ReQuest® MUMPS IgM

REF 01-200M 96 Test Set **IVD**

Intended use:
Immunoenzymatic capture method for the qualitative determination of IgM-class antibodies to mumps virus in human serum.
For in vitro diagnostic use only.

Summary and Explanation of Test
Mumps is a generalized illness characterized by fever, and by inflammation and swelling of the salivary glands, particularly the parotid glands. Mumps is usually not severe in children, but in the adult the inflammation may involve the ovaries or testes (orchitis). Mumps is also one of the most common causes of aseptic meningitis, and encephalitis. The etiological agent is a member of the paramyxovirus group.

Inflammation and swelling of the parotid glands (parotitis) in mumps infection is usually sufficiently diagnostic to preclude serological confirmation. However, inasmuch as one third of mumps infections are subclinical (1), viral isolation and/or some other serological procedure may be required. An example of the latter would be patients presenting with orchitis or meningoencephalitis, two of the most common sequelae of mumps infection, without salivary gland involvement.

Virus isolation is cumbersome, and time consuming, and is usually an impractical procedure for the typical clinical laboratory. Serodiagnosis of mumps infection has been accomplished by: neutralization, hemagglutination-inhibition (HI), indirect immunofluorescence and complement fixation (CF). These methods lack specificity, which limits their usefulness in determining immune status. The HI test also requires pretreatment of test sera to remove nonspecific inhibitors of hemmagglutination.

Enzyme immunoassays (EIA, ELISA) are sensitive and specific for the detection and measurement of serum proteins (2,3,4). Their sensitivity equals that of the neutralization test, and is greater than CF or HI. They are therefore, reliable tests for the determination of immune status.

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.

Reagents
Reagents are sufficient for 96 determinations. Allow reagents to reach room temperature before use.

Microplate.	Wells coated with anti-human IgM monoclonal antibodies. 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.
Positive control	(1 x 1.6 mL) 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.
Cut off control	(1 x 2.5 mL). 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.
Antigen	Freeze-dried powder x 6 vials. 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.
Conjugate.	Monoclonal antibodies labelled with peroxidase, in phosphate buffer with phenol 0.05% and Bronidox 0.02%. Ready for use. The immunocomplex should be prepared about 45 min. before use.
Negative Control	(1 x 1.6 mL) 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 buffer	10X. 1 x 100 mL. 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. Interchangeable lot-to-lot.
Diluent 2.	1 x 100 mL. To be used to dilute samples. Proteic solution concentrated 50 times, with added phenol 0.05% and Bronidox 0.02%. Interchangeable lot-to-lot.
Substrate	12 mL. Ready for use. Tetramethylbenzidine 0.26 mg/mL and hydrogen peroxide 0.01% stabilised in citrate buffer 0.05 mol/L (pH 3.8). Interchangeable lot-to-lot.
Stop Solution	1x16 mL. H_2SO_4 0.3 mol/L, in solution ready for use. Interchangeable lot-to-lot.
Adhesive films	(2).
Polythene bag	(1).

Other Materials Required
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

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Timer
Sodium Hypochlorite solution (5%)
Containers for collection of potentially infectious materials
Absorbent tissue.

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	ready for use, 2 weeks 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

Precautions

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
Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
The following reagents contain low concentrations of harmful or irritant substances:
The Wash Buffer contains detergents
The conjugate contains phenol
The substrate is acid
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.
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.
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.
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.
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
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 (e.g. 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.
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. Open the envelope containing the strips after at least ½ hr at room temperature.
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.
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.
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.
Do not expose reagents to strong light or hypochlorite fumes during storage or during incubation steps.
Do not allow wells to become dry during the assay procedure.
Care must be taken not to cross-contaminate reagents. It is important that pipettes are dedicated for excusive use with the various reagents.
Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not "blow-out" from microplates.
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. CO_2 incubators must not be used.
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.
Use of highly hemolyzed samples, incompletely clotted sera, or samples with microbial contamination may give rise to erroneous results.

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Use of the kit with automatic instruments must be validated by the user.
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.
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Specimen Collection

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. If a new sample cannot be obtained, such samples should be clarified by filtration (0.45 µm) or centrifugation (3000 rpm x 10').
The test is not applicable to human plasma.

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 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.

1. Place 100 µL of diluted samples/controls in the wells of the strips.
2. Incubate for 45 min. at 37°C
3. Wash 4 times (30" soak time; 300 µL)
4. Add 100 µL of immunocomplex to each well
5. Incubate for 45 min. at 37°C
6. Wash 4 times (30" soak time; 300 µL)
7. Add 100 µL of Substrate to each well
8. Incubate for 15 min. at R.T.
9. Add 100 µL of Stop Solution
10. Read absorbance at 450 nm within 30 min

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.

Interpretation of the Results

Calculate the ratio between the average OD of the sample and the OD of the Cut-Off control. 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.

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.

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)

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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.

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:

		REFERENCE	
		+	-
TRITURUS	+	71	3
	-	2	84

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

Precision

"In run" Precision between different lots

Cut off n=12	Lot 025	Lot 026	Lot 027
O.D.	0.454	0.327	0.48
CV%	12	5	1

"Between run" Precision:

	Index				
Sample	Lot n. 025	Lot n. 026	Lot n. 027	Average	CV%
Postiive Control	3.9	4.8	3.7	4.1	14
MPM1	0.2	0.1	0.2	0.2	35
MPM2	1.1	1.0	1.2	1.1	9
MPM3	2.4	2.3	1.9	2.2	12

Trouble Shooting Guide

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

References

G. Berbers et al. Blocking ELISA for detection of mumps virus antibodies in human sera. J. Virol. Methods 42, 155 (1993).

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Manufacturer:
Quest International, Inc.
8127 NW 29th Street
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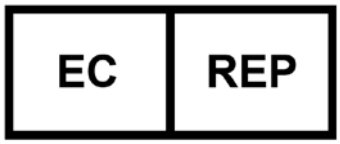
USA



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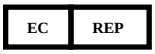
Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.

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