PLATELIA™ Mumps IgM

48 TESTS

IMMUNOENZYMATIC CAPTURE METHOD FOR THE QUALITATIVE DETERMINATION OF IgM-CLASS ANTIBODIES TO MUMPS VIRUS IN HUMAN SERUM
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1. INTENDED USE
IMMUNOENZYMATIC CAPTURE METHOD FOR THE QUALITATIVE DETERMINATION OF IgM-CLASS ANTIBODIES TO MUMPS VIRUS IN HUMAN SERUM

2. INTRODUCTION
Mumps is a frequent childhood disease which is normally diagnosed on the basis of the enlarged salivary glands which constitutes the presenting symptom. However, patients presenting with the most common complications, i.e. orchitis, meningitis or meningoencephalitis, without inflammation of the salivary glands, may require confirmation of the infection by serological methods.

3. PRINCIPLE OF THE TEST
The test for the assay of Mumps IgM is based on the principle of the capture of these immunoglobulins by anti-human IgM monoclonal antibodies found on the solid phase. A subsequent incubation with mumps antigen in a complex with monoclonal antibodies conjugated to horse radish peroxidase selects the IgM antibodies specific for the antigen and is revealed by the addition of the peroxidase substrate. When the enzymatic reaction is stopped by the addition of a sulphuric acid solution, a yellow colouring forms. The colour, which is proportional to the amount of specific antibodies present in the sample, can be read in an ELISA microplate reader.

4. KIT CONTENTS AND REAGENT PREPARATION
- Reagents are sufficient for 48 determinations.

Bring to room temperature before use.

**MT PLATE**
*MICROPLATE. 6x8 wells* coated with anti-human IgM monoclonal antibodies.
*Use:* open the package at the opposite end from the code (M followed by the lot number) which is useful for identification purposes, 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.

**CONTROL +**
*POSITIVE CONTROL (1 x 1.6 mL)*
*Contents:* Diluted human serum containing anti-Mumps IgM antibodies, in Phosphate buffer 0.01 mol/L with BSA 1% and sodium azide 0.09%, liquid, ready for use without further dilution.
*Colour:* the colour is proportional to the relative antibody titer.

**CONTROL CUT OFF**
*CUT OFF CONTROL (1 x 2.5 mL)*
*Contents:* Diluted human serum containing anti-Mumps IgM antibodies, in Phosphate buffer 0.01 mol/L with BSA 1% and sodium azide 0.09%, liquid, ready for use without further dilution.
*Colour:* the colour is proportional to the relative antibody titer.

**Ag**
*ANTIGEN. Freeze-dried powder x 3 vials.*
*Contents:* Purified Mumps virus, inactivated by treatment with beta-propiolactone, in Phosphate buffer containing lactose.
*Preparation:* reconstitute with the conjugate volume shown on the label, mixing by inversion.

**CONJ**
*CONJUGATE (10 mL)*
*Contents:* monoclonal antibodies labelled with peroxidase, in phosphate buffer with phenol 0.05% and Bronidox 0.02%.
*Preparation:* ready for use.
The immunocomplex should be prepared about 45 min. before use.

**CONTROL IgM -**
*IgM NEGATIVE CONTROL (PF93900) (1 x 1.6 mL)*
*INTERCHANGEABLE BETWEEN LOTS*
*Contents:* Diluted human serum in Phosphate buffer 0.01 mol/L with BSA 1% and sodium azide 0.09%, liquid, ready for use without further dilution.
**WASH BUF 10x**  
**WASH BUFFER 10X (PF93603) (1 x 100 mL)**  
**INTERCHANGEABLE BETWEEN LOTS**  
Contents: Phosphate buffered saline, concentrated 10 times; contains Brij 0.5% .  
Preparation: dilute the required volume 1:10 with distilled water in order to obtain the washing buffer ready for use. If crystals are present, they should be dissolved at 37°C before dilution.

**SAMP DIL**  
**DILUENT 2 (PF93611). 1 x 100 mL. For dilution of serum samples. Ready for use.**  
**INTERCHANGEABLE BETWEEN LOTS**  
Contents: Proteic solution in phosphate buffer with sodium azide 0.09% containing methyl orange as dye.

**SUBS TMB**  
**SUBSTRATE (PF93619) (15 mL). Ready for use.**  
**INTERCHANGEABLE BETWEEN LOTS**  
Contents: Tetramethylbenzidine 0.26 mg/mL and hydrogen peroxide 0.01% stabilised in citrate buffer 0.05 mol/L (pH 3.8).

**H₂SO₄ 0.3 M**  
**STOP SOLUTION (PF93602) (1 x 16 mL).**  
**INTERCHANGEABLE BETWEEN LOTS**  
H₂SO₄ 0.3 mol/L, in solution ready for use.

**ADHESIVE FILMS (2)**  
**POLYTHENE BAG (1)**

**MATERIALS REQUIRED BUT NOT PROVIDED.**  
- Incubator at 37°C  
- Microplate reader (wave length 450 or 450/620 nm, with linearity up to OD >= 2000)  
- Microplate washer (preferable) able to dispense volumes in the range 225-375 µL  
- Distilled or deionised water  
- Normal laboratory glassware: cylinders, test-tubes etc.  
- Micropipettes for the accurate collection of 10, 100, 1000 µl solution  
- Disposable gloves  
- Timer  
- Sodium Hypochlorite solution (5%)  
- Containers for collection of potentially infectious materials  
- Absorbent tissue

**5. STORAGE AND STABILITY OF REAGENTS**  
Reagents must be stored at 2/8°C.  
The expiry date is printed on each component and on the box label.

**Reagents have a limited stability after opening and/or preparation**  
**REAGENT**  
**CONDITIONS**  
Microplate  
5 weeks at 2/8°C, polythene bag  
Controls  
5 weeks at 2/8°C  
Conjugate  
5 weeks at 2/8°C  
Reconstituted antigen  
5 days at 2/8°C if reconstituted with Conjugate ( -20°C if reconstituted with Wash Buffer. Avoid repeated freezing/thawing. See “Analytical Precautions” no. 1)  
Substrate  
up to the expiry date at 2/8°C, 1 week at 15-30°C; store in the dark  
Sample Diluent  
up to the expiry date at 2/8°C  
Wash Buffer  
2 weeks at 2/8°C, 5 days at 15/30°C  
Stop Solution  
up to the expiry date at 2/8°C
6. PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE ONLY.

Caution:
This kit contains materials of human origin which have been tested and gave a negative response by
FDA-approved methods for the presence of HbsAg and for anti-HIV-1, anti-HIV-2 and anti-HCV
antibodies. As no diagnostic test can offer a complete guarantee regarding the absence of infective
agents, all material of human origin must be handled as potentially infectious. All precautions
normally adopted in laboratory practice should be followed when handling material of human origin.

Health and Safety Information
1. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and
performing the assay. Wash hands thoroughly when finished.
2. The following reagents contain low concentrations of harmful or irritant substances:
   a) The Wash Buffer contains detergents
   b) The conjugate contains phenol
   c) The substrate is acid
   d) The controls contain 0.09% Sodium Azide which can react with lead and copper in plumbing
       forming highly explosive deposits of metal azides; dilute with large amounts of water to eliminate.
       If any of the reagents come into contact with the skin or eyes, wash the area extensively with water.
3. Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for 1 h at
   121°C; disposables should be autoclaved or incinerated.
4. Sulphuric acid required for the Stop Solution and hydrochloric acid used for washing glassware are
corrosive and should be handled with appropriate care. If they come into contact with the skin or eyes,
wash thoroughly with water.
5. Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of
sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to 1% sodium
hypochlorite may be necessary to ensure effective decontamination.
6. Spillage of potentially infectious materials should be removed immediately with adsorbent paper tissue
and the contaminated area swabbed with, for example, 1.0% sodium hypochlorite before work is
continued. Sodium hypochlorite should not be used on acid-containing spills unless the spill area is first
wiped dry. Materials used to clean spills, including gloves, should be disposed of as potentially
biohazardous waste. Do not autoclave materials containing sodium hypochlorite.

Analytical precautions
1. The antigen reconstituted with conjugate is not stable after freezing. In the case of a reduced
consumption of antigen, proceed as follows: Reconstitute the antigen in 1/10 of the volume
reported on the label with Wash Buffer ready for use (eg. volume reported on the label 3 ml:
reconstitute with 0.3 ml of Wash Buffer). Take the amount of antigen necessary for immediate use
and mix with 10 parts of conjugate. Aliquot and freeze the remaining antigen. At the time of use,
thaw and mix with 10 parts of conjugate.
2. Allow all reagents and samples to come to room temperature (18-30°C) before use. Immediately after
use return reagents to the recommended storage temperature. It is important to work at the correct
temperature. Check that the thermostat does not go below 35°C or over 39°C.
3. Open the envelope containing the strips after at least ½ hr at room temperature.
4. Do not use the reagents beyond the stated expiry date. Microbiological contamination of reagents must
be avoided as this may reduce the life of the product and cause erroneous results.
5. Do not modify the Test Procedure or substitute reagents from other manufacturers or other lots unless
the reagent is stipulated as interchangeable. Do not reduce any of the recommended incubation times.
6. Any glassware to be used with the reagents should be thoroughly washed with 2M hydrochloric acid and
then rinsed with distilled water or high quality deionized water.
7. Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
8. Do not allow wells to become dry during the assay procedure.
9. Care must be taken not to cross-contaminate reagents. It is important that pipettes are dedicated for
exclusive use with the various reagents.
10. Enzyme immunoassays can occasionally exhibit an "edge effect" which must be minimized by increasing
the humidity during incubation steps. Plates must be covered with their covers and incubated at 37°C
either in a water bath with a rack or float to support the plates if necessary, or in an incubator.
Alternatively, plates can be incubated in an approved analyzer. See the appropriate operating manual for
further details. CO₂ incubators must not be used.
11. Ensure that the bottom of the plate is clean and dry, and that no bubbles are present on the surface of the liquid before reading the plate.

12. Use of highly hemolyzed samples, incompletely clotted sera, or samples with microbial contamination may give rise to erroneous results.

13. For each instrument used, read the manufacturer's instructions manual carefully to obtain additional information on the following points:
   - installation and particular requisites
   - operating principles, instructions, precautions and risks
   - manufacturer's specifications and instrument performance
   - servicing and maintenance.

7. **TYPE AND STORAGE OF SAMPLE**

   The sample is composed of serum collected in the normal manner from the vein and handled with all precautions dictated by good laboratory practice. The fresh serum may be stored for 4 days at 2/8°C, or frozen for longer periods at –20°C, and can be thawed a maximum of 3 times. Defrosted samples must be carefully mixed before performing the test. Heat inactivation can lead to erroneous results. The quality of the sample can be seriously affected by microbial contamination which leads to erroneous results. Strongly lipemic, icteric or contaminated samples should be avoided.

   The test is not applicable to human plasma.

8. **TEST PROCEDURE:**

   - Prepare the required number of strips.
   - Prepare the washing buffer by diluting the Wash Buffer 10x (100 ml + 900 mL H₂O).
   - Prepare the antigen by reconstituting the freeze-dried product directly with the conjugate (volume shown on label). In the case of reduced consumption of the Ag, reconstitute with Wash Buffer ready for use (1/10 of the volume shown on the label) and then 1/11 in the conjugate.

   Dilute samples 1:101 distributing 10 µL of serum into 1 mL of diluent; dispense 100 µl of each diluted sample per well (duplicate testing is recommended). Place UNDILUTED controls in a strip (100 µL in each well). The minimum requisite is 1 negative control, 2 cut-off and 1 positive control. Leave one well for the blank, performed using 100 µL of the substrate mixture.

   Wells are covered with protective film and incubated for 45 minutes at 37°C. After washing four times for 30 seconds (300 µL), add 100 µL of immunocomplex (antigen/monoclonal antibodies labelled with POD) to each well except blank and incubate again for 45 minutes at 37°C, covering the wells with the protective film. The plate is washed again 4 times, as described above. Finally, the substrate is distributed, 100 µL/well. After 15 minutes at room temperature the enzymatic reaction is stopped with 100 µL of Stop Solution. The absorbance (O.D.) is read at 450 nm or 450/620 nm within 30 min.
**STEP 1**  Place 100 µL of diluted samples / controls in the wells of the strips  
Incubate for 45 min. at 37°C  
Wash 4 times (30" soak time; 300 µL)

**STEP 2**  Add 100 µL of immunocomplex to each well except the blank  
Incubate for 45 min. at 37°C  
Wash 4 times (30" soak time; 300 µL)

**STEP 3**  Add 100 µL of Substrate to each well  
Incubate for 15 min. at R.T.

**STEP 4**  Add 100 µL of Stop Solution  
Read absorbance at 450 nm within 30 min.

**10. TEST VALIDATION**  
Subtract the value of the blank (<= 0.150) from all the other readings. The OD value of the Cut-Off Control must be within 25% of the average value when tested in triplicate. Discard any anomalous values and recalculate the average. The Positive Control must have an OD of at least 1.5 times the Cut-off value. The ratio between Negative Control and Cut-Off must be less than 0.6. The O.D. Cut-off must be >= 0.2 at 450 nm, and > 0.16 at 450/620 nm.

**11. INTERPRETATION OF THE RESULTS**  
**Qualitative results**  
If the OD of the sample is higher than the Cut-Off the sample is positive for the presence of specific IgM. Calculate the ratio between the average OD of the sample and that of the Cut-Off. The sample will be considered:  
Positive: when the ratio is > 1.2  
Doubtful: ± 20% of the Cut-Off  
Negative: when the ratio is <0.8  
If the result is doubtful, repeat the test. If it remains doubtful, take a new blood sample.

**12. LIMITATIONS OF THE PROCEDURE**  
All positive results must be interpreted with care, as some false-positive results or heterotypical responses of the IgM have been seen in the serum of pregnant women or in patients with an acute infection caused by Cytomegalovirus, Herpes Simplex, Measles, Rubella and Parvovirus. The results must always be interpreted together with other clinical and diagnostic data.

**13. ANALYTICAL SPECIFICITY**  
The following serum samples containing potentially interfering substances were tested:  
- Serum from pregnant women (n=12)  
- Parvovirus IgM (n=3)  
- CMV IgM (n=5)  
- HSV IgM (n=5)  
- VCA IgM (heterophyl Ab) (n=5)  
- Rubella IgM (n=5)  
- Measles IgM (n=5)  
- Varicella (Herpes Zoster) IgM (n=5)  
- Rheumatoid Factor (up to 1080 UI/dl) (n=5)  
- Bilirubin (up to 11 mg/dl)(n=5)  
- Triglycerides (up to 1281 mg/dl) (n=5)  
- Strongly hemolyzed samples (n=3).  
In some cases, interference was found in serum from pregnant women and in serum containing IgM anti-HSV, Rubella, Parvovirus and Measles.
14. **DIAGNOSTIC SENSITIVITY AND SPECIFICITY**

In an external clinical trial, 160 samples were tested with this kit in parallel with the routine method. The results are summarized in the following table:

<table>
<thead>
<tr>
<th></th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Platelia™ Mumps IgM</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

The Platelia™ Mumps IgM kit has a sensitivity of 97.3% and a specificity of 96.6%.

15. **PRECISION**

**“In run” Precision between different lots:**

<table>
<thead>
<tr>
<th>Cut off n=12</th>
<th>Lot 025</th>
<th>Lot 026</th>
<th>Lot 027</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.D.</td>
<td>0.454</td>
<td>0.327</td>
<td>0.48</td>
</tr>
<tr>
<td>CV%</td>
<td>12</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

**“Between run” Precision:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lot n. 025</th>
<th>Lot n. 026</th>
<th>Lot n. 027</th>
<th>Average</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>3.9</td>
<td>4.8</td>
<td>3.7</td>
<td>4.1</td>
<td>14</td>
</tr>
<tr>
<td>MPM1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>35</td>
</tr>
<tr>
<td>MPM2</td>
<td>1.1</td>
<td>1.0</td>
<td>1.2</td>
<td>1.1</td>
<td>9</td>
</tr>
<tr>
<td>MPM3</td>
<td>2.4</td>
<td>2.3</td>
<td>1.9</td>
<td>2.2</td>
<td>12</td>
</tr>
</tbody>
</table>
16. **TROUBLE SHOOTING GUIDE**

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE SOURCE</th>
<th>TEST OR ACTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invalid run (all negative)</td>
<td>One or more reagents not added or added in wrong sequence</td>
<td>Recheck procedure. Check for unused solutions. Repeat test.</td>
</tr>
<tr>
<td>Unreactive plate</td>
<td>Check the code on the package containing the plate (see package insert point 4 for correct code).</td>
<td>Check for moisture in unused plate. (Silica gel dessiccant must be pale yellow). Repeat test</td>
</tr>
<tr>
<td>Invalid run (all positive)</td>
<td>Contamination of substrate</td>
<td>Take new aliquot of substrate.</td>
</tr>
<tr>
<td>Poor precision</td>
<td>Incomplete washing</td>
<td>Ensure that wash apparatus works well.</td>
</tr>
<tr>
<td>Poor precision</td>
<td>Inadequate aspiration of wells</td>
<td>Ensure that wash apparatus works well.</td>
</tr>
<tr>
<td>Poor precision</td>
<td>Pipetting error</td>
<td>Check pipette function.</td>
</tr>
<tr>
<td>Poor precision</td>
<td>Reagent addition too slow</td>
<td>Avoid drying of the plate after washing step. Add reagents immediately</td>
</tr>
<tr>
<td>Presence of bubbles</td>
<td></td>
<td>Avoid air bubbles during pipetting.</td>
</tr>
<tr>
<td>Optical pathway not clean</td>
<td></td>
<td>Check instrument light source and detector for dirt. Wipe bottom of plate with soft tissue.</td>
</tr>
<tr>
<td>Inadequate Color development</td>
<td>Incorrect incubation times or temperature</td>
<td>Check for temperature control and time monitoring.</td>
</tr>
<tr>
<td>Inadequate volume of substrate</td>
<td></td>
<td>Adhere to recommended instruction for use.</td>
</tr>
<tr>
<td>added to the plate</td>
<td></td>
<td>Check pipette function.</td>
</tr>
</tbody>
</table>

17. **REFERENCES**

| **CE** | - CE marking (European directive 98/79/CE on in vitro diagnostic medical devices)  
| **IVD** | - For in vitro diagnostic use  
| **manufacturer** | - In vitro-Diagnostikum  
| **storage temperature limitation** | - Manufacturer  
| **lagerungstemperatur** | - Storage temperature limitation  
| **consult instruction for use** | - Manufacturer  
| **siehe Gebrauchsanweisung** | - Storage temperature limitation  

The other languages which are required in conformity to the European Directive can be obtained from your local Bio-Rad agent.

Les autres langues requises par la Directive Européenne sont disponibles auprès de votre représentant Bio-Rad local.

Los otros idiomas que se requieren para la conformidad de la Directiva Europea puede ser obtenida en su oficina local Biorad.

Die anderen Sprachen, die in Übereinstimmung mit der europäischen IVD Direktive benötigt werden, erhalten Sie über Ihre lokale Bio-Rad Niederlassung.

Le altre lingue che sono richieste in conformità con le Direttive Europee possono essere ottenute dal locale agente Bio-Rad.

As restantes línguas, obrigatórias em conformidade com a Directiva Europeia, podem ser obtidas através da subsidiária Bio-Rad mais próxima de si.

Övriga språk som krävs i enlighet med EG-direktivet kan erhållas från din lokala Bio-Rad-representant.

De øvrige sprog som kræves i henhold til EU direktiv kan fås ved henvendelse til den lokale Bio-Rad leverandør.

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