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 hemolized, 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.

PERFORMANCE CHARACTERISTICS
1. Sensitivity and Specificity
100 human sera were analyzed by this EBV VCA IgG Elisa and an Elisa reference method. Out of 100 samples, 81 were positive for the presence of IgG antibodies to EBV VCA IgG by DIAsource Elisa and reference Elisa showed 81 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>81</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reference</td>
<td>81</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

2. Precision

<table>
<thead>
<tr>
<th>Replicates 10</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ODs)</td>
<td>1.29</td>
<td>0.91</td>
<td>0.23</td>
</tr>
<tr>
<td>SD</td>
<td>0.012</td>
<td>0.009</td>
<td>0.028</td>
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<tr>
<td>CV%</td>
<td>9.37</td>
<td>9.78</td>
<td>10.86</td>
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</table>

2.2 Inter-assay Study

<table>
<thead>
<tr>
<th>No of Replicates</th>
<th>Serum 1</th>
<th>Serum 2</th>
<th>Serum 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ODs)</td>
<td>1.43</td>
<td>0.98</td>
<td>0.26</td>
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<tr>
<td>SD</td>
<td>0.016</td>
<td>0.017</td>
<td>0.019</td>
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<tr>
<td>CV%</td>
<td>6.71</td>
<td>6.83</td>
<td>7.50</td>
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3. Intra-assay study

TMB Substrate

<table>
<thead>
<tr>
<th></th>
<th>Wash Concentrate</th>
<th>Sample Diluent</th>
<th>TMB Substrate</th>
<th>Negative control</th>
<th>Cut off control</th>
<th>Positive control</th>
<th>2nd Antibody Conjugate</th>
<th>Stopping Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ULT</td>
<td>Quantity : 1 plate</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Wash</td>
<td>Gold</td>
<td>Control</td>
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</table>

REFERENCES

Revision Nr : 110531/ 1
MATERIAL 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 capable of maintaining temperature at 37°C

Antigen-Coated Microtitration Strips

One strip holder containing 12x8 (96) microtitration wells coated with EBV capsid 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 polystyrene 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 the wash concentrate 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.

EBV VCA 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.

2nd Antibody Conjugate

One bottle, 12 mL, containing anti-human IgG monoclonal antibodies exoxxid with exoxxidise, in a phosphate buffer solution with 0.02% Proclin. 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 2-8°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 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 Wash buffer and diluent.

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 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 washing 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 anyzuqjgk.

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 ressealed to protect from moisture.

Assay 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. Leave one well for the blank, performed using 100 µL of the substrate mixture.
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.
6. Add 100 µL of Enzyme-Labelled 2nd Antibody 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 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 calibration correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 600 or 620 nm.

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 of all hazardous reagents by flushing with large volumes of water to prevent build up 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.
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<table>
<thead>
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<tbody>
<tr>
<td>Consult instructions for use</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>Storage temperature</td>
<td>Contains sufficient for n tests</td>
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<tr>
<td>Use by</td>
<td>In vitro diagnostic medical device</td>
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<tr>
<td>LOT</td>
<td>Batch code</td>
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Revision date: 2011-05-31