TPHA

Microplate hemagglutination

REF. 2029 100 tests

CE IVD

DNV CERTIFIED COMPANY
UNI EN ISO 9001:2008
UN EN ISO 13485:2012

GIESSE DIAGNOSTICS

INTENDED USE

Qualitative determination of anti-treponema pallidum antibodies.

PRINCIPLE

The TPHA is an indirect hemagglutination test for the qualitative and semi-quantitative detection of specific anti-T. pallidum antibodies in human serum. Stabilized avian erythrocytes sensitised with an antigenic T. pallidum solution, agglutinates in the presence of anti-T. pallidum antibodies to give a characteristic patterns.

SAMPLE

Fresh Serum. Stable 8 days at 2-8°C or 3 months at -20°C.
Samples with presence of fibrin should be centrifuged before testing.
Do not use highly hemolized or lipemic samples.

KIT COMPONENTS

Reagent (A) Test Cells Volume = 7.5 ml	Stabilized avian erythrocytes sensitised with T. pallidum (Nichols) antigens, pH 7.2. Preservative.
Reagent (B) Control Cells Volume = 7.5 ml	Stabilized suspension of avian erythrocytes. pH 7.2 Preservative.
Reagent (C) Diluent Volume = 20 ml	Phosphate buffered, pH 7.2, T. pallidum (Reider) extract. Preservative.
Control (+) Volume = 1 ml	Immune human serum prediluted 1:20 . Preservative.
Control (-) Volume = 1 ml	Animal serum. Preservative.

The Reagents are stable until the expiration date printed on the label, when stored tightly closed at 2-8°C . Once opened, the reagents are stable one month if contamination is avoided. Do not freeze.
Store the vials in vertical position. Horizontal position may cause cellular clusters.

REAGENT PREPARATION

All the kit components are ready to use.

ADDITIONAL EQUIPMENT

U-well microtitration plates.

PRECAUTIONS AND WARNINGS

Biological risk for Control (+)
Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Use the normal precautions required in the laboratory.
Components from human origin have been tested and found to be negative for the presence of HbsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.
Dispose of waste according to local laws.

PROCEDURE

QUALITATIVE METHOD:
Allow the reagents and sample to reach room temperature
Dilute serum 1:20 with Reagent (C) Diluent. (10 µl serum + 190 µl Diluent)
Pipette into adjacent wells of a microtitration plate: (1)

Sample 1:20 or Controls (2)	25 µl	25 µl
Reagent (A) - Test Cells	75 µl	---
Reagent (B) - Control Cells	---	75 µl

Mix thoroughly the microplate till the complete homogenisation of the mixing reaction.
Cover the microplate and incubate at room temperature (15-25°C) for 45-60 minutes. (Keep the microplate away from the vibrations, heat and direct sunlight). Examine macroscopically the agglutination patterns of the cells.

(1) Shake vigorously the vials of both Test and Control Cells immediately before use.
(2) The Control are ready to use, you have not to dilute them.

SEMI-QUANTITATIVE METHOD:
Make two fold dilutions of the prediluted 1:20 sample in Diluent (Reagent C).
Test each dilution as described in the qualitative method.

READING AND INTERPRETATION

Read the results by comparing the agglutination patterns of the Test Cells with the Control Cells (3). Readings are scored and reported according to the following criteria:

Degree of hemagglutination	Reading	Result
Smooth mat of cells covering entire well bottom, sometimes with folded edges.	4+	Reactive
Smooth mat of cells covering part of the well bottom.	3+	Reactive
Smooth mat of cells sorrounded by a red circle.	2+	Reactive
Smooth mat of cells covering less area and surrounded by a smaller red circle.	1+	Reactive
Button of cells having a small hole in centre.		Borderline
Definite compact button of cells, sometimes with a very small hole in the centre.	-	Negative

The Negative Control should not show any agglutination pattern with both Reagent A (Test Cells) and Reagent B (Control Cells).
The Positive Control should only show agglutination patterns with Reagent A (Test Cells).
Any agglutination pattern showed by Control Cells (Reagent B) indicates the presence of non-specific antibodies and cannot be interpreted.
Samples with a borderline pattern should be retested and reported as negatives if the same pattern is reproduced. Reactive samples should be tittered following the semi-quantitative method. The serum titer is defined as the highest dilution showing reactive result.
Clinical diagnosis should not be made on finding of a single test result, but should integrate both clinical and laboratory data.
(3) The agglutination pattern of the Control Cells should not be used as a reference for negative results since Control Cells give more compact button than do the Test Cells.

QUALITY CONTROL

Positive and Negative Controls are recommended to monitor the performance of the reagent and to have a better results interpretation.

PERFORMANCE

Sensitivity: Accurate titer determination of the Reference Material, under the described assay conditions.
Prozone Effect: No prozone effect up to titers 1/163840
Diagnostic sensitivity: 99.5 %
Diagnostic specificity: 100 %
Interferences: bilirubin does not interfere up to 20 mg/dl. Hemoglobin and lipemia do not interfere up to 10 g/l, Rheumatoid factors up to 300 U/ml do not interfere.

METHOD LIMITATIONS

The TPHA test cannot discriminate antibodies anti-T. pallidum from antibodies to other pathogenic treponemas. It is recommended that all positive results be confirmed by alternative procedures.
False Positive Results have been described with sample of patients with mononucleosis, leprosy, autoimmune diseases and drug addiction.
The TPHA test is not useful in determining the effectiveness of the therapy, since the antibodies level remains long time after the disease has been clinically cured and test remains positive.

REFERENCES

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