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 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
92 human sera were analyzed by this HSV 1 IgG Elisa and a commercial Elisa (Test A) as reference method. Out of 92 samples, 69 were positive for the presence of IgG antibodies to Herpes simplex virus by DIAsource Elisa, and commercial Elisa also showed 69 of them as positive. The results are summarized below.

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Negative</th>
<th>FN (false negative)</th>
<th>FP (false positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIA</td>
<td>69</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Test A</td>
<td>69</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2. Precision

<table>
<thead>
<tr>
<th>Replicates</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
<th>Mean (OD's)</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>0.075</td>
<td>0.066</td>
<td>0.077</td>
<td>0.076</td>
<td>0.007</td>
<td>7.4</td>
</tr>
<tr>
<td>Serum 2</td>
<td>0.022</td>
<td>0.096</td>
<td>0.038</td>
<td>0.086</td>
<td>0.004</td>
<td>4.9</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0.006</td>
<td>0.119</td>
<td>0.007</td>
<td>0.008</td>
<td>0.007</td>
<td>7.4</td>
</tr>
</tbody>
</table>

REFERENCES

REAGENTS
The DIAsource Herpes simplex virus 1 IgG ELISA kit contains sufficient reagent for 96 wells. Each kit contains the following reagents:
- HSV 1-Antigen-Coated Microtitration Strip
- Wash Concentrate
- Sample Diluent
- TMB-Substrate
- Negative control
- Cut off control
- Positive control
- 2nd Antibody Conjugate
- Stopping Solution
**HSV 1 Antigen-Coated Microtitration Strips**

One stripholder containing 12x8 (96) microtitration wells coated with Herpes simplex virus 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 absorbent 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.

**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.

**2nd Antibody Conjugate**

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.

**TMB Substrate**

One bottle, 12 mL, containing tetramethylbenzidine (TMB) and hydrogen peroxide stabilized in citrate buffer, pH 3.8. 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. A void exposure of the reagents to excessive heat or sunlight during storage and incubation.

**Streptavidin-HRP**

One bottle, 3.0 mL, containing a streptavidin-HRP complex. Store at 2-8°C when stored in a tightly sealed bottle.

**Microtitration Strips**

For in vitro use. Mark the microtitration strips to be used.

**PREPARATION FOR ASSAY**

1. Pipette 100 µL of serum into 1 mL of Sample Diluent.
2. Pipette 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.
3. Incubate for 45 minutes at 37°C.
4. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using a mechanical pipette. Blot dry and reinject plate on absorbent material.
5. Add 100 µL of Enzyme-Labeled 2nd Antibody-Conjugate into each well.
6. Incubate for 45 minutes at 37°C.
7. Aspirate and wash each well four times for 30 seconds with Washing Solution using a mechanical pipette or manually using a dispenser. Blot dry and reinject plate on absorbent material.
8. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
9. Incubate for 15 minutes at room temperature.
10. 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.

**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 REAGENTS**

Wash Concentrate

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.

**Assay Procedure**

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. Leave one well for the blank, performed using 100 µL of the TMB-substrate at the substrate incubation step.
4. Aspirate and wash each well four (4) times for 30 seconds with Washing Solution using a mechanical pipette. Blot dry and reinject plate on absorbent material.
5. Incubate for 45 minutes at 37°C.
6. Aspirate and wash each well four times for 30 seconds with Washing Solution using a mechanical pipette or manually using a dispenser. Blot dry and reinject plate on absorbent material.
7. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
8. Incubate for 15 minutes at room temperature.
9. Add 100 µL of Stopping Solution to each well using a dispenser.
10. 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><strong>Consult instructions for use</strong></th>
<th><strong>Manufacturer</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage temperature</strong></td>
<td><strong>Contains sufficient for n tests</strong></td>
</tr>
<tr>
<td><strong>Use by</strong></td>
<td><strong>IVD</strong></td>
</tr>
<tr>
<td><strong>Batch code</strong></td>
<td><strong>Catalogue number</strong></td>
</tr>
</tbody>
</table>

Revision date: 2011-05-31