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.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

130 human sera were analyzed by this Mumps IgG Elisa and an Elisa reference method. Out of 130 samples, 92 were positive for the presence of IgG antibodies to Mumps IgG by DIAsource Elisa and reference Elisa also showed 92 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>
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<tbody>
<tr>
<td>DIA</td>
<td>92</td>
<td>38</td>
<td></td>
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<tr>
<td>Reference</td>
<td>92</td>
<td>38</td>
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2. Precision

<table>
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<th>No of 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>
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<tbody>
<tr>
<td>16</td>
<td>0.315</td>
<td>1.92</td>
<td>0.013</td>
<td>0.614</td>
<td>0.004</td>
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<td>0.009</td>
<td>0.012</td>
<td>0.019</td>
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<tr>
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<td>0.010</td>
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REFERENCES


INTENDED USE

The DIAsource KAPRMUG12 Mumps IgG Elisa test system is an Enzyme-Linked Immunosorbent Assay kit providing material for the detection of IgG-class antibodies to the antigen of Mumps virus in human serum. This assay is intended for in vitro use only.

SUMMARY AND EXPLANATION

Mumps is a frequent childhood disease that is normally diagnosed on the basis of the parotitis that constitutes the presenting symptom. However, patients presenting the most common complications, i.e., orchitis, meningitis, meningoencephalitis, without inflammation of the salivary gland may require confirmation of the infection by serological methods.

PRINCIPLE OF THE TEST

The KAPRMUG12 Mumps IgG kit is based on the ELISA technique. In the assay, controls and unknowns are incubated in microtitration wells coated with purified and inactivated Mumps Virus 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-Mumps virus IgG antibodies present.

REAGENTS

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

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

Catalogue Nr : KAPRMUG12
PI Nr : 1701204
Revision Nr : 110531
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 +/- 2°C

Antigen-Coated Microtitration Strips
One stripholder containing 128 (96) microtitration strips coated with Mumps 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 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.

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.

MUMPS 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 labeled with peroxidase, 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 laboratory procedures should be observed:

- Do not eat, drink, smoke or allow 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 of all reagents and materials 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 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 human source material.

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 of the assay. Do not re-use the TMB-Chromogen Concentrate (TMB-Substrate). Do not dilute the TMB-Chromogen Concentrate. Immediately add the appropriate volume of the substrate mixture to each well. Do not adjust the concentration of TMB-Chromogen Concentrate by dilution. The TMB-Chromogen Concentrate is stable for 2 days at 2-8°C when stored in a tightly sealed bottle.

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.

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. Cover the wells with protective film and 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. 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.

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 plating 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.

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.

1. Add 100 µL of Enzyme-Labelled 2nd Antibody into each well.
2. Cover the wells with protective film and incubate for 45 minutes at 37°C.
3. 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.
4. 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.
5. Add 100 µL of TMB Chromogen Solution to each well using a dispenser.
6. Incubate for 15 minutes at room temperature. Avoid exposure to direct sunlight.
7. Add 100 µL of Stopping Solution to each well using a dispenser.
8. 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 ± 2 nm.

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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>
</tr>
<tr>
<td>Use by</td>
<td><strong>IVD</strong> In vitro diagnostic medical device</td>
</tr>
<tr>
<td><strong>LOT</strong> Batch code</td>
<td><strong>REF</strong> Catalogue number</td>
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Revision date: 2011-05-31