

9. Wash the strip five times (see previous page).
10. Dispense 100µl of ready to use TMB Substrate per well. Incubate in the dark for 30 min at room temp (18-23°C).
11. Stop the reaction by adding 100 µl stop solution per well (color shift: blue→yellow). Read the absorbance at 450 nm (optionally with a 620 nm reference filter) within 10 minutes.

INTERPRETATION OF TEST RESULTS

A. Calculations

1. Calculate the mean absorbance value of the cut-off, the positive control, the negative control and patient sera.
2. The value of *T.pallidum*-IgM is expressed in 'Capture Index (CI)' and is calculated as follows:

$$\text{IgM value} = \frac{\text{OD of "X"} \times \text{CI}}{\text{OD of "CO"}}$$

"X" = Controls and Patient Serum
"CO" = Cut-off Serum
"CI" = Capture Index

Use not more than one decimal to express the value. * See also below for validation.

B. Validation of test

1. Cut-off. The absorbance of the cut-off serum should be **between 0.200 and 0.600 OD**
2. Negative control: The *T.pallidum*-IgM value should be < 0.7 CI
3. Positive control: The *T.pallidum*-IgM value should be > 2.0 CI

A test is valid if the above criteria are met. If OD values of a run are out of the indicated range, the validity ranges given for the Capture Units should be considered as the ultimate criteria against which a run is considered valid.

C. Patient sera

Interpretation of IgM test results is as follows:

Positive: A serum should be considered positive for *T.pallidum* specific IgM antibodies when the value is ≥ 1.1 CI. A positive result indicates either that the patient has an active syphilis infection or that the patient was recently treated for syphilis.

Negative: A serum may be considered negative when the *T.pallidum* IgM value is <0.9 CI. A negative result indicates that the patient does not have an active syphilis infection and is unlikely to have recently been treated for syphilis.

Equivocal: A serum may be considered equivocal, if the *T.pallidum* IgM value falls between 0.9 and 1.1 CI. In such case it is advised to confirm the results by testing that serum again in duplicate. In the case the repeated result is again equivocal, a second serum should be tested and judged for a change in result (as expressed in CI).

Manufactured For:

WARRANTY

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QUICK REFERENCE PROTOCOL FOR <i>T.pallidum</i>-IgM EIA	
Preparation of Reagents	Test Procedure
A. Dilute Patient Test Serum Mix 1ml dilution buffer (BLUE) + 10µl patient test serum	
	1. Dispense 100µl per well of each control: Pos (RED) and Neg (GREEN) in duplicate, and Cut-off (YELLOW) in quadruplicate, diluted patient test sera (BLUE) and incubate 60 minutes at 37°C in a resealable bag or in a 100% moist chamber.
B. Prepare diluted conjugate mix per 8-well strip: 1.5ml dilution buffer (BLUE) +15 µl <i>T.pallidum</i> -PO conjugate (100x) +15µl Control Antigen (100x)	
C. Prepare washing buffer mix per 8-well strip 28.5ml distilled water + 1.5ml Washing Buffer (20x).	
	2. Wash 5 times, dispense per well 150µl diluted conjugate (BLUE) and incubate 60 minutes at 37°C in a resealable bag or in a 100% moist chamber.
D. TMB substrate. Ready to use	
	3. Wash 5 times, dispense per well 100µl of substrate and incubate in the dark 30 minutes at room temperature.
	4. Add per well 100µl stop solution and read absorbance at 450 nm (optionally with 620 nm ref).

PHOENIX BIO-TECH CORP.

TREP CHEK™
Anti-Treponema IgM EIA

Catalogue # TPM96

For Investigational Purposes Only

An enzyme immunoassay for detection of *Treponema pallidum* specific IgM antibodies in Human Serum expressed in Capture Index (CI)

INTENDED USE

The *T.pallidum* IgM EIA kit is an enzyme immuno assay for the detection of *T.pallidum* specific IgM antibodies in human serum and is used as an aid in the diagnosis of active *T.pallidum* infections. The assay must be performed strictly in accordance with the instructions set out in this protocol. No responsibility can be held for any loss or damage (except as required by statute) how so ever caused by or arising out of non-compliance with the instructions provided.

INTRODUCTION

Syphilis is caused by *T.pallidum*. During early primary syphilis IgM and IgG class antibodies appear in the blood. In the secondary phase *T.pallidum* antibodies of both IgM and IgG classes reach peak titres. Thereafter the developed *T.pallidum* IgG antibodies remain present in the blood irrespective of the course of the disease. *T.pallidum* specific IgM serum levels are related to disease activity. In the late phases of syphilis serum IgM remain demonstrable. Successful treatment of syphilis in the primary phase shows a more rapid IgM decrease than in a later phase of the disease. A significant decrease in *T.pallidum* IgM titer can be expected within 3-4 months following successful treatment and will disappear usually within 2 years, depending on the assay in use. *T.pallidum* -specific IgM is commonly detected by the fluorescent treponemal antibody test with absorption in combination with removal of IgG (19S IgM FTA-Abs); this test is sensitive, but very laborious. It cannot be automated and only a limited number of samples can be processed at onetime. It requires highly skilled technologists in order to obtain reproducible "readings".

Trep Chek™ *T.pallidum* IgM kit is based on the IgM class capture principle making use of *T.pallidum* antigens covalently labeled with peroxidase. It has the advantages of a modern ELISA: it can be quantitated, it is adapted for “open” automated systems for ELISA processing and large numbers of serum specimens can be run at one time. In combination with Trep Chek™ IgG EIA, the Trep Check *T.pallidum* IgM EIA can be used in the differential diagnosis between past (*T.pallidum* IgM negative and IgG positive) and active (or recent) syphilis infection (*T.pallidum* IgM positive). A decline in IgM level can be used to monitor efficacy of therapy.

PRINCIPLE OF THE ASSAY

The *T.pallidum* IgM EIA is an antibody class capture ELISA for the detection of *Treponema pallidum* specific IgM in human serum. Antibody specific for the μ chain of human antibodies is coated to the wells. These antibodies will bind human IgM present in each serum specifically; capture of the IgM. Purified *T.pallidum* antigens labelled covalently to peroxidase (the conjugate) will bind to captured *T.pallidum*-specific IgM. TMB will induce color proportionally to the amount of *T.pallidum* specific IgM captured.

COMPONENTS PROVIDED IN THE KIT

1. Microtiter plate of twelve 8-well breakaway strips coated with anti-human IgM
2. Positive control (RED, ready-to-use) 1.8 ml
3. Negative control (GREEN, ready-to-use) 1.8 ml
4. Cut-off (YELLOW, ready-to-use) 1.5ml x 2
5. Peroxidase *T.pallidum* conjugate (100x) concentrate 0.25 ml
6. Control Antigen (100 x concentrated) 0.25 ml
7. TMB Substrate solution ready to use 15 ml
8. Dilution buffer (Ready-To-Use) 120 ml
9. Washing buffer (20 x concentrated) 60 ml
10. Stop solution(ready-to-use) 15 ml
- 11.Instruction manual

ADDITIONAL REQUIREMENTS

1. Pipettes to deliver volumes between 10 and 1000 μ l.
2. Volumetric laboratory glassware.
3. Deionised (or distilled) water.
4. Incubator 37°C.
5. Clean disposable tubes for diluting patients sera (capacity appr. 3 ml)

6. Clean disposable tubes for diluting conjugate
7. Disposable absorbent paper towels.
8. Automatic plate washer (optional).
9. Microtiter plate reader, equipped for measuring absorbances at 450 nm (optionally equipped for dual wavelength measurement at 450 and 620 nm).
10. Vortex tube mixer.
11. Timer.

PRECAUTIONS

1. All reagents supplied are for *in vitro* use only.
2. All control sera from human origin, that are provided in this *T.pallidum*-IgM test kit, have been assayed for Hepatitis B antigen, anti-HCV and anti-HIV antibodies and found negative. However, these sera must be considered as potentially infectious, and should be handled accordingly.
3. Avoid contact of substrate, sulfuric acid, washing and dilution buffer with skin and mucous membranes. If these reagents come into contact with skin or mucous membranes, wash with tap water.
4. Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the strips, prevent damage and dirt.
5. Use only components that are provided in this kit: intermixing between kits may cause interpretation problems.
6. The reagents supplied should be used only as indicated in this instruction manual.

COLLECTION, HANDLING AND STORAGE OF SERUM SPECIMENS

To obtain sera for the detection of *T.pallidum* antibodies, patient blood should be drawn and allowed to clot at room temperature. Centrifuge within one day, transfer the serum into a vial. Sera may be stored at 4°C for up to 7 days. If storage time exceeds 7 days, store at –20°C to –70°C.

T.pallidum-IgM EIA PROCEDURE

WASHING PROCEDURE

Efficient washing is a fundamental requirement of EIA's. It is essential that each washing procedure is carried out with care to obtain reproducible inter and intra- assay results. Both manual washing or washing with an automatic plate washer is acceptable.

Manual Washing

1. Empty the contents of each well by turning the strips in the holder upside down followed by a firm short vertical movement. Keep the strips tightened by pressing the sides of the strip holder.
2. Fill all the wells *to the rim* (300-350 μ l) with washing buffer, for instance with a 8-channel pipet. Be aware of carry-over.
3. Turn the strips upside down and empty the wells by a firm short vertical movement.
4. Repeat steps 2,3 *5 (five) times*.
5. Place the inverted plate on absorbent paper towels and tap the plate firmly *to remove residual washing solution from the wells*.
6. Take care that none of the wells dries before the next reagent is dispensed. Therefore, proceed immediately with the next step.

Washing with automatic microtiterplate wash equipment

When using automatic plate wash equipment, check that all wells can be aspirated completely, that the washing buffer is accurately dispensed reaching the rim of each well during each washing cycle. The washer should be programmed to execute *5 (five) washing cycles*. After the last cycle, remove the washing buffer from the wells by tapping the plate firmly on absorbant towels.

ASSAY PROTOCOL

Allow all reagents to come to room temperature.

1. Dilute patients sera (1+100): mix 1 ml dilution buffer with 10 μ l patient serum. Control sera are 'ready-to-use'.
2. Leave as many wells as needed in the holder. Label appropriately. Replace remaining wells in a resealable plastic bag and store at 4°C in the kit box. Resealed wells expire after one month.
3. Use 8 wells for controls: two for positive (RED), four for cut-off (YELLOW) and two for negative (GREEN). Dispense per well 100 μ l of the negative and positive control serum, and of the cut-off serum in (see scheme). Dispense 100 μ l of each diluted patient serum (BLUE) into wells.

A	+	Positive Control Red
B	+	
C	-	Negative Control Green
D	-	
E	+/-	Cut-Off Yellow
F	+/-	
G	+/-	
H	+/-	

4. Incubate the wells in a resealable bag or in 100% moist atmosphere for 1 hr at 37°C.
5. Prepare the *T.pallidum*-peroxidase conjugate: mix per 8-well strip 1.5 ml dilution buffer with both 15 μ l *T.pallidum*-PO conjugate (100x) and Control Antigen (100x). See scheme below.

Dilution scheme for preparation of <i>T.pallidum</i> PO conjugate			
No. of 8 well strips in use	Dilution Buffer	<i>T.pallidum</i> PO Conjugate (100X)	Control Antigen (100X)
1	1.5ml	15 μ l	15 μ l
2	3ml	30 μ l	30 μ l
6	9ml	90 μ l	90 μ l
12	18ml	180 μ l	180 μ l

6. Prepare the washing buffer: for 8-well strip mix 1.5ml washing buffer with 28.5 ml distilled water (1:20). Alternatively, mix the total volume (60 ml) of the washing buffer (20x) with 1140 ml distilled water. Stability at working concentration is one week at room temperature or one month at 4°C.
7. Wash the 8-well strips five times according to the washing protocol (previous page).
8. Dispense 150 μ l per well of diluted conjugate, incubate the strips in a resealable bag or in a 100% moist atmosphere for 1 hour at 37°C.