

Title: ASI TPHA Test Kit		Doc#: 6004-980 CLSI
Effective Date: 01/2024	Supersedes Revision/Date: 12/2008	Revision: 01/2024

For in vitro diagnostic use *Rx Only*
Catalog Number Kit Size
980200E 200 Tests
CPT Code: 86781

INTENDED USE

The **ASI TPHA Test** is a qualitative and semiquantitative test for use in the detection of antibodies to *T. pallidum* in human serum. These materials are intended to be acquired, possessed, and used only by health professionals.

SUMMARY AND EXPLANATION

Syphilis is a chronic infection which progresses through distinct stages of infection: primary, secondary, tertiary and quaternary. These stages produce diverse clinical symptoms, typically producing initial chancres then syphilitic rash followed by long periods of dormancy and may eventually lead to cardiovascular problems and neurosyphilis.

Caused by the spirochete *Treponema pallidum*, infection is usually acquired by sexual contact, and the disease may be transmitted by transfusion of infected blood.

Tests for syphilis fall into four categories: direct microscopic examination; treponemal antibody test; non-treponemal antibody tests and direct antigen tests. Because of the long periods of dormancy and the non-specific nature of non-treponemal tests, methods which detect specific anti-treponemal antibodies in patient samples have become increasingly popular for screening. The ASI TPHA Test is one such test.

PRINCIPLE OF THE PROCEDURE

The ASI TPHA Test uses preserved avian erythrocytes coated with antigens of *T. pallidum* (Nichol's strain) to bind with specific antibodies present in patient sera. The cells are suspended in diluent containing components to eliminate non-specific reactions. Positive reactions are characterized by hemagglutination. Test patterns may be interpreted by eye.

REAGENTS

Materials 200 Test Kit

TPHA TEST CELLS 2 x 7.5 ml

TPHA CONTROL CELLS 2 x 7.5 ml

TPHA SAMPLE DILUENT 4 x 10 ml

TPHA REACTIVE CONTROL 1 x 1 ml

TPHA NONREACTIVE CONTROL 1 x 1 ml

ADDITIONAL MATERIALS REQUIRED

1. Volumetric pipet.
2. Disposable, clear plastic trays with 8 rows of 12 U-shaped wells each.
3. Timing device, minute and second capability.

TPHA TEST CELLS - Preserved avian erythrocytes, treated with tannic acid and coated with antigens to *T. pallidum*. The cells are washed and suspended in a medium containing: sorbent,

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rabbit serum and bovine serum albumin (USA source), 0.002% Gentamycin sulphate and 0.1% sodium azide as preservatives, and Tween 20 surfactant in a phosphate-buffered saline solution. Mix well to resuspend the cells before using.

TPHA CONTROL CELLS - Preserved avian erythrocytes, treated with tannic acid. The cells are washed and suspended in a medium containing: sorbent, rabbit serum and bovine serum albumin (USA source), 0.002% Gentamycin sulphate and 0.1% sodium azide as preservatives, and Tween 20 surfactant in a phosphate-buffered saline solution. Mix well to resuspend the cells before using.

TPHA SAMPLE DILUENT - Phosphate buffered saline solution containing absorbers (used to remove possible cross-reacting heterophile antibodies), ox stroma, rabbit serum, and Tween 20 surfactant in a phosphate-buffered saline solution preserved with 0.1% sodium azide.

TPHA REACTIVE CONTROL - Human defibrinated plasma, as required for titer, in a phosphate-buffered saline solution preserved with 0.1% sodium azide. Prediluted 1:20.

TPHA NONREACTIVE CONTROL - Human defibrinated plasma in a phosphate-buffered saline solution preserved with 0.1% sodium azide.

WARNINGS AND PRECAUTIONS

For In Vitro Blood Screening Use Only

1. **ASI TPHA CELLS, DILUENT and CONTROLS** contain sodium azide. Azides in contact with lead and copper plumbing may react to form highly explosive metal azides. When disposing of reagents containing azide, flush down the drain with large quantities of water to prevent azide buildup.
2. **ASI TPHA TEST CONTROLS** contain human plasma which has been tested at the donor level for HBsAg and for HIV-1, HIV-2 and HCV antibodies and found to be non-reactive. As no known test offers complete assurance that infectious agents are absent, the CONTROLS should be considered potentially infectious and "universal precautions" should be used. REACTIVE AND Nonreactive CONTROL material should be handled in the same fashion as donor samples. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories" describes how these materials should be handled in accordance with Good Laboratory Practice.
3. All reagents should be brought to room temperature (15-30° C) prior to use.
4. Do not pipet by mouth.
5. Do not smoke, eat, drink or apply cosmetics in areas where plasma/serum samples are handled.
6. Any cuts, abrasions or other skin lesions should be suitably protected.

STORAGE INSTRUCTIONS Store the kit contents at 2–8°C in an upright position. Do not freeze reagents. Do not use reagents after the expiration date indicated on the container.

INDICATIONS OF DETERIORATION

1. Precipitation or turbidity in the CONTROLS or DILUENT is indicative of deterioration and the component should not be used.

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2. Hemolysis of the TPHA TEST CELL or TPHA CONTROL CELL reagent is indicative of deterioration and the component should not be used.

SPECIMEN COLLECTION AND STORAGE

1. Use serum or EDTA (1.5 - 2.2 mg/mL) plasma for specimens. Serum is preferred. Specimens should be brought to room temperature (15-30° C) before testing.
2. Specimens from pleural fluid, saliva, cadaveric samples or non human species are not acceptable for testing.
3. Samples should be free from bacterial contamination or hemolysis.
4. Specimens should not contain particulate matter. If necessary before testing, centrifuge the specimens at a force sufficient to sediment cellular components.
5. Samples are unacceptable if they contain greater than the following amounts of:
 - Free hemoglobin 1000 mg/dl
 - Phospholipids 1000 mg/dl
 - Total bilirubin 20 mg/dl
6. If a delay of more than 5 days is anticipated before testing, freeze the specimen at -20° C or below. Frozen specimens should be brought to room temperature (15–30°C) and mixed thoroughly before testing. Do not repeatedly freeze and thaw specimens.
7. Heat-treated specimens and plasma using sodium citrate or heparin as anticoagulants should not be used with the ASI TPHA TEST reagents.

PERFORMANCE OF THE TEST

Handling And Procedural Notes

1. In order to obtain reliable and consistent results, the instructions in this package insert must be strictly followed. Do not modify the handling or storage conditions for the reagents or samples.
2. The microplates must be clean before use. Inadequate washing may cause false negative or false positive results.
3. Do not use past the expiration date indicated on the kit.
4. Do not use reagents from one kit with reagents from a kit with a different lot number.

TEST PROCEDURE

Qualitative Assay

1. Sample Dilution (To 1/20)

- Add 190 µl of TPHA Diluent to a well.
- Add 10 µl of sample to the same well.
- Ensure thorough mixing.

Note: Positive & negative controls are already diluted 1/20.

2. Assay

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- Add 25 µl of diluted sample from step 1. to each of 2 wells.
- Gently mix Test Cells and Control Cells to ensure thorough resuspension.
- Add 75 µl of Test Cells to 1st well.
- Add 75 µl of Control Cells to 2nd well.
- Ensure thorough mixing.

Note: Final sample dilution after addition of cells is 1/80.

- Incubate at room temperature on a vibration free surface for a minimum of 45 mins.
- Read & interpret the settling pattern. See Interpretation.

Semiquantitative Assay

1. Sample Dilution (To 1/20)

- Add 190 µl of TPHA Diluent to a well.
- Add 10 µl of sample to the same well.
- Ensure thorough mixing.

Note: Positive & negative controls are already diluted 1/20.

2. Sample Titration

- Leaving the 1st well empty add 25 µl of TPHA Diluent to remaining 7 wells in an 8 well sequence.
- Add 25 µl from step 1 to the 1st well.
- Add 25 µl from step 1 to the 2nd well and mix - then serially dilute along the well sequence; discard the excess 25 µl from the final well.

3. Assay

- Gently mix the Test Cells to ensure thorough resuspension.
- Add 75 µl of Test Cells to all wells.
- Ensure thorough mixing.

Note: Final sample titration after addition of cells is 1/80 - 1/10,240.

- Incubate at room temperature on a vibration free surface for a minimum of 45 mins.
- Read & interpret the settling pattern. See Interpretation.

QUALITY CONTROL

1. The controls must be run with each batch of samples.
2. The Reactive control should give a clear positive result.
3. The Non-reactive control should give a clear negative result.

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INTERPRETATION OF RESULTS

Degree of hemagglutination	Reading	Results
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
Button of cells having a small hole in center.	±	Borderline
Definite compact button of cells, sometimes with a very small hole in the center.	-	Negative

LIMITATIONS OF THE PROCEDURE

1. Serum must be used for any repeat or confirmatory testing on any indeterminate or reactive plasma.
2. Carryover between specimens is a possible source of interference.
3. A diagnosis of syphilis should be made on the basis of a careful history and physical examination together with laboratory results.

EXPECTED VALUES

Specimens found to be reactive using the ASI TPHA Test Reagents are considered to be reactive for IgG and/or IgM antibodies to *T. Pallidum*. Reactive results may indicate an active, past, or successfully treated infection. A diagnosis should be made with a careful history of the patient and a physical examination as well as pertinent laboratory results.

TEST PERFORMANCE CHARACTERISTICS

Specificity

Two independent studies on 2900 donor sera have shown 100% consensus with existing test methods. Initial reactive rate was 0.1%. Repeat reactive rate was 0%. An independent study on 200 antenatal sera has shown 100% specificity.

Sensitivity

In house studies on 110 characterized positive samples gave 100% positive results. This included 2 samples tested negative by other commercial TPHA tests but confirmed positive and IgM EIA positive. An independent study on characterized sera including positive samples from various stages of syphilis and disease conditions other than syphilis have shown excellent performance characteristics.

Bibliography

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