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TPHA

QUALITATIVE AND SEMIQUANTITATIVE DETERMINATION OF ANTIBODIES ANTI - TREPONEMA PALLIDUM

IVD CE

Packaging
REF. N° HRPR02 **100 Tests**

Reaction principle
TPHA is an indirect haemoagglutination test for the detection of specific antibodies to Treponema Pallidum.
The test use chicken erythrocites coated with components of Treponema Pallidum in the presence of Antibodies anti - Treponema Pallidum the sensitized erytrocites will agglutinate

Reagents composition, Contents and Safety warnings
1. Erytrocites 1x8 ml
Tannate Chicken erythrocytes coated with Treponema Pallidum Antigen cultivated in rabbit testicles - Sodium Azide 0.095%

2. Control Erytrocites 1x5ml
Chicken erythrocytes no treated Sodium Azide 0.095%

3. Diluent 1x25 ml
Saline Solution - Sodium Azide 0.095%

4. Positive Control * 1x0.5 ml
Stabilized solution containing enough human antibody anti - Treponema Pallidum to give visible agglutination - Sodium Azide 0.095%

5. Negative Control 1x 0.5 ml
Protein solution no reactive with the Erythrocytes - Sodium Azide0.095%

6. U - well Microtitration plate 1

The product is not classified as hazardous pursuant to the provisions set forth in EC Regulation 1272/2008 (CLP) (and subsequent amendments and supplements).
Further information on the risks to health and / or the environment are given in the data sheet.

Storage and Stability of Reagents
Store the kit at 2 - 8° C

All the components are stable until the stated expiration date if stored tightly closed and refrigerated

Preparation and Stability of working solution
Erythrocytes	**liquid and ready to use**
Control erythrocytes	**liquid and ready to use**
Diluent	**liquid and ready to use**
Positive and Negative Control	**liquid and ready to use**

Gently mix the Erythrocytes and the Control erythrocytes to obtain homogeneous suspensions before use.

Keep reagents at Room Temperature before use

Samples
Fresh serum or Citratated plasma

The sample may be stored at 2 - 8°C for 7 days, for longer storage should be at - 20°C.
(After thawing and before use the samples must be mixed with great care)

Caution
BIOLOGICAL RISK
* Each unit of source material used in the preparation of Positive Control has been tested by a licensed method and found non reactive for HbsAg and negative for Antibodies to HCV and HIV 1/2.
However no known test can offer complete assurance that products derived from human blood will not transmit Hepatitis, AIDS or other infectious diseases. This product, like all materials of human origin, should be handled as potentially infectious biological material

Safety Precautions
For in vitro diagnostic use only
Do not pipette by mouth
Exercise the normal precautions required for handling laboratory reagents

Qualitative Procedure
Reagents	Well 1	Well 2	Well 3
Diluent	40 µl	25 µl	25 µl
Sample	10 µl	25 µl from well 1	25 µl from well 2
Discard 25 µl from the well 3			
Erythrocytes	-	-	75 µl

Shake the plate gently to ensure that the contents are perfectly mixed.
Place the plate on a flat surface, away from vibrations and direct sunlight, and let stand for 60 minutes before reading the results

Results
Negative: Presence of a definite button of non - agglutinated cells

Positive: Presence of smooth mat of agglutinated erytrocites
(Positive samples should be titrated (see semiquantitative procedure)

Indeterminated: Presence of a ring of agglutinated cells with a button in the centre

Note:
In case of positive results should control that aren't false positive by adding 75 µl of Control Erythrocytes into the Well 2

If well 2 give Negative result, the sample is sample for testing and real positive
If well 2 give Positive result, instead, the sample is not suitable for testing due to the presence of non specific agglutinins.
To eliminate non specific agglutinins mix 25 µl of sample with 500 µl of Control Erythrocytes, incubate for 30 minutes and than centrifuge for 5 minutes.
Repeat the test using 25 µl of the supernatant

Semi-quantitative Procedure
Reagents	Well 1	Well 2	Well 3	Well 4	...	Well 10
Diluent	40 µl	25 µl	25 µl	25 µl	...	25 µl
Sample	10 µl	25 µl from well 1	25 µl from well 2	25 µl from well 3	...	25 µl from well 9
discard 25 µl from the well 10 and then add 75 µl of Erythrocytes to each well containing 25 µl of diluted sample from well 3 to well 10						
Erythrocytes	-	-	75 µl	75 µl	...	75 µl
Control Erythrocytes	-	75 µl	-	-	...	-
TITRE*	-	-	1:80	1:160		1:10240

*** Therefore the Titre of the sample can be between 1:80 (well 3) and 1:10240 (well 10)**

Shake the plate gently to ensure that the contents are perfectly mixed.
Place the plate on a flat surface, away from vibrations and let stand for 60 minutes

Results
See qualitative procedure and consider the last well showing visible agglutination.

Note: The results are reliable if the negative control shows negative result and the positive control shows a titre between 640 and 2560

Performance Characteristics
A. PRECISION
N = 10 (positive sample)
CV = 8.1%

B. SENSIBILITY
A study on 110 positive samples gave 100% of positive results

C. ACCURACY
Comparison between this method and another commercial one on 2900 samples gave 100% correlation

D. INTERFERENCES
False positive results can be caused by some diseases such as Leprosy, IM and connective Tissue disorders

Notes
1. False positive results can happen in the case of treated syphilis
2. The final diagnosis should not be made using a single test but should be based on more than one result and confirmed by clinical data

Quality control
Positive and Negative Control Sera should be run with each serie.
Their results should be compared with those of unknown specimen to distinguish possible agglutination of the reagent

Bibliography
1. Tomizawa T. et all.: Jap. J. Sci. Biol. 19, 305 (1966)
2. Sequiera P. J. L. et all : Brit. J. Vener. Dis. 49, 242 (1972)