Mumps IgG Capture EIA

An Enzyme Immunoassay for the detection of human IgG antibodies to mumps virus in oral fluid, serum and plasma samples

Cat. No: MuVG012

For *in-vitro* diagnostic use CE IVD

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INTENDED USE AND APPLICATION

An enzyme Immunoassay (EIA) for the detection of human IgG antibodies to mumps virus in serum, plasma and oral fluid. 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, practised as either single mumps vaccine, a combination measles and mumps vaccine (MM) or the measles, mumps and rubella vaccine (MMR), is high [(2)].

Antibody responses after mumps infection or vaccination are predominantly to the nucleoprotein (NP) and the haemagglutinin-neuraminidase (HN) of mumps virus. Detection of IgM antibodies to NP has been shown to be useful in diagnosing recent mumps infections [(3-4)]. The detection of mumps specific IgG can aid laboratory diagnosis of acute infection and can be used to confirm exposure to mumps virus caused by past infection or vaccination.

TEST PRINCIPLE

In the Microimmune Mumps IgG Capture EIA, oral fluid extract, diluted serum or diluted plasma is added to an anti-human IgG coated microtitre well. IgG in the specimen binds to the well and after washing, recombinant mumps nucleoprotein (rMuNP) antigen is added. Mumps specific IgG in the sample, if present, binds the rMuNP. After washing the wells, a peroxidase-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 IgG 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 IgG 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 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 of 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 any unused reagent - 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 IgG PLATE: PN 2109j, 8 × 12 microwell strips coated with anti-human IgG antibody packed in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edge and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2-8°C. Strips should 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 2157, 1.4 mL: one vial containing pre-diluted serum positive for mumps IgG antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
5. NEGATIVE CONTROL PN 2159, 1.9 mL: two vials containing pre-diluted serum negative for mumps IgG antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
6. MUMPS ANTIGEN 100× PN 2153, 125 µL: one vial containing 100× recombinant mumps nucleoprotein antigen in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and yellow dye. Dilute in antigen diluent before use.

7. CONJUGATE PN 2155, 12 mL: one bottle containing peroxidase-conjugated anti-mumps nucleoprotein antibody in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and purple 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 2105, 12.5 mL: one bottle containing phosphate buffered saline, protein stabiliser, detergent and antimicrobial agent.

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 purified 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 results are obtained with specimens taken more than four days and up to four weeks after the onset of rash.

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.

Thoroughly mix the vial containing 100× rMuNP Antigen (Reagent 6) and dilute into Antigen Diluent (Reagent 10) before use. For example, dispense 10 µL of 100× rMuNP Antigen into 990 µL of Antigen Diluent. The diluted antigen should be yellow in colour. Alternatively add the entire contents of the unused Mumps Antigen 100× to the unused bottle of Antigen Diluent and mix well. Diluted antigen may be stored up to 7 days at 2-8°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 60 ± 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 diluted Mumps Antigen (see reagent preparation) to each well, cover plate and incubate at 37 ± 2°C in a moist chamber for 60 ± 2 minutes.
5. Wash the wells four times with wash buffer as in step 7.
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 wells four times with wash buffer as in step 7.
8. Pipette 100 µL of TMB Substrate (Reagent 8) to each well. This is best performed with a multichannel pipette. Incubate for 10 ± 1 minutes, protected from strong light at room temperature (18-25°C).
9. Pipette 100 µL of Stop Solution (Reagent 9) to each well. 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 (OD) 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 0.04 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 $\overline{NC}$. 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 IgG Positive, Negative or Equivocal.

Mumps specific IgG Positive

Specimen OD $\geq \overline{NC}$ x 1.4

Mumps specific IgG Negative

Specimen OD $< \overline{NC}$

Equivocal for mumps specific IgG

Serum: $\overline{NC} \leq$ Specimen OD $< \overline{NC}$ x 1.4

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 14 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 IgG negative.

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 erroneous results.

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

Mumps specific maternal antibodies may be detected in sera or oral fluids from infants of twelve months or younger, leading to false positive results.

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

TEST PERFORMANCE

The performance of the Microimmune Mumps IgG Capture EIA was evaluated on panels of 122 subject matched sera and oral fluids collected by a reference laboratory (Virus Reference Department, VRD, Health Protection Agency, Colindale, UK). These were predominantly from healthy adults (between the ages of 20 and 65). An additional 30 sera from an adult cohort that tested negative in a competitor assay were used to assess the specificity of the Microimmune EIA. Specificity of the Microimmune EIA on oral fluid samples was assessed on 44 specimens taken from infants between twelve to fifteen months old with a negative history for MMR and sent to the reference laboratory for rubella investigation.

Evaluation of Microimmune Mumps IgG Capture EIA on serum samples

One hundred and nineteen (119) of the 122 sera from subjects with matched oral fluids were available for testing by the Microimmune EIA. The sera had been tested by the reference laboratory for mumps IgG using two competitor tests. The results of testing using the Microimmune EIA are shown in Table 1.

Table 1. Mumps specific IgG detection in sera by Microimmune and competitor assays

MICROIMMUNE EIA	COMPETITOR TEST 1				COMPETITOR TEST 2			
	POS	EQV	NEG	TOTAL	POS	EQV	NEG	TOTAL
POS	83	14	4	101	101	0	0	101
EQV	0	4	3	7	7†	0	0	7
NEG	0	1	10	11	4	1	6	11
TOTAL	83	19*	17#	119	112	1	6	119

Concordant Microimmune results were obtained for 97/119 (81.5%) sera compared to Test 1 and for 107/119 (89.9%) of sera compared to Test 2. All 19 sera (*) giving equivocal in Test 1 were positive in Test 2 whereas, 14/19 tested positive in the Microimmune EIA. Of the 17 sera (#) that were negative in Test 1, four sera were positive by the Microimmune EIA and three were negative, all seven of these sera were positive in Test 2. Of the 7 positive sera in Test 2 for which Microimmune equivocal results were obtained (†), 4 were equivocal and three were negative in competitor Test 1. Excluding equivocal results, compared to competitor Test 1 and Test 2 the sensitivity of the Microimmune EIA was as follows,

Sensitivity (Test 1): 100% (83/83, 95% CI 95.7% to100.0%).

Sensitivity (Test 2): 96.2% (101/105, 95% CI 90.5% to 99.0%).

There were too few negatives in the above panel to allow estimation of specificity with respect to the two assays. In order to assess the specificity of the Microimmune EIA with respect to test 1, 12 mumps IgG negative sera received for routine testing, 17 mumps IgG negative sera from cases that were received prior to vaccination during an outbreak and the 17 sera negative for mumps IgG from the adult cohort were tested in the Microimmune kit. The specificity of Microimmune EIA with respect to test 1 was 84.7% (39/46) and after excluding equivocal results 90.7% (39/43).

Since there is no gold standard for mumps IgG [6], the relative sensitivity and specificity between assays is not a good indicator of the performance of the tests. Test 1 has a cut-off

that favours specificity over sensitivity whereas the cut-off for Test 2 favours sensitivity over specificity. Analysis of the reactivity of all the sera tested in the Microimmune EIA indicated that the sera distributed into two clear groups that could be separated into positive and negative populations by using the cut-off indicated.

Evaluation of the Microimmune Mumps IgG Capture EIA on oral fluids.

Of the 122 serum samples taken from healthy adults and tested in Competitor Test 1, 119 sera were also available for testing in the Microimmune EIA. Matched oral fluid samples from these subjects were also available for testing in the Microimmune EIA.

Table 2. Mumps specific IgG detection in oral fluid in relation to matched serum results in competitor Test 1 and Microimmune serum test.

MICROIMMUNE EIA	COMPETITOR TEST 1				COMPETITOR TEST 2			
	POS	EQV	NEG	TOTAL	POS	EQV	NEG	TOTAL
POS	81	11	0	92	89	0	1	90
EQV	4	8	3	15	9*	5	1	15
NEG	0	0	15	15	3	2	9	14
TOTAL	85	19	18	122	101	7	11	119

Compared to the matched serum results in Test 1, there was agreement for 104 of the 122 (85.2%) oral fluid tested in Microimmune EIA. The discordant results were mainly for oral fluids for which the serum Test 1 result was equivocal. As indicated in the evaluation of sera, all Test 1 equivocal sera were positive in Test 2 and the corresponding Microimmune EIA result on the matched oral fluid samples were 11 positive and 8 equivocal. There was agreement for 103 of the 119 (86.5%) oral fluids and sera pairs tested using Microimmune EIA. Oral fluids from 2 out of 7 Microimmune serum test equivocal specimens were negative and the remaining 5 were equivocal. Excluding equivocal results, sensitivity of the Microimmune oral fluid test compared to competitor Test 1 on the matched serum was as follows,

Sensitivity (Test 1): 100% (81/81, 95% CI 95.5 to 100.0%).

The sensitivity of Microimmune oral fluid result compared to Microimmune serum result, excluding the 9 equivocal oral fluid results (*) was as follows,

Sensitivity (Oral fluid/Sera): 96.7% (89/92, 95% CI 90.8 to 99.3%).

There were not enough matched oral fluids samples to assess the specificity of Microimmune oral fluids compared to serum results in this study. Therefore, in order to assess the specificity of Microimmune EIA for oral fluid samples an additional 44 samples were tested. These samples collected for rubella surveillance from twelve to fifteen month old infants with a negative history for MMR vaccination and were therefore assumed to be negative for mumps IgG. All except one oral fluid sample gave negative results for mumps IgG in Microimmune test. Assuming that oral fluids from subjects with a negative mumps IgG result for their serum samples are truly negative for mumps IgG, then the specificity of Microimmune EIA on oral fluids was 93.5% (58/62) and after excluding equivocal results 98.3% (58/59, 95% CI 90.9 to 100.0%).

Analysis of the reactivity of all the oral fluids tested in Microimmune EIA indicated that the oral fluids distributed into two clear groups that could be separated into positive and negative populations by using the cut-off indicated.

REFERENCES

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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.

SUMMARY OF ASSAY PROTOCOL

Bring All Reagents to Room Temperature

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

Dilute Mumps 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	60 ± 2 min @ 37 ± 2°C
3. Wash with diluted Wash Buffer	4 × 350 µL	30 second soak and aspiration after each wash.
4. Pipette Antigen	100 µL	60 ± 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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