INSTRUCTIONS FOR USE

TPHA TEST

HEMAGGLUTINATION TEST (IHA) FOR QUALITATIVE AND SEMIQUANTITATIVE DETERMINATION OF ANTIBODIES TO *TREPONEMA PALLIDUM*

1 – CLINICAL SIGNIFICANCE AND INTENDED USE

For ***in Vitro*** diagnostic use only

Syphilis, also known as lue is an infectious disease mainly sexually transmitted. Is caused by a bacterium, *Treponema pallidum*, Spirochetes' genus, which appears under a microscope as a small spiral shaped filament. 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. The TPHA test kit is designed for the detection of antibodies to *Treponema Pallidum* in human serum and plasma.

2 - PRINCIPLE OF THE METHOD

TPHA TEST, Haemaglutination Treponema pallidum (TPHA), is an indirect hemagglutination test for the detection and titration of antibodies against the causative agent of syphilis. Stabilized avian erythrocytes sensitized with an antigenic T. pallidum solution, agglutinates in the presence of anti-*T. pallidum* antibodies to give a characteristic patterns.

3 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
TPHA TEST CND: W0105010302 EDMA: 15.01.03.90; RDM: 1425048/R	Latex agglutination test	**UC80500** (100 tests)	1 plastic bottle containing Test Cells: stabilized avian erythrocytes sensitised with *T.pallidum* (Nichols) antigens, preservative, pH 7,2 (8 mL = 100 tests) 1 plastic bottle containing Control Cells: stabilized suspension of avian erythrocytes, preservative, pH 7,2 (8 mL = 100 tests) 1 plastic bottle containing Diluent: Phosphate buffered saline, pH 8,2, T. pallidum (Reiter) extract, preservative, (20 mL) 1 glass bottle containing Positive Control: immune human serum prediluted 1:20. Preservative (0,5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (0,5 mL) Secondary packaging: cardboard box.
TPHA TEST CND: W0105010302 EDMA: 15.01.03.90; RDM: 1425059/R	Latex agglutination test	**UC80515** (300 tests)	1 plastic bottle containing Test Cells: stabilized avian erythrocytes sensitised with *T.pallidum* (Nichols) antigens, preservative, pH 7,2 (24 mL = 100 tests) 1 plastic bottle containing Control Cells: stabilized suspension of avian erythrocytes, preservative, pH 7,2 (24 mL = 100 tests) 3 plastic bottles containing Diluent: Phosphate buffered saline, pH 8,2, T. pallidum (Reiter) extract, preservative, (60 mL) 1 glass bottle containing Positive Control: immune human serum prediluted 1:20. Preservative (1,5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (1,5 mL) Secondary packaging: cardboard box.
TPHA CONTROLLI CND: W0105080801 EDMA: 15.50.01.01; RDM: 1555769/R	Controls for latex agglutination test	**UD80502** (2x0,5 mL)	1 glass bottle with Positive Control: immune human serum prediluted 1:20. Preservative. (0,5 mL) 1 glass bottle containing Negative Control: stabilized liquid control, contain Sodium azide <0.1% (0,5 mL) Secondary packaging: cardboard box.

Positive Control

Warning
H317
P261; P272; P501
(2-methyl-2H-isothiazol-3-one (Proclin 950)

4 - MATERIALS REQUIRED BUT NOT PROVIDED

U-well microtitration plates (REF. UE91600), Timer or clock. *Pipettes 25-75 µL*

5 - PRECAUTIONS AND WARNINGS

- TPHA TEST is a kit for *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel.
- 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.
- Do not pipette by mouth.
- The sensitivity of the test may be reduced at low temperatures. Allow the reagents and samples to reach room temperature (15-30ºC/59-86ºF) before use.
- Do not use after expiration date or if the packaging is damaged. The quality of the reagent cannot be guaranteed beyond their shelf-life date or if the reagents are stored under inappropriate conditions.
- Normal precautions exercised in handling laboratory reagents should be followed. Dispose of waste observing all local, state, provincial or national regulations. Refer to Material Safety Data Sheet for any updated risk, hazard or safety information.
- All operations linked to the use of the test must be performed in accordance with Good Laboratory Practices.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent.
- This product is classified as dangerous according to current European legislation (view above table and consulting the MSDS).
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.masciabrunelli.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.
- Notify Mascia Brunelli Spa and the Relevant Authorities of any serious incidents occurring in connection with the in vitro diagnostic device. complaint@masciabrunelli.it

6 - STORAGE CONDITIONS AND SHELF LIFE

All the kit components will remain stable until the expiration date printed on the label, *when stored tightly closed* at 2-8ºC *and contaminations are prevented during their use*. Do not freeze: *frozen reagents could change the functionality of the test*.
Always keep vials in vertical position. Horizontal position may cause cellular clusters. If the position is changed, gently mix to dissolve aggregates that may be present.
Reagents deterioration: Presence of clusters, particles and turbidity.

7 – SPECIMENS COLLECTION

Fresh serum or plasma. Stable *7 days* at 2-8ºC or *3 months at –20ºC*. The samples should be thawed and mixed prior to testing. Do not use highly hemolysed or lipemic samples. *Samples with presence of fibrin should be centrifuged before testing*.

8 - TEST PROCEDURE

Allow the components of the kit to reach to room temperature (15-30ºC/59-86ºF) prior to testing.

Qualitative test

1.Dilute sample 1:20 with Diluent (10 µL sample + 190 µL Diluent)
2.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

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

Semiquantitative test

Follow for each samples the scheme using all the 7 wells. Test each dilution as described in the qualitative method.

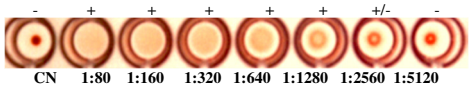
Well	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Diluent (µl)			25	25	25	25	25
Serum 1:20 or Controls (µl)	25	25	25	25 from well 3	25 from well 4	25 from well 5	25 from well 6, than discard 25µl
Control Cells (µl)	75	---	---	---	---	---	---
Test Cells (µl)	---	75	75	75	75	75	75
Titre	Control	1 : 80	1 : 160	1 : 320	1 : 640	1 : 1280	1 : 2560

9 – 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+	Positive
Smooth mat of cells covering part of the well bottom	3+	Positive
Smooth mat of cells surrounded by a red circle	2+	Positive
Smooth mat of cells covering less area and surrounded by a smaller red circle	1+	Positive
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 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 nonspecific 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.

10 – CHARACTERISTICS

- **Analytical sensitivity:** 0,1 IU/mL against the 1st International Standard for human syphilitic plasma IgG and IgM NIBSC 05/132.
- **Diagnostic sensitivity:** 100%
- **Diagnostic specificity:** 100%
- **Interferences:** the follow substances not interfere: bilirubin (20 mg/L), hemoglobin (10 g/L), lipemia (10 g/L) and rheumatoid factors (300 IU/mL). Other substances may interfere[4].

11 – NOTES

1. Mix vigorously or on a vortex mixer the vials of both Test and Control Cells immediately before use.
2. Keep the microplate away from the vibrations, heat and direct sunlight.
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.
4. Sera with a high level of antibodies may give agglutination patterns with very folded edges.

12 – LIMITATIONS OF THE METHOD

- 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.
- As with all diagnostic tests, a final diagnosis cannot rely on the outcome of a single test and must be supported by other clinical parameters.
- The components of this I.v.D. were always tested together without compatibility with components from other manufacturers. While not excluding the possibility that these components can be used with components of the same formulation but produced by other companies, there is no experimental evidence of such compatibility.

13 – REFERENCES

1. Larsen S.A. et al., Clin.Microbiol.Rev., 1995.
2. M.Janier et al., European Guideline on the Management of Syphilis, 2014.
3. Ratnam S. et al., Can J Infect Dis Med Microbiol, 2005.
4. Young DS. Effects of drugs on clinical laboratory test, 4th ed AACC Press, 1995.

TABLE OF APPLICABLE SYMBOLS

IVD	In Vitro Diagnostic Medical Device		Temperature limitation	LOT	Batch code (DXXX)		Manufacturer		Keep dry	UDI	Unique device identifier
	Consult Instructions for use		Use by (year/month)	REF	Catalogue number		Do not reuse		Fragile, handle with care		Keep away from heat

REVISION HISTORY

Version	Description of changes	Date
Instructions for Use (IFU) - Revision 12	Updated layout and content	2022/04

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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