

TmpA IgG (EIA-2054)

SUMMARY AND EXPLANATION OF THE TEST:

The serological diagnosis of syphilis is performed by demonstrating the presence of significant levels of specific *Treponema pallidum* antibodies in the serum sample.

The reference method used is the FTA-ABS technique, which allows the detection of both IgG and IgM. However, its execution is laborious and the interpretation of the results is not simple; alternative methods have therefore been introduced to simplify the procedure. ELISA gives completely automated reproducible results, and for this reason is favoured by many operators.

The specific assay of IgM as opposed to IgG is of particular importance in the diagnosis of congenital syphilis.

PRINCIPLE OF THE TEST

The test is based on the ELISA technique (Enzyme linked Immunosorbent Assay) (1-6).

The purified and inactivated *Treponema pallidum* antigen is bound to the solid phase (8-well strips). The specific immunoglobulins are bound to the antigen through incubation with diluted human serum.

After washings to eliminate the proteins which have not reacted, incubation is performed with the conjugate, composed of human IgG monoclonal antibodies labelled with peroxidase.

The unbound conjugate is eliminated and the peroxidase substrate is added. The colour which develops is proportional to the concentration of specific antibodies present in the serum sample.

KIT CONTENTS AND REAGENT PREPARATION:

- Reagents are sufficient for 96 determinations.
- Reagents are stored between 2-8°C. Bring to room temperature before use.
- The expiry date is printed on each component and on the external package.

1. MICROWELL STRIPS (12x8 wells) coated with a pool of recombinant proteins of *Treponema pallidum*.
Use: remove the support and strips to be used from the foil package, and place the unused strips in the polythene bag with the silica gel, expel the air and seal by pressing the closure.
Stability: once opened the product is stable for 4 weeks at 2/8°C.
2. RINSING BUFFER 10X. 1 x 100 ml.
Contents: Phosphate buffered saline, concentrated 10 times; contains Brij 0.5% w/v.
Preparation: dilute 1:10 with distilled water in order to obtain the washing buffer ready for use. If crystals are present, they should be dissolved at 37°C before dilution.
Stability: stable for 5 days at room temperature and 2 weeks at 2/8°C.
3. DILUENT 100 ml. For dilution of serum samples.
Contents: Proteic solution in phosphate buffer with sodium azide 0.09%.
Preparation: Ready for use.
Stability: Once prepared ready for use, it is stable for 2 weeks at 2/8°C.
4. CONTROL SERA: POSITIVE (1.6 ml), NEGATIVE (1.6 ml) AND CUT-OFF (2.0 ml) SERUM
Contents: Diluted human serum, containing known amounts of anti T-pallidum IgG, in Phosphate buffer 0.01 mol/l with BSA 1% and sodium azide 0.09%, liquid, ready for use without further dilution.

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Stability: The products are stable up to the expiry date when stored unopened at 2/8°C.

Colour: the colour of the calibrators is proportional to the relative antibody titer.

5. CONJUGATE 1x16 ml
Contents: monoclonal antibodies labelled with Peroxidase, in phosphate buffer with phenol 0.05% and Bronidox 0.02%. Ready for use without further dilution.
Stability: The product is stable up to the expiry date when stored at 2-8°C.
6. IGG NEGATIVE CONTROL, 1.6 ml.
Contents: Diluted human serum in Phosphate buffer 0.01 mol/L with BSA 1% and sodium azide 0.09%, liquid, ready for use without further dilution.
Stability: The product is stable up to the expiry date when stored unopened at 2-8°C.
7. SUBSTRATE-HS 12 ml. Ready for use.
Contents: Tetramethylbenzidine 0.26 mg/ml and hydrogen peroxide 0.01% stabilized in citrate buffer 0.05 mol/L, pH 3.8.
Stability: The product is stable up to the expiry date when stored at 2/8°C.
8. STOP SOLUTION: 1x16 ML:
H₂SO₄ 0.3 mol/L, in solution ready for use.
9. ADHESIVE FILMS (2).
POLYTHENE BAG (1).
10. MATERIALS REQUIRED BUT NOT PROVIDED:
 - Incubator at 37° C
 - Microplate reader
 - Microplate washer (preferable)
 - Distilled water
 - Normal laboratory glassware: cylinders, test-tubes etc.
 - Micropipettes.

PRECAUTIONS

For in vitro diagnostic use only.

All materials of human origin gave a negative response both for the presence of HbsAg and for anti-HIV and anti-HCV antibodies. Nevertheless, all precautions normally adopted in laboratory practice should be followed when handling material of human origin.

Control sera contain Sodium Azide 0.09% as preservative which may react with copper and lead in plumbing to form potentially explosive metal azides. On disposal, flush with large volumes of water to prevent azide build-up.

When the kit is used with the Labotech instruments, regulate the incubation temperature at 40°C.

SAMPLES:

Fresh or defrosted serum. Samples may be stored at 2/8°C for 2 days. For longer storage, freeze at -20°C.

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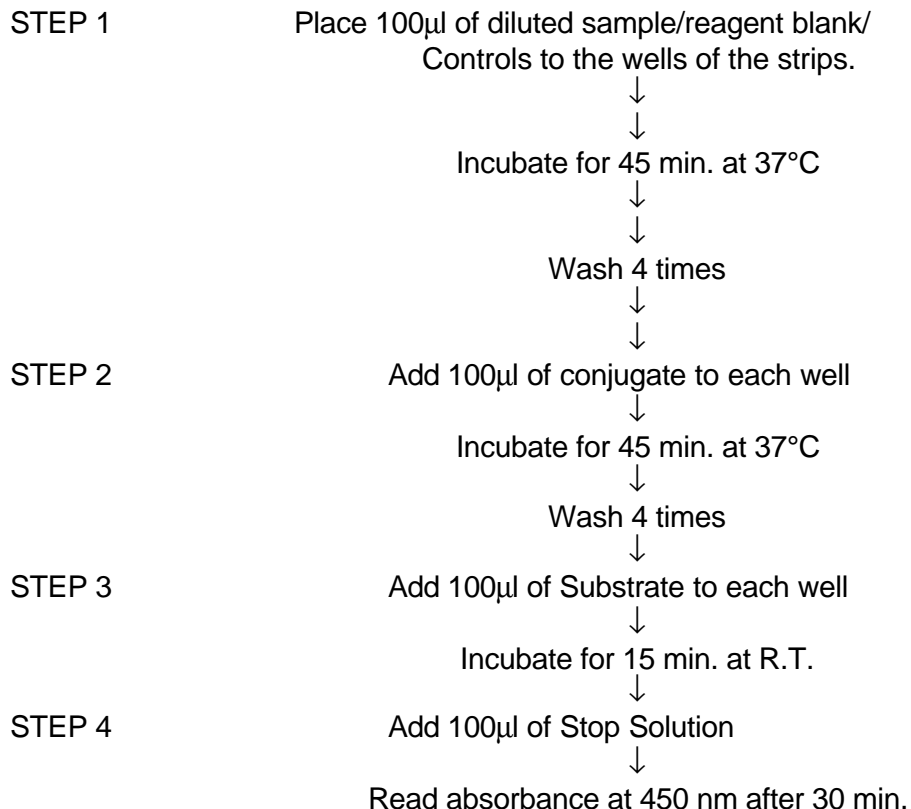
TEST PROCEDURE:

Manual Technique:

1. Prepare the required number of strips.
2. Prepare the washing buffer by diluting the Rinsing Buffer 10x (100 ml + 900 ml H₂O).
3. Dilute samples 1:51 distributing 20 µl of serum into 1 ml of diluent. Dispense 100 µl of each diluted sample per well (duplicate testing is recommended). Place **undiluted** controls in a strip (100 µl in each well). The minimum required is 1 negative control, 2 cut-off and 1 positive control.
4. Leave one well for the blank performed using 100µl of the substrate mixture.
5. Wells are covered with protective film and incubated for 45 minutes at 37°C. After washing four times for 30 seconds, add 100µl of conjugate to each well and incubate again for 45 minutes at 37°C, covering the wells with the protective film. The plate is washed again 4 times, as described above.
6. Finally, the substrate is distributed 100 µl/well.
7. After 15 minutes at room temperature the enzymatic reaction is stopped with 100 µl of Stop Solution.
8. The adsorbance (O.D.) is read at 450 nm after 30 min.

TEST PROCEDURE FOR TREPONEMA PALLIDUM IgG

Manual Technique



QUALITY CONTROL VALUES

Subtract the value of the blank from all the other readings. The O.D. values of the control Cut-off serum must be within 25% of the mean value. Disregard any abnormal value and recalculate the

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mean. The Positive control must have an O.D. at least 1.5 times that of the Cut-Off serum. The ratio between Negative Control and Cut-off must be less than 0.6.

INTERPRETATION OF THE RESULTS

1. Qualitative results

If the adsorbance of the sample is higher than that of the Cut-Off the sample is positive for the presence of specific IgG

Calculate the ratio between the average O.D. value of the sample and that of the Cut-off. The sample is considered:

Positive: if the ratio is > 1.1.

Doubtful: = 10% of the Cut-Off.

Negative: if the ratio is <0.9.

If the result is doubtful, repeat the test. If it remains doubtful, collect a new serum sample.

2. Quantitative results

Arbitrary Units (AU)

Positive results can be expressed in AU by applying the following formula:

$$AU = \frac{\text{O.D. sample} - \text{O.D. Cut-Off}}{\text{O.D. positive} - \text{O.D. Cut-Off}}$$

International Units (IU)

The IU/ml are reported for each batch of negative, positive and cut-off control serum. A graph can be constructed by plotting the IU/ml against the average O.D. of the control sera; when the O.D. of the sample is reported on the graph, the IU/ml contained in the serum sample can be calculated.

LIMITATIONS OF THE PROCEDURE

A serum sample obtained during the acute phase of infection, when only IgM antibodies are present, may be negative by this procedure.

The Treponema Pallidum IgM level should be determined using DRG Treponema Pallidum IgM kit. Alternatively, a second serum sample obtained 8-14 days later, should be tested in parallel to determine an increase in the IgG antibody level.

The test result should be used in conjunction with information available from the evaluation of other diagnostic procedures.

Although the control sera used in the kit are calibrated to WHO reference serum, certain discordances of results may be observed when the same serum is tested by different serological techniques

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PERFORMANCE CHARACTERISTICS **SENSITIVITY AND SPECIFICITY**

In a clinical trial performed in a hospital laboratory, 205 samples were analyzed. The TPHA method was used for comparison, while the reference method was the FTA-ABS technique. 51 samples were positive to the TPHA test (titer 1/160), and 100% agreement was found on these samples between the two methods.

Of 24 samples with titre of 1/80, the ELISA method gave a positive result in 19 cases: two were doubtful and 3 were negative. The reference method confirmed the 3 negative samples found with ELISA.

The DRG TmpA IgG kit offers 97.4% sensitivity and 100% specificity when compared with the FTA-ABS reference method and considering doubtful results as positive.

REFERENCES

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