RESULTS

Calculate the mean absorbance for each controls 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 IgG.

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.

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 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

80 human sera were analyzed by this Syphilis IgG ELISA and a commercial Elisa (Test A) as reference method. Out of 80 samples, 9 were positive for the presence of IgG antibodies to Treponema pallidum by Aviva Elisa and commercial Elisa showed 10 of them as positive. The results are summarized below.

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
<th>FN (false negative)</th>
<th>FP (false positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIA</td>
<td>9</td>
<td>71</td>
<td>0</td>
</tr>
<tr>
<td>Test A</td>
<td>10</td>
<td>70</td>
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2. Precision

<table>
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<tr>
<th>Replicates 16</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Mean (OD’s)</th>
<th>SD</th>
<th>CV%</th>
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<td>Replicates 16</td>
<td>Serum 1</td>
<td></td>
<td></td>
<td>Mean (OD’s)</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
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<tr>
<th>No of Replicates 16</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Mean (OD’s)</th>
<th>SD</th>
<th>CV%</th>
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<td>0.53</td>
<td>1.81</td>
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<td>0.063</td>
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<td>2.7</td>
<td>9.7</td>
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<td>3.88</td>
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</table>

REFERENCE


Catalogue Nr : KAPRSPG16  
PI Nr : 1701208  
Revision Nr : 090916/1

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**Syphilis IgG Elisa**

Catalog No. OKDA00115

Aviva Systems Biology  
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San Diego, CA 92121  
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**INTENDED USE**

The AViva OKDA00115 Syphilis IgG Elisa is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to Treponema pallidum in human serum.

**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 symptoms 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**

The OKDA00115 Syphilis IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Treponema pallidum antigen. After incubation and washing, the wells are treated with the conjugate, composed of anti-human IgG antibodies labeled with peroxidase. After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by wavelength absorbance measurement at 450 nm. The absorbance measured is directly proportional to the concentration of anti-*Treponema pallidum* IgG antibodies present.

**REAGENTS**

The AvivaSyphilis IgG ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:

- Treponema-Antigen-Coated Microtitration Strip
- Wash Concentrate
- Sample Diluent
- TMB Substrate
- Negative control
- Positive control
- 2nd Antibody Conjugate
- Stopping Solution

For further details and instructions, please refer to the product manual supplied with the kit.
**Treponema-Antigen-Coated Microtitration Strips**
One strip holder containing 12x8 (96) microtitration wells coated with Treponema pallidum 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 polyethylene 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% per weight by volume (w/v). Dilute with deionized/distilled water prior to use. Store at 2-8°C until expiration date.

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

**Stopping Solution**
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.

**Sample Diluent**
One bottle, 100 mL, containing a protein solution with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

**Treponema IgG Controls**
Three vials, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.

**PRECAUTIONS**
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic material is being handled. Do not pipet by mouth. Wear lab coats, negative, cut off and positive, each 2 mL of human serum in a 0.01 M phosphate buffer with 0.09% sodium azide as a preservative. Store at 2-8°C until expiration date.
- Patient samples at a Biosafety Level 2, as recommended for any potentially hazardous biologic material.

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**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. To minimize potential assay drift due to variation in the substrate incubation times, 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 enzymes 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 energy 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. 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 resalable pouch with a desiccant pack. The pouch must be resealed to protect from moisture.

**Assay Procedure**

**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.

**SAMPLE RECEPTION AND PREPARATION**

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**ASSAY PROCEDURE**

**For 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. **Pipeette 100 µL of each diluted serum sample and ready to use controls to the appropriate wells. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.**
4. **Incubate for 45 minutes at 37°C.**
5. **Aspirate and wash each well four (4) times for 30 seconds with Wash Solution using an automatic microplate washer or manually using a dispenser. Blot and dry by inverting plate on absorbent material.**
6. **Add 100 µL of Enzyme-Labeled 2nd Antibody Conjugate into each well.**
7. **Incubate for 45 minutes at 37°C.**
8. **Aspirate and wash each well four times for 30 seconds with Wash Solution using an automatic microplate washer or 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.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Text</th>
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<tbody>
<tr>
<td>📚</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>🏷️</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>📜</td>
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<td>Contains sufficient for n tests</td>
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Revision date: 2009-09-16

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