

## **TmpA IgM (EIA-2055)**

### **INTENDED USE:**

IMMUNOENZYMATIC CAPTURE METHOD FOR THE DETERMINATION OF IgM-CLASS ANTIBODIES TO TREPONEMA PALLIDUM IN HUMAN SERUM

### **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 patient's serum.

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 for the assay of *Treponema pallidum* IgM is based on the principle of the capture of these immunoglobulins and the subsequent identification of those, which are specific, making

use of their ability to bind an antigen conjugated to Peroxidase. The capture is performed using monoclonal antibodies bound to the solid phase (microtiter wells). The antigen is composed of purified, inactivated and sonicated *Treponema pallidum* labelled with peroxidase bound to specific anti *Treponema pallidum* monoclonal antibodies (1-6).

### **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. STRIPS COATED WITH ANTI-HUMAN IgM MONOCLONAL ANTIBODIES.  
12x8wells.  
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, expell 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 10X. 1x15ml. For dilution of serum samples.  
Contents: Proteic solution with added merthiolare 0.05% w/v, BSA 1% for use. conc. 10 times.  
Preparation: Dilute 1:10 in the washing buffer to obtain the diluent ready for use.  
Stability: Once prepared ready for use, it is stable for 2 weeks at 2/8°C.

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4. CONTROL SERA: POSITIVE (1.6 ml), NEGATIVE (1.6 ml) AND CUT-OFF (2.0ml.)  
SERUM  
Contents: Human serum in Phosphate buffer 0.01 mol/L, liquid, ready for use without further dilution.  
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. ANTIGEN: Freeze-dried powder x 4 vials.  
Contents: Purified Treponema pallidum in Phosphate buffer 0.04 mol/L, pH 7.2.  
Preparation: reconstitute with the diluent volume shown on the label, mixing by inversion.  
Stability: the reconstituted product is stable for 2 hours.
6. CONJUGATE 100 X concentrated, 0.2 ml  
Contents: monoclonal antibodies labelled with Peroxidase, in phosphate buffer solution.  
Preparation: dilute 1:100 in the reconstituted antigen solution.  
Stability: The unopened product is stable up to the expiry date. The immunocomplex should be prepared about 45 min. before use, and remains stable for 1 day when stored at 2-8° C. For longer storage freeze at -20°C.
7. SUBSTRATE 12 ml. Ready for use.  
Contents: Tetramethylbenzidine and hydrogen peroxide stabilized in citrate buffer, pH 3.8.  
Stability: The product is stable up to the expiry date when stored at 2/8°C.
8. STOP SOLUTION: 1X16 ML:  
H<sub>2</sub>SO<sub>4</sub> 1N, in solution ready for use.
9. ADHESIVE FILMS (2).  
POLYTHENE BAG (1).
10. MATERIALS REQUIRED BUT NOT PROVIDED:
  - 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 buildup.

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



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### **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 mean. The positive control must have an O.D. at least 1.5 times that of the Cut-Off serum. The ration 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 IgM  
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:

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

### **LIMITATIONS OF THE PROCEDURE**

The results of this test should be interpreted in conjunction with other clinical and laboratory data.

As in the IgM response in other infectious diseases, a small number of syphilis patients may present persistent low levels of IgM to T. pallidum even after the normal maximum period of 1-2 years.

In cases of suspected very early syphilis in which the test may result negative, these patients should be retested about 1 week later.

### **PERFORMANCE CHARACTERISTICS**

In a clinical trial performed in a hospital laboratory, 180 samples were analyzed, only 5 of which proved positive. The samples were analyzed with another commercial immunoenzymatic method: 100% agreement was found between the two methods, both in the positive and the negative samples.

The TmpA IgM kit offers 100% sensitivity and specificity when compared with another commercial kit used as reference.

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### **REFERENCES**

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