AXIS-SHIELD

MICROSYPH™ TPHA1000

IVD

REF FTPHA1000

Σ 1000

For professional use only

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ENGLISH: INTENDED USE

MICROSYPH™ TPHA1000 is a rapid assay for the detection of specific antibodies to *Treponema pallidum* in human serum or plasma (either di-potassium EDTA, sodium citrate or lithium heparin) by indirect haemagglutination. The kit is intended to be used as an initial screening test.

INTRODUCTION

Syphilis is a venereal disease caused by the spirochaete microorganism *Treponema pallidum*. As this organism cannot be cultured *in vitro* the diagnosis of syphilis depends on the correlation of clinical data with the specific antibody demonstrated by serological tests. Serological screening tests for syphilis utilising cardiolipin and lecithin as antigens are simple to perform but biological false positive (BFP) reactions occur frequently because these tests use non-treponemal antigens[1]. The TPI and FTA-ABS tests utilise pathogenic *T. pallidum* as the antigen but these tests present some difficulties for routine serodiagnosis. The TPI test requires living pathogenic *T. pallidum* and the FTA-ABS test requires a fluorescence microscope. Both tests require a high level of expertise.

TPHA assays have been shown to be a convenient and specific test for the diagnosis of treponemal infection, having a specificity similar to that of the TPI test[6] and a sensitivity comparable to that of the FTA-ABS test[7]. It requires minimal laboratory equipment and is very simple to perform. It can be used in conjunction with automated liquid handling systems for improved throughput in the busy laboratory.

PRINCIPLE OF THE ASSAY

The MICROSYPH™ TPHA1000 test detects human (serum/plasma) antibodies to *T. pallidum* by means of an indirect haemagglutination (IHA) method. Preserved avian erythrocytes are coated with antigenic components of pathogenic *T. pallidum* (Nichol's strain) [2-5]. These Test Cells agglutinate in the presence of specific antibodies to *T. pallidum*, and show characteristic patterns in microwell plates.

Antibodies to non-pathogenic treponemes are absorbed by an extract of Reiter's treponemes included in the cell suspension. Test results are obtained in 60 minutes and the cell agglutination patterns are easily read and stable.

To facilitate the necessary dilution step a blue dye has been added to the Diluent. This changes colour when the sample is added.

KIT COMPONENTS

TEST CELLS	2 × 40mL	Preserved avian erythrocytes coated with sonicated *T. pallidum* antigen in buffer. ❑ **The Test Cells must be thoroughly re-suspended before use.** ❑ **The Test Cells settle out when stored.** ❑ **It is important that settled cells are covered with the buffer during storage at 2-8°C.**	
DIL	1 × 250mL	Buffer containing blue dye. Contains 0.1% sodium azide as preservative. ❑ **Ready-to-use.**	
CONTROL +	1 × 0.5mL	Defibrinated human syphilitic plasma containing antibodies to *T. pallidum.* The human plasma used is non-reactive for hepatitis B surface antigen, HCV, HIV antigen and HIV antibodies when tested by FDA-cleared assays. ❑ **Dilute before use.**	
CONTROL −	1 × 0.5mL	The human serum used is non-reactive for hepatitis B surface antigen, HCV, HIV antigen and HIV antibodies when tested by FDA-cleared assays. Contains 0.1% sodium azide as preservative. ❑ **Dilute before use.**	

STORAGE OF REAGENTS

The reagents in each kit have been matched to produce the appropriate reaction and reagents must not be interchanged with those from other batches.

The kit should be stored **upright** at 2-8°C at all times. Do not use reagents beyond their expiry date. Reagents should be discarded if they become contaminated or do not demonstrate correct activity with the Reactive or Non-Reactive Controls.

A kit was opened and reused in five occasions over a 52 week period with no adverse effect on performance.

SAMPLE AND STORAGE

Serum or plasma samples may be used. Store at 2-8°C if a preservative such as 0.1% azide is added prior to storage. For long term storage, samples should be stored at -20°C. Any visible particulate matter should be removed by centrifugation prior to assay.

SAMPLE DILUTION

Samples, Reactive Control and non-Reactive Control must be diluted 1 in 20 in Diluent. The Diluent contains a blue dye that visibly changes colour from blue to pale green/yellow when the specimen is added.

For spectrophotometric confirmation of sample addition, dilute the samples according to steps 1-2 of the Assay Protocol. Before proceeding to step 3, read the microwell plate in a plate reader at 450nm using 690nm as the reference wavelength if available. If the optical density (O.D.) is less than 0.2 insufficient sample volume should be suspected and a fresh dilution prepared.

Please note that the Reactive and Non-Reactive Controls may give O.D. readings of less than 0.2 due to their composition so extra care must be taken to ensure the Control is added.

Dilutions should be used only on the day of preparation.

WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use only.

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions.
2. Strictly avoid contaminating any of the reagents or sample dilutions with saliva as this will cause confusing patterns similar to a positive result with samples that should be negative.
3. Controls contain human serum or plasma tested by FDA-cleared assays for hepatitis B surface antigen, HCV, HIV antigen and HIV antibodies and found to be non-reactive/negative. As no known test offers complete assurance that infectious agents are absent, Controls should be considered potentially infectious and handled with the same precautions as any other potentially biohazardous material. US Department of Human Health Services "*Biosafety in Microbiological and Biomedical Laboratories*", 5th Edition, Washington, DC: US Government Printing office, January 2007,[14] describes how these materials should be handled in accordance with Good Laboratory Practice.
4. Do not pipette by mouth.
5. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
6. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
7. The Diluent and Non-Reactive Control contain 0.1% sodium azide:

Diluent **Negative Control**	**EUH032**	Contact with acids liberates very toxic gases.

Although the concentration of azide present is low, to prevent the accumulation of explosive lead or copper salts, these materials should not be disposed of via sinks with metal traps or drain lines. All drains should be flushed through with water after use.

8. Material safety data sheets for all components contained in this kit are available on request from Axis-Shield Diagnostics.

PREPARATION

Materials/Equipment Required but not Provided

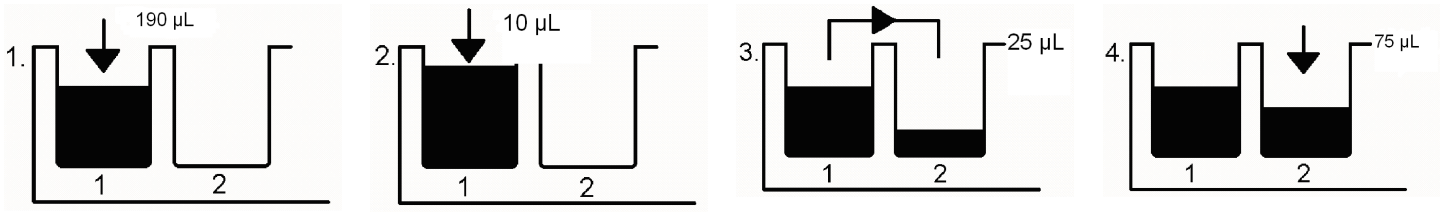
1. Accurate and properly maintained pipettes for delivering 10, 25, 75 and 190 microlitres.
2. Rigid microwell plates with "U"-shaped wells.
3. Microwell plate reader system and/or automated processors (optional). All instruments and interpretation software must be validated before use and operated, maintained and calibrated in accordance with the manufacturers' instructions.

Materials Provided

FTPHA1000 contains sufficient reagents to perform 1000 tests manually. The number of tests obtained using automated systems will depend on the characteristics of the system.

ASSAY PROTOCOL

1. Each test requires 2 wells of a microwell plate. Wipe the microwell plate with a clean damp cloth or tissue to remove any static charge. Add 190µL of Diluent to Well 1.
2. Add 10µL of Sample to Well 1. Using a pipette mix the contents of Well 1. Note: Two further sets of wells are required for the Reactive and Non-Reactive Controls. The Controls should be treated exactly the same as the samples.
3. Transfer 25µL to Well 2.
4. Add 75µL of fully re-suspended Test Cells to the wells containing 25µL of diluted sample or Control.



5. Mix the contents of the plate by tapping all four sides of the plate.
6. Incubate at room temperature for a minimum of 60 minutes.
   Caution: Keep the plate away from heat, direct sunlight and any source of vibration.
7. Read results. If using a reader, read the plate visually first as the reader may agitate the plate when it is ejected from the instrument.

READING THE RESULTS

Visually

Positive Result

A strong positive will appear as a smooth mat of cells on the bottom of the well, sometimes with folded edges. With less strongly reacting samples, this mat will be smaller and may be surrounded by a ring of cells.

Negative Result
A negative result is indicated by a compact button of cells with or without a very small hole in the centre.

Indeterminate Result
An indeterminate result is seen as a button of cells having a small hole in the centre giving the appearance of a well defined dense ring with a fairly clear background around this ring.

Collapsed result
Some very strongly positive samples may give collapsed patterns when tested at a 1/80 dilution. These patterns are similar to an indeterminate result but the dense ring may have a ragged appearance.

Spectrophotometrically

Results obtained spectrophotometrically must also be checked manually.

QUALITY CONTROL

The Non-Reactive Control should not cause agglutination, whilst the Reactive Control should cause agglutination in the test. If this is not the case, this renders the assay invalid and patient sample results should not be reported. If repeating the test, prepare a fresh dilution of each sample and each Control.

INTERPRETATION OF RESULTS

Any sample giving a positive result in the test should be considered reactive in the test. Unless local procedures state otherwise, such samples should be re-tested in duplicate using the original sample. Samples that are reactive in at least one of the duplicate tests are considered repeatedly reactive in the MICROSYPH™ TPHA1000 assay. Such samples should be further investigated and the results from the assay considered with any other clinical and/or assay information.

A negative result indicates the absence of antibodies to *T. pallidum*. In some very early cases of syphilis a negative result may be obtained (see **Limitations of Use**).

An indeterminate results result may indicate a low level of antibody in early syphilis, an old treated syphilis or yaws. In such cases the sample should be re-tested. If this is not possible a fresh sample should be collected as soon as possible and the test repeated, with the patient's clinical condition being taken into consideration.

LIMITATIONS OF USE

1. The MICROSYPH™ TPHA1000 kit does not contain Control Cells. A positive result may therefore be due to a non-specific reaction of the sample with the cells. In order to exclude this possibility any sample reactive in the test should be re-tested using the MICROSYPH™ TPHA200 kit (FTPHA200).

   For confirmation of a positive result the FTA-ABS test should be used, since it allows a differentiation between IgG and early IgM antibodies. The FTA-ABS test is also useful in very early syphilis where the haemagglutination test may be negative.

   For therapeutic control it is advisable to use a quantitative test such as an RPR test. This reagent is available from Axis-Shield Diagnostics Ltd.
2. Although the MICROSYPH™ TPHA1000 test is highly specific, false positive results have been known to occur in patients suffering from leprosy, infectious mononucleosis and connective tissue disorders.
3. Serological tests, including MICROSYPH™ TPHA1000, cannot distinguish between syphilis and other forms of pathogenic treponemal infections[8], e.g. yaws[7]. Clinical evidence should be used to determine which condition is present.
4. Syphilis antibodies detected in the MICROSYPH™ TPHA1000 test persist after successful treatment. Therefore, a positive test may indicate past or present infection [6,7,9,10].

5. Following infection with *T. pallidum*, antibodies (both anti-lipoidal and anti-treponemal) may not appear until 1 to 4 weeks after the characteristic syphilis lesion (chancre) has formed. Thus, in early primary syphilis, tests such as MICROSYPH™ TPHA1000 may give a negative result for some samples[11,12,13]. In late latent/treated syphilis infections antibody levels may drop below the limit of detection of the MICROSYPH™ TPHA1000 assay and therefore may give a negative results. In these cases, alternative testing procedures e.g. microscopic identification of *T. pallidum* should be used.
6. Results obtained using plate reading systems must be checked manually. Depending on the reading parameters some indeterminate or collapsed patterns may be misread as borderline or negative.
7. This test is to be used only with individual (unpooled) serum or plasma samples.
8. Use of haemolysed samples, incompletely clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.

P E R F O R M A N C E C H A R A C T E R I S T I C S

Specificity

1000 donor samples (500 serum and 500 plasma) were assayed in-house with one lot of reagents and a further 1000 donor samples (500 serum and 500 plasma) were assayed with a second lot of reagents, the results are presented below.

No. of Samples	Reagent Lot	No. Samples Positive or indeterminate		Specificity
		Initial	Repeat	
500 Serum	1	0	0	100%
500 Plasma	1	2	0	100%
500 Serum	2	0	0	100%
500 Plasma	2	0	0	100%

Specificity with potentially cross-reactive samples

71 potentially cross-reactive samples were assayed in-house with one lot of reagents and a further 72 samples assayed in-house with a second lot of reagent, the results are presented below.

No. of Samples	Reagent lot	No. Samples Positive or indeterminate	Specificity
71 (see note 1)	1	0	100%
72 (see note 2)	2	0	100%

Note 1 : 18 Rheumatoid Factor positive, 9 Lyme Disease positive, 5 Anti-Cardiolipin positive, 16 antenatal, 12 HCV positive, 6 HIV positive and 5 HBV positives samples

Note 2 : 18 Rheumatoid Factor positive, 9 Lyme Disease positive, 5 Anti-Cardiolipin positive, 16 antenatal, 12 HCV positive, 6 HIV positive and 6 HBV positives samples

Sensitivity

137 samples found to be positive in using ELISA assays were assayed in-house with two reagent lots, the results are presented below.

No. of Samples	Reagent Lot	No. Samples Negative	Sensitivity
137	1	3	97.8%
137	2	2	98.5%

S T A N D A R D I S A T I O N

The MICROSYPH™ TPHA1000 test has been shown to give a 50% agglutination reaction with the WHO 3-1980 reference preparation at a titre of between 1/2560 to 1/10240 using three reagent lots and five operators.

R E F E R E N C E S

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S Y M B O L S

IVD	For *in vitro* diagnostic Medical Device
REF	Catalogue number
LOT	Lot
Σ 1000	Sufficient for 1000 tests
	Caution, consult accompanying documents
	Use by
+8°C +2°C	Store at 2-8°C
CONTROL +	Positive (Reactive) Control
CONTROL −	Negative (Non-Reactive)
TEST CELLS	Test Cells
DIL	Diluent
GTIN	Global Trade Item Number
	Manufacturer

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