# TPHA Syphilis Test

IVD CE

## Hemagglutination test for detection of *Treponema pallidum* specific antibodies

Instructions for Use (IFU)

**ORDERING INFORMATIONS**

| Format | Code | Composition | |
|---|---|---|---|
| Kit 100 test | **REF. CSI087244** | **n° 1 vl. x 7,5 ml** | (Test Cells) |
| | | **n° 1 vl. x 7,5 ml** | (Control Cells) |
| | | **n° 2 vl. x 10 ml** | (Diluent) |
| | | **n° 1 vl. x 1 ml** | (Positive Control) |
| | | **n° 1 vl. x 1 ml** | (Negative Control) |

**INTENDED USE**

In vitro diagnostic medical device for the execution of Hemagglutination test for the qualitative and semi-quantitative detection of *Treponema pallidum specific* antibodies. Test results should always be interpreted in relation to the clinical context. For professional use only.

**CLINICAL SIGNIFICANCE**

Syphilis is a world-wide chronic systemic infection caused by *Treponema pallidum*, a spirochete. It is mainly transmitted through sexual intercourse. Lues connata is caused by diaplacental infection of the fetus from a mother whose disease is either manifest or latent. Infection through transfused fresh blood is possible.

**PRINCIPLE**

TPHA is a set of reagents for detection of syphilis antibodies. The main constituent of the kit is fixed chicken erythrocytes sensitized with *Treponema pallidum* antigens. This kit is designed to be used with microtiter technique.

**Storage and Stability**

= **Storage temperature 2-8 °C**

Store reagent and controls at 2-8° C avoiding direct light. Do not freeze. If stored as described, reagents are stable until the expiry date printed on the label.

**KIT CONTENTS AND COMPOSITION**

| | | |
|---|---|---|
| **Test Cells** | (Yellow Cap) | Stabilized avian erythrocytes sensitized with *Treponema pallidum* Antigens. |
| **Control Cells** | (Green Cap) | Stabilized suspension of avian erythrocytes |
| **Diluent** | (White Cap) | Phosphate buffer, pH 7.2 |
| **Positive Control** | (Red Cap) | Immune human serum prediluted 1:20 |
| **Negative Control** | (Blue Cap) | Animal serum not reactive |

All the reagents listed above, contain Sodium azide 0.95g/L as preservative.

**OTHER REQUIRED MATERIALS, BUT NOT SUPPLIED**

- Micropipettes, variable volume from 10 to 200 µl
- 96-wells Microplate, U bottom

**PRECAUTIONS AND WARNINGS**

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. In addition to any risk claims relating to active components, reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. All human samples must be handled and disposed as potentially infectious materials. Components from human origin have been tested and found to be negative for the presence of HbsAg, HCV, and antibodies to HIV (1/2). However, handle cautiously as potentially infectious (Biosafety Level 2)
5. The kit should only be used by qualified and properly trained technical personnel.
6. Diagnoses shall only be carried out by authorised and qualified personnel.
7. It is recommended to handle the reagent according to the rules of good laboratory practice and to use appropriate personal protective equipment.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that comply with current standards.
10. Laboratory standards for protection against infection shall be used.

**Reporting of serious incidents**

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

**PROCEDURE**

**Quality Control**

Positive and negative controls should be performed in each run. Negative control must clearly exhibit a button in every well. Positive control must exhibit a uniform agglutination. The hemoagglutination degree must decrease from well 3 to 9 in a progressive way.

**Calibration**

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

**SPECIMEN COLLECTION AND PREPARATION**

Use fresh serum or plasma samples. Sample is 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.

**PROCEDURE**

1 - Allow reagents and samples to reach room temperature before use
2 - Mix well erythrocytes suspension before use

For each specimen 3 wells of a micro titer plate are required for qualitative test and 10 wells for quantitative determination.
Positive and negative controls should be performed in each run.

**Sample treatment**

Pipette 190 µL diluent into well no. 1 of the micro titer plate.
Only for the quantitative test pipette 25 µL diluent from well no.4 to 10.
Add 10 µL of patient's serum into well no. 1, mix, and transfer 25 µL from well no.1 into wells 2 and 3 (qualitative test) or 2, 3 and 4 (quantitative test).
Only for the quantitative test after mixing transfer 25 µL of diluted serum from well no. 4 to no. 5, etc. Discard the final residual 25 µL.

**Control treatment**

For both tests (qualitative and quantitative) pipette 25 µL of positive and negative controls into adjacent wells (11-12).

**Test Execution**

Add 75 µL control erythrocytes into well no.2.
Add 75 µL test erythrocytes into well no. 3 (qualitative test) or 3-10 (quantitative test).

**Incubation**

Mix plate for 1-2 minutes on an orbital plate shaker or by gently tapping each side of the plate several times. Take care not to cross contaminate the well's contents.
Cover the plate to avoid evaporation and leave it on a horizontal surface away from vibration for 45-60 minutes at room temperature and away from sunlight.

**Results and interpretation**

After 45-60 minutes read the results against a white background or using a microtitre plate viewing mirror. The results are read in comparison to controls.
**Positive control**: agglutination
**Negative control**: no agglutination
The titre is expressed as the highest dilution which still gives clear cut agglutination.
The read antibody-titre does not refer to the given serum dilution but to the final dilution of serum in the total volume (including red cells). A positive serum reacts with the sensitized erythrocytes but does not react with control erythrocytes.

| Degree of hemagglutination | Reading | Result |
|---|---|---|
| A smooth mat of cells covering the whole bottom of the wells | +++/+++++ | Reactive |
| Smaller smooth mat, margin wrinkled or red ring with clear center | ++/+++ | Reactive |
| Red ring of cells with clear center definitely larger than that of controls | + | Reactive |
| Button or button with small "hole" | +/-- | Borderline |
| Button with definite margin | - | Negative |

If the patient serum shows agglutination with the control erythrocytes, it must be absorbed with the control erythrocytes to remove the excess of heterophil antibodies. Add 25µL of patient serum to 0,5mL of diluted control erythrocytes. Incubate the mixture for 30 minutes. Centrifuge cells for 5 minutes
The absorbed supernatant must be tested again by mixing 25 µL supernatant with 75 µL sensitized erythrocytes. Continue and read as for the qualitative test.

**PERFORMANCES CHARACTERISTICS**

1. **Analytical sensitivity**: Accurate titer determination of the Reference Material, under the described assay conditions (see Chapter: Calibration).
2. **Prozone effect**: No prozone effect was detected for titers up to 1/163.840.
3. **Diagnostic sensitivity**: 99,5%
4. **Diagnostic specificity**: 100%

**INTERFERENCES**

Hemoglobin (≤10 mg/dL), Bilirubin (≤ 20 mg/dL) e Rheumatoid Factors (≤ 300 IU/mL) do not interfere. Other substances may interfere.

**NOTES**

To get confirmation additional tests are recommended in all those specimens showing a positive result. TPHA can persist as positive in treated syphilis patients. False positive results have been found in patients affected by leprosy, infectious mononucleosis and autoimmune diseases. Diagnosis must be based on the correlation among tests' results and all other clinical contexts.

**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.***


| Symbols used for IFU and Packaging | |
|---|---|
| IVD In vitro diagnostics medical device | Manufacturer |
| REF Catalog number | Instruction for Use |
| LOT Lot Number | Storage Temperature |
| Expiration Date | Biological Risk |

| REVISION | DATE | CHANGES |
|---|---|---|
| D | 10-2022 | Modified for IVDR Compliance |


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