Measles IgM Capture EIA

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

Cat. No: MeVM110

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 measles virus in serum, plasma and oral fluid. This product is for *in-vitro* diagnostic use by trained laboratory personnel. Testing of oral fluid samples is recommended for surveillance of measles. Positive IgM results should be confirmed with other tests, such as PCR on well-timed specimens.

SUMMARY AND EXPLANATION

Measles is a severe disease causing extensive morbidity and mortality in large parts of the world. Children under the age of 5 are at most risk. It is highly infectious, being transmitted from person to person by respiratory droplets: there is no known animal reservoir. Measles transmission can be interrupted by immunisation and high profile campaigns in many countries involving the use of measles vaccines - delivered as a single component or combined with rubella (MR) or mumps and rubella (MMR) - have led to encouraging drops in transmission. In 2001 the Measles and Rubella initiative was launched by the American Red Cross, United Nations Foundation, CDC, UNICEF and WHO with the aim of eliminating measles mortality in children and congenital rubella syndrome. All WHO Regions have now established goals to eliminate measles by 2020 [(1)]. It is estimated that in 2014 there were over 20 million cases and was the cause of death of 114,900 people.

Surveillance based on the clinical diagnosis of measles is unreliable and in countries approaching measles elimination only a small proportion of clinically diagnosed cases are confirmed on laboratory testing [(2)]. Laboratory testing is therefore essential for measles surveillance once the control phase of measles elimination is established.

High compliance rates are essential for effective surveillance. To this effect, oral fluid has a number of advantages over serum and plasma for laboratory testing. Collection is simple and cheap, it can be performed by children or their parents at home, it is painless, it is non-invasive, and the risk of exposure to HIV and hepatitis associated with use of needles is low. Compliance amongst patients of all age groups is correspondingly high. Tests using oral fluid require high sensitivity since the total antibody concentration is lower than in serum [(3)]. Measles IgM capture radioimmunoassay (MACRIA) has been demonstrated to detect IgM in oral fluid specimens from clinically diagnosed and seropositive confirmed cases [(4)], however radioimmunoassay technologies cannot easily be transferred to other laboratories. The Microimmune Measles IgM Capture EIA solves this problem as it has both the required sensitivity and is based on enzyme-immunoassay technology.

TEST PRINCIPLE

In the Microimmune Measles 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 measles nucleoprotein (rMeNP) antigen is added. Measles specific IgM in the sample, if present, binds the rMeNP. After washing the wells, an enzyme-conjugated monoclonal antibody to rMeNP is added. After washing, TMB Substrate is added to initiate an enzyme reaction with a coloured end-point. The presence of measles-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 measles 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 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 IgM PLATE: PN 2103B, 12 × 8-well breakaway strips coated with anti-human IgM antibody packed in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edges and separating the re-sealable joint. Return unused wells to the pouch with desiccant and store at 2-8°C. Wells 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 2122, 1.9 mL: one vial containing pre-diluted serum positive for measles IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.

5. NEGATIVE CONTROL PN 2124, 3 × 1.9 mL: three vials containing pre-diluted serum negative for measles IgM antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
6. MEASLES ANTIGEN PN 2126, 12 mL: one bottle containing recombinant measles nucleoprotein antigen in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and green dye.
7. CONJUGATE PN 2125, 12 mL: one bottle containing peroxidase conjugated anti-measles 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, pink colorant and stabilisers.
9. STOP SOLUTION PN 2031, 14 mL: one bottle containing 0.5M hydrochloric acid.

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, e.g. 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 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.

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 wells 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 wells 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 each assigned well, 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 are 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 Measles Antigen (Reagent 6) 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 wells four times with working strength Wash Buffer as in step 3.
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 the previous step 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 measles specific IgM Reactive, Non-Reactive or Equivocal.

Measles specific IgM Reactive

Serum: Specimen OD $\geq (\overline{NC} + 0.15) \times 1.1$

Oral Fluid: Specimen OD $\geq \overline{NC}$

Measles specific IgM Non-Reactive

Serum: Specimen OD $\leq (\overline{NC} + 0.15) \times 0.9$

Oral Fluid: Specimen OD $\leq \overline{NC} \times 0.8$

Equivocal for measles specific IgM

Serum: $(\overline{NC} + 0.15) \times 0.9 <$ Specimen OD $< (\overline{NC} + 0.15) \times 1.1$

Oral Fluid: $\overline{NC} \times 0.8 <$ Specimen OD $< \overline{NC}$

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 measles 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 erroneous results. Some serum or plasma 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 Measles IgM Capture EIA.

The Microimmune Measles IgM Capture EIA detects antibodies specifically to measles nucleoprotein antigen. Antibodies to other virus proteins are not detected in this assay.

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

TEST PERFORMANCE

The performance of the Microimmune Measles Capture EIA was evaluated on serum and oral fluid samples that had been previously tested by a monoclonal antibody capture radioimmunoassay method (MACRIA).

Evaluation of Microimmune Measles IgM Capture EIA on Serum Samples

A total of 80 serum samples were tested. Specimens included 30 sera received for routine testing in a measles reference laboratory, 40 sera from a WHO panel for testing and 10 sera from the measles AccuPanel (Quest Biomedical, UK). In addition, 37 parvovirus B19 IgM positive sera were tested.

Thirty-six of 37 parvovirus B19 IgM positive sera were negative in the Microimmune Measles IgM Capture EIA. One serum gave a high reading in the control antigen wells of a commercial indirect ELISA and also gave a high reading in the Microimmune Measles IgM capture EIA in the absence of added rMeNP antigen.

Sensitivity of the test was 100% compared to MACRIA after excluding the four specimens, two of which were negative by other measles IgM tests and two were patients with other diseases. Compared to MACRIA, the specificity of the test was 96.1% (49/51) and 96.6% (85/88) including the parvovirus B19 specimens.

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

MACRIA	Microimmune Measles IgM Capture EIA			
	POS	EQV	NEG	TOTAL
POS	23	2*	2**	27
EQV	0	0	2**	2
NEG	0	2**	49	51
TOTAL	23	4	53	80

* one specimen was positive for rubella IgM and the other contained RF.
** these specimens were negative in a commercial indirect measles IgM ELISA.

Evaluation of Microimmune Measles IgM Capture EIA on 15 patients enrolled in a clinic-based study of measles virus infection.

In this small number of samples there was complete agreement in the results obtained with oral fluid and serum.

Specimens included 21 sera and 25 oral fluids collected from the 15 patients recruited. Fourteen patients had detectable measles IgM in serum by both MACRIA and Microimmune EIA. In all the IgM seropositive subjects, IgM was detected in the oral fluids by both assays. In three subjects, serum had been taken on the first day of rash and was IgM positive. In two cases an oral fluid specimen was also available on the first day of rash: both of these were

IgM positive in MACRIA and Microimmune EIA. In one subject, IgM was not detected in either serum or oral fluid taken two days after onset of rash by either MACRIA or Microimmune EIA and measles virus was detected by RT-PCR.

REFERENCES

1. WHO (2015). Measles Fact sheet 286. Accessed 04-Dec-2015 at http://www.who.int/mediacentre/factsheets/fs286/en/.
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4. Perry KR, Brown DWG, Parry JV, Panday S, Pipkin C and Richards A (1993). Detection of measles, mumps and rubella antibodies in saliva using antibody capture radioimmunoassay. J. Med. Virol. 40, 235-240.
5. Medical training video: oral fluid samples. Public Health England (2013). Accessed 04-Dec-2015 at https://www.youtube.com/watch?v=6wDDLp_OaTc.

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

	Volume per well	**Incubation Time and Temperatures**
1. Assemble required number of coated wells 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 Measles 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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