



Mumps IgG capture EIA
An Enzyme Immunoassay for the detection of mumps virus specific IgG in
human serum / plasma samples

Cat. No: MuVG012

For *in-vitro* diagnostic use.

IVD

Distributed by



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INTENDED USE AND APPLICATION

Enzyme Immunoassay (EIA) for the detection of human IgG antibodies to mumps virus in serum / plasma 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, 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 either through past infection or vaccination.

TEST PRINCIPLE

In the Microimmune Mumps IgG capture diluted serum / plasma samples are added to anti-human IgG coated microtitre wells and incubated. The IgG in the specimen binds to the anti-human IgG coated wells. The wells are then washed and recombinant mumps nucleoprotein antigen (rMuNP) is added. If mumps specific IgG is present in the sample it will bind the rMuNP. After washing the wells, a monoclonal antibody to the rMuNP conjugated to horseradish peroxidase (HRP) is added. After washing, tetramethylbenzidine (TMB) substrate is added which reacts with HRP. If mumps specific IgG is present the substrate changes from colourless to blue, which then turns yellow on terminating the enzymatic reaction. The colour change and intensity are monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650nm. The presence of mumps specific IgG is indicated by optical density values above the cut-off.

MATERIALS PROVIDED

Each kit contains sufficient materials for 96 tests.

1. ANTI-HUMAN IgG PLATE PN 2109: 8x 12 microwell strips coated with anti-human IgG 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 (100 mL) PN 2040: One bottle containing phosphate buffered saline, protein stabilizer, detergent and red dye. **Ready to use.**
3. WASH BUFFER, 10x (100 mL) PN 2024: One bottle containing 10x phosphate buffered saline, detergent and preservative. Dilute 1 in 10 with good quality deionized or distilled water.
4. POSITIVE CONTROL (0.6mL, Mumps IgG capture EIA) PN 2157: One vial containing pre-diluted serum positive for mumps IgG antibody in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to use. DO NOT DILUTE.**
5. NEGATIVE CONTROL (1.5mL, Mumps IgG capture EIA) PN 2159: One vial containing pre-diluted serum negative for mumps IgG antibody in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to use. DO NOT DILUTE.**
6. ANTIGEN, 100 X (0.1 mL) (Mumps IgG capture EIA) PN 2153 : One vial containing 100 x concentrated recombinant mumps nucleoprotein antigen in phosphate buffered saline containing protein stabilizers, detergent, antimicrobial agent and yellow dye. **DILUTE IN ANTIGEN DILUENT BEFORE USE.**
7. CONJUGATE (10mL) (mumps IgG capture EIA) PN 2155: One vial containing anti-mumps NP IgG antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and purple dye. **Ready to Use.**

8. **TMB SUBSTRATE** (10mL) **PN 2030a**: One vial containing 3,3',5,5'-tetramethylbenzidine. **Ready to Use.**
9. **STOP SOLUTION** (10mL) **PN 2031a**: One vial containing 0.5M hydrochloric acid. **Ready to Use.**
10. **ANTIGEN DILUENT (10mL) PN 2105**: One vial containing phosphate buffered solution with protein stabilizers, detergent, antimicrobial agent.

MATERIALS REQUIRED BUT NOT PROVIDED

- Good quality deionized or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate seals / cover.
- Micropipettes and disposable tips for delivering 1000 µL, 100 µL 10µL and 5 µL volumes.
- Waste discard container with disinfectant.
- EIA plate reader capable of reading optical density at 450nm (and 620-650nm).
- Incubator, 37°C.

REAGENT PREPARATION

Mix the ANTIGEN 100X (PN 2153, Reagent 6) and dilute in ANTIGEN DILUENT (PN 2105, Reagent 10) before use. For example, dispense 10µL of ANTIGEN 100X into 990µL of ANTIGEN DILUENT, **OR**, add the entire contents of the unused ANTIGEN 100 X to the unused vial of ANTIGEN DILUENT and mix well. The diluted antigen should be yellow in colour. The diluted antigen may be stored up to 7 days at 2-8°C.

Warm the WASH BUFFER 10x (Reagent 3) to re-dissolve any salts that may have formed on storage. Prepare working strength wash buffer by adding 1 part WASH BUFFER, 10x to 9 parts distilled or deionized water. It is recommended that working strength buffer be prepared as required on the day of use. Remaining Wash Buffer, 10x should be stored at 2..8°C. Enough Wash Buffer, 10x has been provided to enable four washes of each well.

All other reagents are provided ready to use.

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

WARNINGS AND PRECAUTIONS

- The POSITIVE CONTROL serum and NEGATIVE CONTROL serum are not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen. The controls should be handled and disposed of as though potentially infectious.
- The TMB SUBSTRATE solution containing 3,3', 5,5-tetramethylbenzidine 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.
- The STOP SOLUTION contains hydrochloric acid (0.5M). Contact with skin and mucous membranes should be avoided. If the STOP SOLUTION comes into contact with these sites, rinse with copious amounts of water.
- Wear disposable gloves and safety spectacles 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 0.9-1.0mL per strip) for dispensing into wells. Discard unused reagents - do not return to containers.

SPECIMEN COLLECTION

Handle all serum and plasma as potentially infectious material.

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

procedure.

STABILITY AND STORAGE

When stored unopened at 2-8°C, the kit is stable up to the expiration date printed on the kit label.

ENZYME IMMUNOASSAY PROCEDURE

1. Bring all reagents to room temperature (18-25°C) before use.
2. Dilute serum/plasma samples 1/201 in SERUM DILUENT (PN 2040, Reagent 2). Dispense 5µL of specimen into a labelled tube and add 1mL of SERUM DILUENT.
3. Assign one well for the POSITIVE CONTROL, three wells for the NEGATIVE CONTROL and one well for each of the specimens to be tested. A minimum of **four** control wells must be included in each test run.
4. Remove and assemble the required number of microwell strips from the ANTI-HUMAN IgG PLATE (PN 2109) to perform the test. Return unused microwell strips and the desiccant to the foil pouch and reseal.
5. Pipette 100 µL/well of POSITIVE CONTROL (PN 2157, Reagent 4) into one well, NEGATIVE CONTROL (PN 2159, Reagent 5) into three wells and each of the test specimens (diluted serum) to assigned wells. (Note: Only test the number of samples, in a single test run, that can be dispensed within ten minutes. Alternatively use a low binding microtitre plate to pre-dispense samples and then transfers to a test plate using a multichannel pipette. Cover microtitre plate with lid or sealing tape. Incubate at $37 \pm 2^\circ\text{C}$ in a moist chamber for 60 ± 2 minutes.
6. Wash wells four times with diluted 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 30seconds and aspirate. Repeat the wash cycle three further times. Alternatively an automatic plate washer may be used. After washing tap wells dry on absorbent paper.
7. Pipette 100 µL/well of the diluted antigen (yellow in color, see REAGENT PREPARATION) into all wells. This is best performed with a multichannel pipette. Incubate at $37 \pm 2^\circ\text{C}$ in a moist chamber for 60 ± 2 minutes.
8. Wash wells four times with wash buffer as in step 7.
9. Pipette 100 µL/well of the conjugate (PN 2155, Reagent 7, purple in colour) to the wells. This is best performed with a multichannel pipette. Cover plate and incubate at $37 \pm 2^\circ\text{C}$ in a moist chamber for 30 ± 2 minutes.
10. Wash wells four times with wash buffer as in step 7.
11. Pipette 100 µL/well of the TMB SUBSTRATE (PN 2030a, 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.
12. Pipette 100 µL/well of the STOP SOLUTION (Reagent 9). This is best performed with a multichannel pipette. The stop solution should be added using the same timing and sequence used to add the substrate solution.
13. Read optical density at 450nm (set the reference wavelength at 620, or between 615 and 650nm, if available on the plate reader) using an EIA plate reader within 10 minutes of adding the STOP SOLUTION.

QUALITY CONTROL

The optical density ($OD_{450/620nm}$) of the Positive Control should be > 0.4

The val $OD_{450/620nm}$ ue for each of the three Negative Control (NC) wells should be >0.04 and ≤ 0.25

CALCULATIONS

Calculate the mean of $OD_{450/620nm}$ the three Negative Control wells (\bar{x}_{NC}). The val $OD_{450/620nm}$ ues of the individual wells should not differ by more than 30% from the \bar{x}_{NC} . If one of the three val $OD_{450/620nm}$ ues differs by more than 30%, it should be omitted and the mean value re-calculated.

INTERPRETATION OF RESULTS

The following criteria are required for a specimen to be identified as mumps specific IgG Positive, Negative or Equivocal.

Mumps specific IgG Positive: OD_{450/620nm} specimen is \geq xNC x 1.4

Mumps specific IgG Negative: OD_{450/620nm} specimen is $<$ xNC

Mumps specific IgG Equivocal: OD of specimen is \geq xNC and $<$ xNC x 1.4

The interpretation of a **positive** mumps specific IgG result is that the patient has either had past infection with mumps virus, has been vaccinated against mumps or has received immunoglobulin. In infants under one year, it may indicate the presence of maternal antibody in the sample.

A **negative** result indicates that mumps specific IgG was not detected in the sample. This indicates that the patient has not been exposed to mumps either through vaccination or exposure / contact with circulating mump virus. Alternatively, antibodies to mumps nucleoprotein may have waned or that the sample was taken very soon after exposure to mumps before mumps specific IgG had formed (see LIMITATIONS OF THE TEST).

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 IgG negative.

LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

The Microimmune Mumps IgG capture EIA detects antibodies specifically to mumps nucleoprotein antigen. Antibodies to other mumps virus proteins are not detected in this assay. Mumps specific maternal antibodies may be detected in sera from infants of 12 months or younger, leading to false positive results.

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

TEST PERFORMANCE

The performance of the Microimmune Mumps IgG capture EIA (MI) was evaluated on panels of 119 sera collected by a reference laboratory (Virus Reference Department, VRD, Health Protection Agency, Colindale, U.K.). These were predominantly from healthy adults (age $>$ 20 and $<$ 65). An additional 30 sera from an adult cohort that tested negative in a competitor assay was used to assess the specificity of MI.

EVALUATION OF MICROIMMUNE MUMPS IgG CAPTURE EIA ON SERUM SAMPLES

The sera had been tested by the reference laboratory for mumps IgG using two competitor tests. The results of testing using MI are shown in Table 1.

Table 1: Mumps specific IgG detection in **sera** by MI and competitor assays.

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

Concordant MI 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 MI test. Of the 17 sera that were negative in Test 1, four sera were positive and 3 equivocal by MI. All seven of these sera were positive in Test 2. Of the seven competitor Test 2 positive sera for which MI equivocal results were obtained, four were equivocal in competitor TEST 1.

Excluding equivocal results, compared to competitor Test 1 and Test 2 the sensitivity of MI was 100% (95% CI 95.7% to 100.0%) and 96.2% (95% CI 90.5% to 99.0%) respectively. 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 MI 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 MI. The specificity of MI with respect to test 1 was 39/46 (84.7%) and excluding equivocal results, the specificity was 39/43 (90.69%).

Since there is no gold standard for mumps IgG (5), 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 MI indicated that the sera distributed into two clear groups that could be separated into positive and negative populations by using the cut-off indicated

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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.

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