 µl	25 µl	25 µl	…	25 µl
Sample	10 µl	25 µl from well 1	25 µl from well 2	…	25 µl from well 9 (reject 25 µl)
Erythro-cytes test	--	--	75 µl	…	75 µl
Erythro-cytes control	--	75 µl	--	…	--
Titre	…	…	1 : 80	…	1 : 10240
Final specimen dilution range after addition of cells is 1:80 – 1:10240.					

Shake sweetly the microplate to obtain a mix of reagents into the wells. Incubation sheltered from the vibrations for 60 minutes.

RESULTS INTERPRETATION
According to illustrated modalities, value the last well that shows a clear agglutination.
For the results to be valid the negative control must give a negative result and the positive control must give a titre of 640-2560.

NOTE
- Test gives positive results in case of treated syphilis too.
- Human sera used in controls have been found negative in the reaction with HIV and HbsAg. However, they should be handled with care.
- If the results are incompatibles with clinical presentation, they have to be evaluated within a total clinical study.
- For in vitro diagnostic use only.

CALIBRATION
Positive and Negative control sera should be always used to distinguish an eventual background's agglutination of reactive.

TEST PERFORMANCE
Precision
n = 10 assays of a positive sample
CV = 8.1%
Accuracy = -2.5%

Specificity
Two independent studies on 2900 donor sera have shown 100 % consensus with existing test methods.
An independent study on 200 antenatal sera has shown 100 % specificity.

Sensibility
A study on 110 characterised positive samples gave 100% positive results. This included 2 samples tested negative by other commercial TPHA tests but confirmed FTA positive and IgM EIA positive.

Interferences
False positivity has been verified in case of Leprosy, Infectious Mononucleosis and connective's disease.

WASTE DISPOSAL
Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGEING
CODE AK00601	**(100 TESTS)**
Test cells	1 x 8 ml
Control cells	1 x 5 ml
Diluent	1 x 25 ml
Positive control	1 x 0.5 ml
Negative control	1 x 0.5 ml

CODE AK00600	**(200 TESTS)**
Test cells	1 x 16 ml
Control cells	1 x 10 ml
Diluent	1 x 25 ml
Positive control	1 x 0.5 ml
Negative control	1 x 0.5 ml

REFERENCES
Tomizawa T. e coll. – Jap. J. Sci. Biol. 19; 305 (1966).
Sequiera P.J.L. e coll. – Brit. J. Vener. Dis. 49; 242 (1972).

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SYMBOLS
IVD — Only for IVD use
LOT — Lot of manufacturing
REF — Code number
Storage temperature interval
Expiration date
Warning, read enclosed documents
Read the directions
Biological risk

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