SYPHILIS TPHA

Qualitative and semi-quantitative determination by passive hemagglutination of IgG and IgM anti-*Treponema pallidum* antibodies

TEST SUMMARY

Syphilis is an infection caused by the spirochete *Treponema pallidum* and usually transmitted sexually. The disease can also be transmitted by transfusion with infected blood or during pregnancy from mother to unborn child. Syphilis infection is a chronic condition that progresses through several stages. These stages produce different clinical symptoms. Initially there is an ulcer (syphiloma) usually located on the genitals, subsequently, skin rashes appear followed by long periods of dormancy.
If not treated properly, the infection can cause problems in various organs, with the most serious manifestations affecting the cardiovascular system and the central nervous system. Diagnosing the infection is based on the detection of antibodies in the blood that appear soon after the initial infection.

PRINCIPLE OF THE TEST

Anti-*Treponema pallidum* antibodies, contained in the serum or plasma of the sample, cause agglutination of avian erythrocytes coated with extracted antigens of *Treponema pallidum* (Nichols strain). During incubation the agglutinated particles settle on the bottom of the well forming a characteristic precipitate. Nonspecific reactions are eliminated with the use of absorbents. During the incubation the agglutinated particles are deposited on the bottom of the well forming a characteristic precipitate. Nonspecific reactions are eliminated with the use of sorbents.

SAMPLES

Fresh serum or plasma.
Stability 7 days at 2-8°C. For longer storage periods, store at -20°C or lower. Frozen samples must be thawed and mixed thoroughly before use.

REAGENTS

Test cells: Avian erythrocytes coated with *Treponema pallidum* antigens.

Control cells:
Uncoated Avian erythrocytes coated.

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
Protein solution not reactive with suspension.

PRECAUTION

All Reagents contain <0.1% sodium azide.
All human source materials included in this kit have been tested and found negative or non-reactive for HBsAg, HIV 1 Ag (or HIV PCR (NAT)), HIV 1/2 antibody, HCV antibody, and HCV PCR (NAT).
However, they should be considered as potentially dangerous.

REAGENTS PREPARATION AND STORAGE

The reagents are ready to use.
Stability: stored at 2-8°C until the expiry date indicated on the label, do not use beyond that date. Do not freeze.
The suspensions of Test and Control Cells must be made homogeneous by shaking them many times by gentle inversion. Bring all reagents and samples to room temperature before use.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal equipment of laboratory, Microplate with U bottom, micropipette, centrifuge, test-tube for centrifuge.

NOTE

- As with any diagnostic procedure, if the results are inconsistent with the clinical presentation, the physician should evaluate the data obtained using this test together with other clinical information.
- For in vitro diagnostic use only.

WASTE DISPOSAL

The product is intended for use in professional analysis laboratories. For correct waste disposal, refer to current legislation and the safety information sheets. All human specimens should be handled and disposed of according to local guidelines.

QUALITATIVE PROCEDURE

Prepare dilutions of the sample by pipetting diluent and serum into a "U" bottom microplate, according to the scheme reported.
Using the same pipette (aspirating and discharging several times) thoroughly mix the contents of the well before transferring it to the next well. Discard 25 µL from the last well of each series.
The TPHA kit is for many samples and the packs include a small amount of Control Cells. Screening must be performed the first time using only Test Cells, then it must be repeated with Control Cells for samples that are positive in the previous step.

Reagents	Well 1	Well 2
Diluent	90 µL	25 µL
Sample	10 µL	25 µL from well 1 (Discard 25 µL)
Test Cells	--	75 µL

Gently shake the microplate to obtain a correct mixing of the reagents in the wells.
Incubate at 15-30°C protected from vibration for 60 minutes.

In case of a positive or doubtful result, repeat the analysis using also the Control cells as described below.

Reagents	Well 1	Well 2	Well 3
Diluent	90 µL	25 µL	25 µL
Sample	10 µL	25 µL from well 1 (Discard 25 µL)	25 µL from well 1 (Discard 25 µL)
Test Cells	--	--	75 µL
Control Cells	--	75 µL	--

Gently shake the microplate to obtain a correct mixing of the reagents in the wells.
Incubate at 15-30°C protected from vibration for 60 minutes.

The final dilution of the sample in both well 2 and well 3 is 1/80.

RESULTS INTERPRETATION

The negative result is evidenced by the appearance of a net precipitate of non-agglutinated erythrocytes (bottom), while the positive result is characterized by the presence of a hemagglutinate uniformly distributed over the entire bottom of the well. Intermediate results, for example a hemagglutination ring with a central cap, indicate equivocal results.

Positive **Equivocal** **Negative**

A sample that has not reacted with the Test Cells is to be considered negative for Treponema pallidum.
Reactivities lower than a equivocal result are to be considered negative.
A sample that reacts with the Test Cells indicates the presence of anti-Treponema pallidum antibodies deriving from a syphilis infection, therefore it must be considered positive for *Treponema pallidum*.
A sample which gave equivocal results twice must be considered positive.

Sample that reacts with both Test Cells and Control Cells: if the agglutination is higher in the Test Cells than in the Control Cells it is considered positive.
If the agglutination is equal or greater in the Control Cells compared to the Test Cells, the sample must be treated with the following procedure:

1. In a centrifuge tube Add 10 µL of sample to 190 µL of control cells, mix thoroughly and leave to rest for 30 minutes.
2. Centrifuge to deposit cells at a minimum of 1500 rpm for 3 minutes.
3. Pipette 25 µL of supernatant each into 2 wells.
4. Add 75 µL of Control Cells to the first well and 75 µL of Test Cells to the second well.
5. Shake well and incubate at 15-30°C protected from vibration for 60 minutes and read the results

CALIBRATION / CONTROL

The kit is supplied with controls which should always be used in order to distinguish any background agglutination of the reagent. The controls must be used in the same way as the sample.
The controls must give the correct result; Negative Control is negative and Positive Control is positive. When the Positive Control is titrated the final dilution must be between 1/640 and 1/2560.

SEMIQUANTITATIVE PROCEDURE

Follow the scheme below starting the dilutions from well 3 and continuing to well 10.

Reagents	Well 1	Well 2	Well 3	Wel .4	---	Well 10
Diluent	90 µl	25 µL	25 µL	25 µL	---	25 µL
Sample	10 µl	25 µL **from well 1**	25 µL **from well1**	25 µL from well3	---	25 µL from well 9
Discard 25 µL from well 2 and from well 10						
Test Cells	--	--	75 µL	75 µL	--	75 µL
Control Cells	--	75 µL	--	--	--	--
Titre	--	1/80	1/80	1/160	…	1/10240

Gently shake the microplate to obtain a correct mixing of the reagents in the wells.
Incubate at 15-30°C protected from vibration for 60 minutes.

The final dilution of the sample after Test Cells dispensing ranges from 1/80 in well 3 to 1/10240 in well 10. Evaluate the last well that shows discernible hemagglutination.

TEST PERFORMANCE

Specificity
A study on 300 donor serum showed 100% specificity. (95% confidence limit 98.8 – 100%).
A study on 300 donor EDTA plasma showed 100% specificity. (95% confidence limit 98.8 – 100%).

Sensitivity
A study on 100 syphilis positive samples showed 100% sensitivity (95% confidence limit 96.6 – 100%).

Analytical sensitivity
The test has a sensitivity between 0.1 and 0.025 UI/mL calibrated against 1st International Standard for human syphilitic plasma IgG and IgM NIBSC code: 05/132.

Interference
No interfering substances were identified.
Cross reactions may occur with other treponemal infections such as Treponema pertenue and Treponema carateum, therefore positive results must be confirmed by another method.
Occasionally specific antibodies in an early primary syphilis may not be detected.

PACKAGING

	(100 TESTS)	(200 TESTS)
CODE	**AK00601**	**AK00600**
Test Cells	1 x 8 mL	2 x 8 mL
Control Cells	1 x 5,5 mL	2 x 5,5 mL
Diluent	1 x 25 mL	1 x 25 mL
Positive Control	1 x 0.5 mL	1 x 0.5 mL
Negative Control	1 x 0.5 mL	1 x 0.5 mL

REFERENCES

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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

NOTE: MODIFICATIONS HIGHLIGHTED WITH GRAY BACKGROUND