CHRONOLAB

CE
IVD

TPHA

Microplate hemagglutination

Qualitative determination of anti-Treponema pallidum antibodies

PACKAGING

Ref.: 101-0389	Cont.: 100 tests
Ref.: 101-0177	Cont.: 200 tests

Store at 2 - 8º C.

CLINICAL SIGNIFICANCE

Syphilis is a venereal disease caused by *T. pallidum* infection. *T. pallidum* transmission occurs by direct contact with a productive lesion. The incubation period is about 20 days and the disease progress trough 3 different stages with different symptomatology. The anti-*T. pallidum* antibodies appears in the first stage and may persist in the 85 – 90 % of treated patients after they have been treated and cured.

PRINCIPLE OF THE METHOD

The TPHA (Treponema Pallidum Hemagglutination) 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.

REAGENTS

R1: Test Cells (TC)	Stabilized avian erythrocytes sensitised with T.pallidum (Nichols) antigens, Preservative, pH 7.2.
R2: Control Cells (CC)	Stabilized suspension of avian erythrocytes, Preservative, pH, 7.2.
R3: Diluent (DIL)	Phosphate buffered saline, pH 7.2, T.pallidum (Reiter) extract, Preservative.
Control +	Immune human serum prediluted 1:20. Preservative
Control -	Animal serum, Preservative

PRECAUTIONS

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.

CALIBRATION

The reagent sensitivity is calibrated against the 1st International Standard for Syphilitic serum (WHO).

STORAGE AND STABILITY

All the kit components will remain stable until the expiration date printed on the label, when stored tightly closed at 2 - 8º C and contaminations are prevented during their use. Do not freeze: frozen reagents could change the functionality of the test.

Store the vials in vertical position. Horizontal position may cause cellular clusters.

Reagents deterioration: Presence of clusters, particles and turbidity.

ADDITIONAL EQUIPMENT

- U-well microtitration plates.
- Pippetes 25 - 75 µL.

SAMPLES

Fresh serum or plasma. Stable 8 days at 2 - 8º C or 3 months at –20º C. The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolized or lipemic samples.

PROCEDURE

Notes:

Mix vigorously or on a vortex mixer the vials of both Test and Control Cells immediately before use.

Keep the microplate away from the vibrations, heat and direct sunlight.

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.

Sera with a high level of antibodies may give agglutination patterns with very folded edges.

Qualitative method

1. Allow the reagents and sample to reach room temperature.
2. Dilute serum 1:20 with Diluent (10 µL serum + 190 µL Diluent)
3. Pipette into adjacent wells of a microtitration plate (Note 1):

Sample 1:20 or Controls (µL)	25	25
Control Cells (µL)	75	--
Test Cells (µL)	--	75

4. Mix thoroughly the microplate till the complete homogenisation of the mixing reaction.
5. Cover the microplate and incubate at room temperature for 45 - 60 min. (Note 2).
6. Examine macroscopically the agglutination patterns of the cells.

Semi-quantitative method

1. Make two fold dilutions of the prediluted 1:20 sample in Diluent.
2. Test each dilution as described in the qualitative method.

Limitations of the procedure

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 as FTA-Abs.

False positive results have been described with samples of patients with mononucleosis, leprosy, borreliosis, 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 the test remains positive.

READING AND INTERPRETATION

Read the results by comparing the agglutination patterns of the Test Cells with the Control Cells (Note 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 surrounded 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

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The Negative Control should not show any agglutination pattern with both Test Cells and Control Cells.
The Positive Control should only show agglutination patterns with Test Cells.
Any agglutination pattern showed by Control Cells 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 findings of a single test result, but should integrate both clinical and laboratory data.

QUALITY CONTROL
Positive and Negative controls are recommended to monitor the performance of procedure, as well as a comparative pattern for a better result interpretation.
All result different from the negative control result, will be considered as a positive.

PERFORMANCE CHARACTERISTICS
Analytical sensitivity: Accurate titer determination of the Reference Material, under the described assay conditions (see, Calibration).

Prozone effect: No prozone effect was detected up to titers ≥ 1/163840 (Note 4).

Diagnostic sensitivity: 99.5 %

Diagnostic specificity: 100 %

INTERFERENCES
Bilirubin (20 mg/dL), hemoglobin (10 g/L), lipids (10 g/L) and rheumatoid factors (300 IU/mL), do not interfere. Other substances may interfere[5].

BIBLIOGRAPHY
1. Paris hamelin et al. Feuillets de Biologie 1983: 24(133): 35-42.
2. Tomizawa T et al. Jap L Med Sci Biol 1966; 19: 305-308.
3. Tomizawa T et al. Jap L Med Sci Biol 1969; 22: 341.
4. Sandra A Larsen et al. A manual of Test for Syphilis American Public Health Association 1990: 1-192.
5. Young DS. Effects of drugs on clinical laboratory test, 4th ed AACC Press, 1995.

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