Mumps IgM capture EIA

An Enzyme Immunoassay for the detection of mumps virus specific IgM in human oral fluid, serum and plasma samples

Cat. No: MuVM004

For *in-vitro* diagnostic use CE IVD

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INTENDED USE AND APPLICATION

An Enzyme Immunoassay (EIA) for the detection of human IgM antibodies to mumps virus in serum, plasma and oral fluid specimens. This product is for in-vitro diagnostic use by trained laboratory personnel.

SUMMARY AND EXPLANATION

Mumps is an acute contagious viral disease caused by mumps virus, a member of the genus Rubulavirus in the family Paramyxoviridae. The disease is usually mild with asymptomatic infections occurring in 15-20% of infected individuals. Mumps usually presents as parotitis about 16 to 18 days after infection. Symptoms include fever, headache, malaise, myalgia and anorexia. Orchitis affects up to 38% of post-pubertal men with mumps. Aseptic meningitis occurs in approximately 10% of patients and mumps meningoencephalitis is seen in a small number (0.25%) of mumps cases [(1)].

Mumps is transmitted through saliva by direct contact or through droplet spread. Transmission can occur one or two days before the onset of parotitis and up to three days after parotitis has subsided.

Epidemiological surveys have indicated that the incidence of mumps disease is dramatically reduced in countries where vaccination coverage is high, practised as either single mumps vaccine, a combination measles and mumps vaccine (MM) or the measles, mumps and rubella triple vaccine (MMR) [(2)].

The antibody response after a mumps infection or vaccination is predominantly to the nucleoprotein (NP) and the haemagglutinin-neuraminidase (HN) of the mumps virus. Detection of IgM antibodies to NP has been shown to be useful in diagnosing recent mumps infections [(3-4)].

TEST PRINCIPLE

In the Microimmune Mumps IgM Capture EIA, oral fluid extract, diluted serum or diluted plasma is added to an anti-human IgM coated microtitre well. IgM in the specimen binds to the well and after washing, recombinant mumps nucleoprotein (rMuNP) antigen is added. Mumps specific IgM in the sample, if present, binds the rMuNP. After washing the well, an enzyme-conjugated monoclonal antibody to rMuNP is added. After washing, TMB Substrate is added to initiate an enzyme reaction with a coloured end-point. The presence of mumps-specific IgM results in a blue coloured product which becomes yellow on adding the acid Stop Solution. The yellow-coloured solution is measured using a photometric plate reader at 450 nm with background correction set between 620 and 650 nm.

The presence of mumps-specific IgM is indicated by optical density values above the cut-off.

WARNINGS AND PRECAUTIONS

- Although the serum used to prepare the positive and negative controls were not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen, the Positive Control and Negative Control should be handled and disposed of as though potentially infectious.
- TMB Substrate solution contains 3,3',5,5'-tetramethylbenzidine and has been reported to be non-carcinogenic. Contact with skin and mucous membranes should be avoided.

Wear latex gloves when dispensing and using this reagent. If TMB Substrate comes into contact with skin and mucous membranes, rinse with copious amounts of water.

- Stop Solution contains 0.5 mol/L hydrochloric acid. Contact with skin and mucous membranes should be avoided. If Stop Solution comes into contact with these sites, rinse with copious amounts of water.
- Wear disposable gloves when handling clinical specimens and kit components. Treat all clinical specimens and controls and any materials coming into contact with them as potentially infectious.
- Dispose clinical material and potentially infected materials in accordance with local regulations.
- Do not mix components of one lot of kits with components from other lots.
- Avoid microbial contamination of reagents. Do not use reagents that show signs of contamination.
- Good laboratory procedure should be employed to avoid cross contamination of samples and reagents. Take out only the required volume of reagent from the original container (usually 0.9 - 1.0 mL per strip) for dispensing into wells. Discard unused reagents - do not return to the container.

MATERIALS PROVIDED

Each kit contains one 96 well microplate and has sufficient materials to run up to 92 tests. The kit is stable up to the expiration date printed on the kit label if stored at 2-8°C.

1. ANTI-HUMAN IgM PLATE: PN 2103, 8 × 12 microwell strips coated with anti-human IgM antibody in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edges and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2-8°C. Strips must be used within 3 months of initial opening.
2. SERUM DILUENT PN 2040, 100 mL: one bottle containing phosphate buffered saline, protein stabiliser, detergent and red dye.
3. WASH BUFFER 10× PN 2024, 100 mL: one bottle containing 10× phosphate buffered saline, detergent and preservative. Dilute 1 in 10 with purified water.
4. POSITIVE CONTROL PN 2141, 1.4 mL: one vial containing pre-diluted serum positive for mumps IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
5. NEGATIVE CONTROL PN 2142, 1.9 mL: two vials containing pre-diluted serum negative for mumps IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
6. rMuNP ANTIGEN 100× PN 2136, 125 µL: one vial containing concentrated recombinant mumps nucleoprotein antigen in phosphate buffered saline containing protein stabilisers, detergent and antimicrobial agent. Dilute in antigen diluent before use.

7. CONJUGATE PN 2139, 12 mL: one bottle containing peroxidase conjugated anti-mumps nucleoprotein antibody in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and blue dye.
8. TMB SUBSTRATE PN 2030a, 13 mL: one bottle containing 3,3',5,5'-tetramethyl-benzidine, a peroxide source and stabilisers.
9. STOP SOLUTION PN 2031, 14 mL: one bottle containing 0.5M hydrochloric acid.
10. ANTIGEN DILUENT PN 2137, 12.5 mL: one bottle containing phosphate buffered solution with protein stabilisers, detergent, antimicrobial agent and yellow dye.

MATERIALS REQUIRED BUT NOT PROVIDED

- Oral Fluid collection device e.g. Oracol or other similar swab (see Specimen Collection).
- Buffer for extracting Oral fluid from an oral fluid collection device.
- Laboratory grade deionised or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate sealer.
- Micropipettes and disposable tips capable of delivering 1000 µL, 100 µL, 10 µL and 5 µL volumes.
- Waste discard container with disinfectant.
- ELISA plate reader capable of reading optical densities at 450 nm and 635 ± 15 nm.
- Incubator set to 37 ± 2°C.

SPECIMEN COLLECTION

Handle all oral fluid, blood, serum and plasma as potentially infectious material.

Optimal performance is obtained with specimens taken between seven days and up to four weeks after onset of symptoms.

Oral fluid specimens should be collected as described on the outer package of the Oracol collection device. Other oral fluid collection devices should be validated in the assay before use.

Oral fluids should be eluted into transport medium, a buffer of neutral pH containing between 3-10% (v/v) foetal bovine serum, 0.2-0.5% (v/v) Tween-20 and antibacterial and antifungal reagents. The procedure for processing Oracol swabs used to collect oral fluid has been described in a video [5].

Serum and plasma (EDTA, citrated or heparinised) samples are suitable specimens for the test and should be obtained using standard procedure.

REAGENT AND SAMPLE PREPARATION

Bring all reagents to room temperature (18-25°C) prior to use.

If necessary, warm the Wash Buffer 10× (Reagent 3) to re-dissolve any salts that may have formed on storage. Prepare working strength wash buffer by adding 1 part Wash Buffer 10× to 9 parts distilled or deionised water. It is recommended that working strength buffer be

prepared as required on the day of use. Remaining Wash Buffer 10× should be stored at 2-8°C. Enough has been provided to enable 3 × 4 washes of each well.

Dilute the 100× rMuNP Antigen (Reagent 6) in Antigen Diluent (Reagent 10) before use. For example, add 10 µL of the 100× rMuNP Antigen to 990 µL of Antigen Diluent. Alternatively, the entire contents of the unused 100× rMuNP Antigen can be rinsed into the unused bottle of Antigen Diluent. Mix well. The diluted antigen should be orange in colour. Diluted antigen may be stored for up to 7 days at 2 to 8°C.

All other reagents are provided ready to use.

Dilute serum and plasma samples 1/201 in Serum Diluent (Reagent 2) e.g. dispense 5 µL of specimen into a labelled tube and add 1 mL of Serum Diluent.

Oral Fluid samples extracted into transport medium should not be diluted.

ENZYME IMMUNOASSAY PROCEDURE

1. Remove and assemble the required number of microwell strips to perform the test. A minimum of 4 wells is needed for the controls which must be included in each test run. Return unused microwell strips and the desiccant to the foil pouch and reseal.
2. Pipette 100 µL of the Positive Control (Reagent 4) and Negative Control (Reagent 5) to assigned wells, one well for the positive control and three wells for the negative control.

   Pipette 100 µL of the oral fluid or diluted serum specimens to assigned wells. Only test the number of samples in a single test run that can be dispensed within ten minutes. Cover microwell plate with lid or sealing tape. *Note: this step can be accomplished more quickly if controls and test samples pre-dispensed into microplate compatible tubes or a microtitre holding plate then transferred to the test plate using a multichannel pipette*.

   Incubate at 37 ± 2°C in a moist chamber for 30 ± 2 minutes.
3. Wash wells four times with working strength wash buffer (see reagent preparation). The wash cycle is carried out as follows: aspirate the contents of the well and dispense 350 µL/well of diluted wash buffer, leave to soak for approximately 30 seconds and aspirate. Repeat the wash cycle three further times. It is recommended to use an automatic plate washer for this procedure. Tap the wells dry on absorbent paper
4. Pipette 100 µL of the diluted rMuNP antigen solution (orange in colour, see reagent preparation) to each well, cover plate and incubate at 37 ± 2°C in a moist chamber for 30 ± 2 minutes.
5. Wash the wells four times with working strength Wash Buffer as in step 3.
6. Pipette 100 µL of Conjugate (Reagent 7) to each well, cover plate and incubate at 37 ± 2°C in a moist chamber for 30 ± 2 minutes.
7. Wash the wells four times with working strength Wash Buffer as in step 3.
8. Pipette 100 µL/well of the TMB substrate (Reagent 8). This is best performed with a multichannel pipette. Incubate for 10 ± 1 minutes at room temperature (18-25°C) protected from strong light.
9. Pipette 100 µL of Stop Solution (Reagent 9). This reagent should be added to wells in the same order as step 12 so that the timing is accurate.

10. Read the optical densities at 450 nm in an ELISA plate reader. If the feature is available, set the reference wavelength between 620 and 650 nm.

QUALITY CONTROL.

The optical density $OD_{450\text{-}620\ nm}$ of the Positive Control should be greater than 0.4.

The $OD_{450\text{-}620\ nm}$ of each of the three Negative Control (NC) wells should fall between 0.05 and 0.25.

INTERPRETATION OF RESULTS

Calculate the mean OD of the three Negative Control wells ($\overline{NC}$). The OD values of the individual wells should not differ by more than 30% from the $\overline{NC}$. If one of the three OD values differs by more than 30%, it should be omitted and the mean value re-calculated.

The following criteria are required for a specimen to be identified as mumps specific IgM Reactive, Non-Reactive or Equivocal.

Mumps specific IgM Reactive

Serum: OD of specimen is $\geq \overline{NC} \times 3.5$

Oral Fluid: OD of specimen is $\geq \overline{NC} \times 2.0$

Mumps specific IgM Non-Reactive

Serum: OD of specimen is $< \overline{NC} \times 3.0$

Oral Fluid: OD of specimen is $< \overline{NC} \times 1.5$

Equivocal for mumps specific IgM

Serum: OD of specimen is $\geq \overline{NC} \times 3.0$ and $< \overline{NC} \times 3.5$

Oral Fluid: OD of specimen is $\geq \overline{NC} \times 1.5$ and $< \overline{NC} \times 2.0$

A sample giving an equivocal result should be re-tested. If the equivocal status cannot be resolved on re-testing, follow up samples taken between 7 and 21 days after the initial sample should be tested in parallel with a further retest of the first sample. If an equivocal result is obtained on re-testing a follow up sample, it should be reported as mumps IgM negative.

Positive oral fluid results should be confirmed. Confirmation can be by PCR on well-timed specimens or by testing oral fluid for specific IgM on samples collected seven to ten days later. In addition, results may be confirmed by testing matched serum samples for specific IgM.

LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

Oral fluid samples with low total immunoglobulin concentration (less than 10 µg/mL) are not suitable for use in this test and may give rise to false negative results. Some serum specimens with rheumatoid factor (RF) can give false positive results in the test. If RF is suspected,

remove the RF using a commercially available RF absorbent and retest in the Microimmune Mumps IgM capture EIA.

The Microimmune Mumps IgM capture EIA detects antibodies specifically to mumps nucleoprotein antigen. Antibodies to other mumps virus proteins are not detected in this assay.

Patient's profile, epidemiological data and the test results should be considered when making a diagnosis.

TEST PERFORMANCE

The performance of the Microimmune Mumps IgM capture EIA was evaluated on panels of sera and oral fluids received by a reference laboratory (Enteric, Respiratory and Neurological Virus Laboratory ERNVL, Health Protection Agency, Colindale, UK) for routine investigation and collected as part of a sero-epidemiological study. Serum and oral fluid samples received by the reference laboratory for mumps investigation had been tested by an IgM antibody capture radioimmunoassay (MACRIA) [6].

Evaluation of Microimmune Mumps IgM Capture EIA on serum samples.

A total of 150 sera were tested. These included 126 sera received in ERNVL for routine mumps investigation by MACRIA, 8 sera from the MMRV IgM Serum Standard Panel (consisting of 2 IgM positives for measles, mumps, rubella and varicella zoster obtained from Quest Biomedical, UK), 8 parvovirus B19 IgM positive samples and 8 RF positive serum samples. The results are summarised below in table 1.

Table 1. Evaluation of mumps specific IgM by MACRIA and Microimmune EIA.

MACRIA	Microimmune Mumps IgM Capture EIA			
	POS	NEG	EQV	TOTAL
POS	71	4**	0	75
NEG	3*	71		74
EQV			1*	1
TOTAL	74	75	1	150

* RF positive samples.
** One of the 4 discordant samples was also RF positive.

There was good agreement between MACRIA and the Microimmune EIA with discordant results obtained for only seven of the 150 sera tested. Of these 7 samples, 3 MACRIA negative, Microimmune EIA positive serum samples were RF positive and represent Microimmune EIA false positives.

Of the four MACRIA positive, Microimmune EIA negative samples, one was an RF sample which was also positive in a parvovirus B19 IgM capture EIA. Two of these samples were from a 40 year old female with polyarthritis and a 37 year old male without clinical details, suggesting that these may be MACRIA false positives. The last discordant result was obtained on a sample from a 14 year-old male with clinical details given as "unilateral mass on the left side".

Sensitivity and specificity of Microimune EIA compared to MACRIA was

Sensitivity: 94.7%, (71/75, 95% CI 86.9% to 98.5%).

Specificity: 95.9%, (71/74, 95% CI 88.6% to 99.2%).

Evaluation of Microimmune Mumps IgM Capture EIA on serum / oral fluid pairs.

Test performance with oral fluids was evaluated by comparison of results from patient-matched sera and oral fluids. The panel included 44 oral fluid/serum pairs collected from mumps IgG serum-positive, IgM serum-negative, healthy individuals and 26 oral fluids taken from 17 mumps IgM seropositive subjects.

Fourty two of the 44 oral fluids from the mumps IgG seropositive subjects were negative in the Microimmune EIA. One oral fluid tested positive and one gave equivocal results by Microimmune EIA. 24 of the 26 oral fluids from mumps IgM (MACRIA and Microimmune EIA) seropositive subjects were positive in the MACRIA, one oral fluid was equivocal and one was negative. The 26 oral fluids were positive by Microimmune EIA. The two discrepant oral fluids were from one subject and were collected 31 days after immunisation with a mumps containing vaccine. The serum sample from the same subject, collected on the same day, was positive by MACRIA and Microimmune EIA.

The sensitivity and specificity of the Microimmune EIA on oral fluid specimens compared to serum IgM results was

Specificity: 95.5% (42/44)

Sensitivity: 100% (26/26)

Comparison of results obtained for the Microimmune Mumps IgM Capture EIA and MACRIA on oral fluids.

Seventy oral fluids received in ERNVL for routine mumps investigation over a week in March 2003 were used in the evaluation. The results are shown in table 2 below.

Table 2. Comparison of Microimmune Mumps IgM capture EIA and Mumps MACRIA on oral fluid samples.

MACRIA Oral Fluid result	Microimmune Mumps IgM capture EIA			
	Positive	Equivocal	Negative	Total
Positive	25	1	1	27
Negative			43	43
Total	25	1	44	70

The evaluation of MACRIA and Microimmune EIA gave discrepant results for two specimens that could not be resolved. These were for two MACRIA positive samples, one of which was equivocal and the other negative in the Microimmune EIA. In this set of samples, the sensitivity and specificity of the Microimmune Mumps IgM capture EIA for oral fluid samples compared to MACRIA was,

Sensitivity: 92.6% (25/27).

Specificity: 100% (43/43).

Additionally, 11 oral fluids previously found to be rubella specific IgM positive and 15 oral fluids found to be measles specific IgM positive (an extra 26) were also tested by Microimmune Mumps IgM capture EIA and were all negative for mumps IgM confirming the high specificity of the test, 100% (69/69).

REFERENCES

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5. Medical training video: oral fluid samples. Public Health England (2013). Accessed 04-Dec-2015 at https://www.youtube.com/watch?v=6wDDLp_OaTc.
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WARRANTY

The product is warranted to perform as described in the labelling and in the product insert when used as instructed. NO WARRANTY EXTENDS BEYOND THIS. MICROIMMUNE LTD DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. MICROIMMUNE'S sole obligation and the purchaser's exclusive remedy for breach of this warranty shall be at the option of Microimmune Ltd to replace the products. In no event shall Microimmune be liable for any proximate, incidental or consequential damage in connection with this product.

The use of results from ERNVL does not constitute endorsement of the product by ERNVL.

SUMMARY OF ASSAY PROTOCOL

Bring All Reagents to Room Temperature

Dilute Wash Buffer 10× in water (1 + 9) as required.

Dilute rMuNP Antigen 100× in Antigen Diluent (1 + 99) as required

Dilute Test Serum or Plasma in Serum Diluent (1 + 200)

	Volume per well	**Incubation Time and Temperatures**
1. Assemble required number of coated strips into plate frame		
2. Pipette Controls, 1 × PC, 3 × NC, and all oral fluid extract, diluted serum and plasma test specimens. Complete this step within 10 minutes.	100 µL	30 ± 2 min @ 37 ± 2°C
3. Wash with diluted Wash Buffer	4 × 350 µL	30 second soak and aspiration after each wash.
4. Pipette diluted rMuNP Antigen	100 µL	30 ± 2 min @ 37 ± 2°C
5. Wash with diluted Wash Buffer	4 × 350 µL	30 second soak and aspiration after each wash.
6. Pipette Conjugate	100 µL	30 ± 2 min @ 37 ± 2°C
7. Wash with diluted Wash Buffer	4 × 350 µL	30 second soak and aspiration after each wash.
8. Pipette TMB Substrate	100 µL	10 ± 1 mins protected from light @ room temperature
9. Pipette Stop Solution	100 µL	
10. Read Optical Density @ 450 nm with reference set to 635 ± 15 nm		

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