Syphilis Total
ELISA TEST SYSTEM

INTENDED USE
The Reactiva Search.'s Syphilis Total ELISA Test System is an EnzymeLinked Immunosorbent Assay kit providing material for the detection of IgM, IgG and IgA class Antibodies to Treponema pallidum in human serum or plasma. This assay is intended For in vitro use only.

SUMMARY AND EXPLANATION
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 total antibodies of IgG, IgM and IgA is becoming the Gold standard for the diagnosis of syphilis.

PRINCIPLE OF THE TEST
The Syphilis Total kits use recombinant antigens in a sandwich test to produce a test that is both highly specific and sensitive. The antigens will detect Treponema pallidum (Syphilis) -specific IgG, IgM, and IgA enabling the test to detect antibodies during all stages of infection. The plastic wells are coated with a mixture of recombinant antigens of Treponema. Specific antibodies in serum or plasma specimens combine with these antigens and with the same antigens conjugated to horseradish peroxidase, when conjugate is added to a well in which thespecimen has been incubated. After unreacted material has been removed by washing, the presence of bound enzyme indicating the presence in the specimen of specific antibodies is revealed by a colour change in the TMB substrate. The intensity of the colour is compared to that in control wells to determine the presence or absence of specific antibody.

MATERIALS AND COMPONENTS PROVIDED

• Antigen Coated Microtitration Strip	One Plate
• Wash Concentrate	One Bottle
• Sample Diluent	One Bottle
• TMB-Substrate	One Bottle
• Negative Control	One Vial
• Cut Off Control	One Vial
• Positive Control	One Vial
• Syphilis-HRP Conjugate	One Vial
• Stopping Solution	One Bottle
• Conjugate Diluent	One Vial

MATERIALS REQUIRED BUT NOT PROVIDED
- Microtitration plate reader capable of absorbance measurement at 450 nm
- 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 material for blotting the strips
- Incubator

REAGENTS PROVIDED
• **Antigen Coated Microtitration Strips:** One strip holder containing 12x8 (96) microtitration wells coated with recombinant 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 Total Controls:** Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer containing BSA with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
• **Syphilis - HRP Conjugate:** One bottle, 3 mL, containing Syphilis- conjugate, in a phosphate buffer solution with 0.02% Proclin. Store at 2-8°C until expiration date.
• **Conjugate diluent :** One bottle, 13 ml, containing a BSA solution with 0.02% Proclin as a preservative. Store at 2-8°C until expiration date.
• **TMB-Substrate:** One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. Store at 2-8°C until expiration date.
• **Stopping Solution:** One bottle, 15 mL, containing 0.3 M H2SO4 in solution. Store at 28°C until expiration date.

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 materials in compliance with applicable regulations.
This kit may contain some reagents made with human source material (e.g., serum or plasma) or used in conjunction with human source materials. The material in this kit has been tested by CE marked 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," 4th 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 venepuncture 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.

ASSAY PREPARATION
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. To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed to add the TMB Chromogen Solution. Avoid microbial contamination of reagents, especially of the conjugate, wash buffer and diluent. Avoid contamination of the TMB Chromogen Solution with the 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 TMB 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.

REAGENT PREPARATION
• **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.
• **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.

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moisture.
• **Conjugate preparation:** Add 150 ul of Syphilis -HRP conjugate in to 1 ml conjugate diluent (suffiecient for one strip) to make a working solution. Should be prepared 30 mins before the using.

TEST PROCEDURE
All specimens and reagents to reach room temperature (~25°C) before use. Serum Samples and Controls 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 ready to use controls to the appropriate wells.
4. Incubate for 45 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 300 uL of the Wash Solution into each well, and (c) repeat step (a) and (b) four times.
6. Add 100 µL of -HRP-Conjugate into each well.
7. Incubate for 45 minutes at 37°C.
8. Aspirate and wash each well four times for 30 seconds with Washing Solution using an automatic microplate washer or 1. manually using a dispenser. Blot and dry by inverting plate on absorbent material.
9. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
10. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
11. Add 100 µL of Stopping Solution to each well using a dispenser.
12. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

CALCULATION OF RESULTS
Calculate the mean absorbance for each control and unknown.
Qualitative results:
If the absorbance of the sample is higher than that of the Cut-Off, the sample is positive for the presence of specific antibody to T.Pallidum. Calculate the ratio between the average OD 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.

LIMITATION OF THE PROCEDURE
• A serum sample obtained during the very early phase of infection, before antibody development, 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
The OD values of Cut off control must be at least 0.2. Positive control must have an OD at least 1.5 times that of Cut off control.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity : 104 human sera were analysed by this Syphilis Total Elisa and a commercial Elisa (Test A) as reference method. Out of 104 samples, 9 were positive for the presence of I antibodies to T. Pallidum (Syphilis) by Reactiva Search Elisa and commercial Elisa showed 9 of them positive.

	Positive	Negative	FN (false negative)	FP (false positive)
Reactiva Search	9	95	0	0
Test A	9	95	0	0

2. Precision

Inter-Assay Study

No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.315	1.92	0.013
SD	0.004	0.0074	0.001
CV %	1.3	3.8	8.15

Inter-Assay Study

No of Replicates 16	Serum 1	Serum 2	Serum 3
Mean	0.261	0.55	0.036
SD	0.009	0.015	0.019
CV %	3.6	2.78	7.8

3. Interferences Study: Interferences with lipemic, hemolytic or icteric sera are not observed up to a concentration of 5 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

REFERENCES

1. S.A. Lukehart: Identification and characterization of Treponema pallidum antigens by monoclonal antibodies. in: Monoclonal Antibodies, Academic Press 1986, p. 1.
2. L. Lewis et al.: Evaluation of immunoglobulin M Western blot analysis in the diagnosis of congenital syphilis. J. Clin. Microbiol. 28: 296 (1990).
3. M. Norgard et al.: Sensitivity and specificity of monoclonal antibodies directed against antigenic determinants of Treponema pallidum Nichols in the diagnosis of syphilis. J. Clin. Microbiol. 20: 711 (1984).

PRESENTACIÓN:

CONT. 96 TEST CODIGO: RSET59-2