



7018 Owensmouth Ave. Suite 103  
 Canoga Park, CA, 91303  
 Phone: 818-710-1281  
 Fax: 818-936-0121  
 Email: [Info@immunospec.com](mailto:Info@immunospec.com)  
[www.immunospec.com](http://www.immunospec.com)

## Syphilis IgM CLIA

**REF**

Catalog No. C30-911

**IVD**

*For In Vitro Diagnostic Use Only*

### INTENDED USE

Immunospec Syphilis IgM CLIA kit provides material for the detection of IgM-class antibodies to *Treponema pallidum* in human serum or plasma using chemiluminescence detection technology. This assay is intended for *in vitro* use only.

### SUMMARY AND EXPLANATION

*Immunospec Treponema pallidum* is the causative agent of Syphilis, a contagious and infectious systemic disease characterized by periods of active florid manifestations and by years of symptomless latency. Syphilis is traditionally classified as acquired or congenital, each being further subdivided on the basis of the natural course of the disease. In acquired syphilis, infection is usually transmitted by sexual intercourse. The incubation period of syphilis can vary from 1 to 13 weeks, but usually from 3 - 4 weeks. Untreated patients with primary or secondary syphilis having active lesions are the most infectious, and the risks of contagion are greatest during the first 2 years of infection. Virtually every organ and tissue of the body is affected, including most body fluids. Over 80% of patients have mucocutaneous lesions, 50% have generalized enlargement of the lymph nodes, and about 10% have lesions of the eyes, bones and joints, meninges, liver, and spleen. Mild constitutional symptoms of malaise, headache, anorexia, nausea, aching pains in the bones, and fatigability are often present. Congenital Syphilis is the result of passage of *T. pallidum* across the placenta. Clinical manifestations may be present at birth but are more often seen at 3 weeks to 6 months of age. Two types of antibodies are produced by *T. pallidum*: nontreponemal antibodies (reagin) and treponemal antibodies. ELISA for detection of IgG and IgM antibodies is becoming the Gold standard for the diagnosis of syphilis.

### PRINCIPLE OF THE TEST

*Syphilis IgM* kit is based on the chemiluminescence immunoassay technique. In the assay, Calibrators and Unknowns are incubated in microtitration wells coated with recombinant protein of *T. pallidum* as antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgM monoclonal antibodies labeled with peroxidase. After a second incubation and washing step, substrate solution is added to the wells and read. The degree of enzymatic turnover of the substrate is determined by relative light unit (RLU) measurement using luminometer. The RLU measured is directly proportional to the concentration of anti-Syphilis IgM antibodies presence.

### REAGENTS

*Immunospec Syphilis IgM* CLIA kit contains sufficient reagents for 96 wells. Each kit contains the following reagents:

MATERIAL PROVIDED	QUANTITY
<i>Syphilis</i> Antigen-Coated Microtitration Strip	One Plate
Wash Concentrate	One Bottle
Sample Diluent	One Bottle
Substrate A	One Bottle
Substrate B	One Bottle
Neg. cont	One Vial
Cut off cont	One Vial
pos cont	One Vial
2 <sup>nd</sup> Antibody Conjugate	One Bottle
Sorbent M	One Bottle

### MATERIAL NOT PROVIDED

- Microtitration plate luminometer
- Deionized/Distilled water
- Precision pipette to deliver 10 µl, 100 µl, and 1 ml
- Semi-automatic pipette to deliver 100 µl
- Automatic microtitration plate washer
- Absorbent materials for blotting the strips
- Incubator

### Antigen-Coated Microtitration Strips:

One strip holder containing 12x8 (96) microtitration wells coated with recombinant protein of *T. pallidum* as antigen. Store at 2-8°C until expiration date. 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. Once opened, the product is stable for 4 weeks at 2-8°C.

### Wash Concentrate:

One bottle, 100 ml, containing a phosphate buffered saline, concentrated 10-fold containing 0.5% Brij weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

### Sample Diluent:

One bottle, 100 ml, containing a BSA solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

### Syphilis IgM Controls:

Three vials, each 2 ml of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. The value for. Store at 2-8°C until expiration date.

### 2nd Antibody Conjugate:

One bottle, 12 ml, containing anti-human IgM monoclonal antibodies labeled with peroxidase, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.

### Substrate A:

containing Substrate A. Store at 2-8°C until expiration date.

### Substrate B :

containing Substrate B. Store at 2-8°C until expiration date.

**Sorbent M :** One Bottle, 4 ml, containing protein solution in a phosphate buffer solution with 0.02% proclin. Store at 2°-8° C.

### PRECAUTIONS

*For in vitro* use

The following universal Good Laboratory Practices should be observed:

Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats and disposable gloves when handling immunodiagnostic material. Wash hands thoroughly afterwards. Cover working area with disposable absorbent paper. Wipe up spills immediately and decontaminate affected surfaces. Avoid generation of aerosols. Provide adequate ventilation. Handle and dispose all reagents and material in compliance with applicable regulations.

### WARNING: POTENTIAL BIOHAZARDOUS MATERIAL

This kit may contain some reagents made with human source material (e.g. serum or plasma) or used in conjunction with human source material. The material in this kit has been tested by CE recommended methods and found to be non-reactive for HIV-1/2 Antibodies, HCV and HBsAg. No available test method can offer complete assurance of eliminating potential biohazardous risk. Handle all reagents and patient samples at a Biosafety Level 2, as recommended for any potentially infectious human material in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories," 4<sup>th</sup> Edition, April 1999.

### WARNING AND PRECAUTION:

Some of the reagents in this kit contain sodium azide as a preservative at concentrations below the regulatory limit of < 0.1%. Although significantly diluted, concentrated sodium azide is an irritant to skin and mucous membranes, and may react with lead and copper plumbing to form explosive metal azides, especially if accumulated. Additionally, TMB and Sulfuric Acid, in concentrated amounts are also irritants to skin and mucous membranes. These substances are in diluted form and therefore may minimize exposure risks significantly but not completely. Provide adequate ventilation. Avoid contact with skin, eyes and clothing. In case of contact with any of these reagents, wash thoroughly with water and seek medical advice. Dispose all nonhazardous reagents by flushing with large volumes of water to prevent buildup of chemical hazards in the plumbing system.

For further information regarding hazardous substances in the kit, please refer to the component specific MSDS by request.

### SPECIMEN COLLECTION AND HANDLING

Serum should be used, and the usual precautions for venipuncture should be observed. Specimens may be stored at 2-8°C for 2 days. For longer periods, store at -20°C. Do not use hemolyzed or lipemic specimens. Avoid repeated freezing and thawing of samples.

### PREPARATION FOR ASSAY

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. Do not mix various lots of any kit component within an individual assay. Do not use any component beyond the expiration date shown on its label. Incomplete washing will adversely affect the outcome and assay precision. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination with Substrate Solution and Conjugate. Use a clean disposable pipette tip for each reagent. Avoid pipettes with metal parts. Containers and semi-automatic pipette tips used for the Conjugate and Substrate can be reused provided they are thoroughly rinsed with deionized/distilled water and dried prior to and after each usage. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Use high quality water. Avoid exposure of the reagents to excessive heat or sunlight during storage and incubation.

#### PREPARATION OF REAGENTS:

##### Wash Solution:

Dilute 1:10 with deionized/distilled water prior to use. If crystals are present, they should be dissolved at 37°C before dilution. Pour 100 ml of the Wash Concentrate into a clean container and dilute by adding 900 ml of deionized/distilled water. Mix thoroughly by inversion. The wash solution is stable for 5 days at room temperature and 2 weeks at 2-8°C when stored in a tightly sealed bottle.

##### Microtitration Strips:

Select the number of coated strips required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

Substrate : At least 30 minutes prior to use mix 1 part of Substrate A in 1 part of Substrate B ( Example : 1ml of Substrate A and 1 ml Substrate B ).The two components should be mixed thoroughly by inversion and store the substrate mixture at RT, avoid exposure to light.

#### Assay Procedure:

All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Calibrators should be assayed in duplicate. .

1. Mark the microtitration strips to be used.
2. Dilute serum samples 1:101 distributing 10 µL of serum into 1 mL of Sample Diluent.
3. Pipette 100 µL of each diluted serum sample and Calibrators to the appropriate wells. Leave one well for the blank.
4. Incubate for 15 minutes at 37°C.
5. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.  
*NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, (a) completely aspirate the liquid from each well, (b) dispense 0.35 mL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.*
6. Add 100 µL of Enzyme-Labeled 2<sup>nd</sup> Antibody into each well.
7. Incubate for 15 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 50 µL of substrate mixture to each well using a dispenser.
10. Shake the wells for 10 sec.
11. Read the wells within 4 minutes using a microplate luminometer.

#### RESULTS

Calculate the mean RLU for each calibrator and unknown.

##### Qualitative results:

The Cut-off control corresponds to Calibrator 1.

If the RLU 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 RLU value of the sample and that of the Cut-Off.

The sample is considered:

Positive: if the ratio is > 1.1.

Doubtful: if +/- 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.

#### LIMITATIONS OF THE PROCEDURE

- A serum sample obtained during the early phase of infection, when only IgM antibodies are present, may be negative by this procedure.
- The test result should be used in conjunction with information available from the evaluation of other clinical and diagnostic procedures.
- Avoid repeated freezing and thawing of reagents and specimens.
- Grossly hemolyzed, icteric or lipemic specimens should be avoided.

- Heat inactivated sera should be avoided.

#### QUALITY CONTROL

Subtract the value of the blank from all the other readings. The RLU values of Calibrator 1 must be at least >100. Calibrator 2 must have a RLU at least 3 times that of Calibrator 1.

#### PERFORMANCE CHARACTERISTICS

##### 1. Sensitivity and Specificity

80 human sera were analyzed by this Syphilis IgM CLIA and a commercial ELISA as reference method. Out of 80 samples, 3 were positive for the presence of IgM antibodies to Syphilis by this CLIA and reference method showed 3 of them as positive. The results are summarized below.

	Positive	Negative	FN (false negative)	FP (false positive)
This assay	3	77	0	0
Test A	3	77	0	0

#### REFERENCES

1. G.B. Wisdom: Enzyme-Immunoassay. Clin. Chem. 22: 1243 (1976).
2. L. Schaefer, J. Dyke et al. Evaluation of microparticle enzyme immunoassays for immunoglobulins G and M to Rubella virus and Toxoplasma gondii on the Abbott IMx automated analyzer. J. Clin. Microbiol. 27: 2410 (1989).
3. Chaye, HH, et al: Cellular and humoral immune responses to Rubella virus structural proteins E1, E2, and C. J. Clin Microbiology, Washington, DC, 1992, pp. 596-599.
4. Mahony, JB, and Chernesky, MA: Rubella virus. In Rose, NR, et al (eds): Manual of Laboratory Immunology, ed. 4. ASM, Washington, DC, 1992, pp. 600-605.



#### Manufacturer:

IMMUNOSPEC CORPORATION

7018 Owensmouth Ave.  
Canoga Park, CA. 91303 USA  
(818) 710-1281

PIC30-912

Effective date: 04/2010