



Measles IgM capture EIA
An Enzyme Immunoassay for the detection of human IgM antibodies to
measles virus in human serum / plasma samples

Cat. No: MeVM010

For *in-vitro* diagnostic use.

IVD



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INTENDED USE AND APPLICATION

Enzyme Immunoassay (EIA) for the detection of human IgM antibodies to measles virus in serum and plasma. This product is for *in-vitro* diagnostic use by trained laboratory personnel.

SUMMARY AND EXPLANATION

Measles is a severe vaccine preventable disease causing extensive morbidity and mortality in large parts of the world. It is transmitted from person to person by respiratory droplets and there is no known animal reservoir. Measles transmission can be interrupted by immunization. Despite the widespread use of measles vaccine, however, either as a single antigen vaccine or as a component of the triple vaccine against measles, mumps and rubella (MMR), there are still an estimated 30-40 million reported measles cases and 770,000 deaths per year globally (1). In 1997 there was an estimated 151,000 measles cases and 6,500 deaths in the European Region (2). The World Health Organization (WHO) has targeted the elimination of measles in Europe by the year 2007 as part of a program for the global eradication of the disease. The strategy to achieve this aim is based on political commitment to achieve and maintain high vaccination coverage. A key component of the plan is surveillance to monitor progress.

Surveillance based on the clinical diagnosis of measles is unreliable and in countries approaching measles elimination only a small proportion of clinically diagnosed cases can be confirmed by laboratory testing (3). Thus laboratory testing is essential for measles surveillance once the control phase of measles elimination is established.

TEST PRINCIPLE

In the Microimmune Measles IgM capture EIA, diluted serum / plasma is added to anti-human IgM coated microtitre wells. IgM in the specimen binds to the wells and after washing, recombinant measles nucleoprotein (rMVN) antigen is added. Measles specific IgM in the sample, if present in the sample, binds the rMVN. After washing the wells, a monoclonal antibody to the rMVN conjugated to horseradish peroxidase is added. After washing, tetramethylbenzidine (TMB) substrate is added to reveal the presence of specific IgM. The presence of measles specific IgM results in a color change in the TMB from colorless to blue and then yellow on terminating the enzymatic reaction. The color change and optical density are monitored using a spectrophotometric plate reader set at 450nm with a correction filter between 620 and 650nm. The presence of measles specific IgM is indicated by optical density values above the cut-off.

MATERIALS PROVIDED

Each kit contains sufficient materials for 96 tests. The shelf life of each kit is as indicated on the kit label fixed to the outer box.

1. ANTI-HUMAN IgM PLATE PN 2103: 8x 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 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.
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 (0.6 mL Measles IgM only) PN 2122: One vial containing pre-diluted serum positive for measles IgM antibody, in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to Use. DO NOT DILUTE.**
5. NEGATIVE CONTROL (1.8 mL Measles IgM only) PN 2124: One vial containing pre-diluted serum negative for measles IgM antibody, in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to Use. DO NOT DILUTE.**
6. MEASLES ANTIGEN (Measles IgM EIA only, 10 mL) PN 2126: One vial containing recombinant measles nucleoprotein antigen in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and green dye. **Ready to Use.**
7. CONJUGATE (Measles IgM EIA only, 10 mL) PN 2126: One vial containing anti-measles NP IgG antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and blue 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.**

MATERIALS REQUIRED BUT NOT PROVIDED

- Good quality deionized or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate seals / covers.
- Micropipettes and disposable tips capable of delivering 1000 µL, 100 µL 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

Warm the WASH BUFFER, 10x 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 HIV 1 and 2, HCV and for Hepatitis B surface antigen. The controls should be handled and disposed 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 these sites, 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 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 -1.0mL 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. Optimal performance is obtained with specimens taken more than four days and up to four weeks after onset of rash. Serum and plasma (EDTA, citrated or heparinized) samples are suitable specimens for the test and should be obtained using standard laboratory procedure.

STABILITY AND STORAGE

When stored 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. This is very IMPORTANT.
2. Dilute serum/plasma samples 1/201 in SERUM DILUENT (PN 2040, Reagent 2 – red color). Dispense 5 µL of specimen into a labelled tube and add 1mL of SERUM DILUENT.

3. Remove and assemble the required number of microwell strips from Anti-Human IgM plate, PN 2103, to perform the test. A minimum of 4 wells is need for the controls which must be included in each test run. Return unused microwell strips and the desiccant to the foil pouch and reseal.
4. Pipette 100 µL/well of the POSITIVE CONTROL (PN 2122; Reagent 4 – red color) and NEGATIVE CONTROL (PN 2124; Reagent 5 – red color) controls to assigned wells. Use one well for the POSITIVE CONTROL and three wells for the NEGATIVE CONTROL.
5. Pipette 100 µL/well of diluted serum specimens to assigned wells. 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 transfer to a test plate using multichannel pipette. Cover microtitre plate with lid or sealing tape and incubate at 37±2°C in a moist chamber for 30±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 at least 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 the wells dry on some absorbent paper
7. Pipette 100 µL/well of the MEASLES ANTIGEN(PN 2126; Reagent 6, green color) to the wells. This is best performed with a mulitchannel pipette. Cover plate and incubate at 37±2°C in a moist chamber for 30±2 minutes.
8. Wash the well four times with wash buffer as in step 6.
9. Pipette 100 µL/well of the CONJUGATE (PN 2125; Reagent 7, blue color) to the wells. This is best performed with a mulitchannel pipette. Cover plate and incubate at 37±2°C in a moist chamber for 30±2 minutes.
10. Wash wells four times with wash buffer as in step 6.
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 protected from strong light.
12. Pipette 100 µL/well of the STOP SOLUTION (PN 2031a; 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 STOP SOLUTION.

QUALITY CONTROL.

The optical density, OD_{450/620nm} of the POSITIVE CONTROL should be > 0.4

The OD_{450/620nm} value 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 (xNC). The OD_{450/620nm} values of the individual wells should not differ by more than 30% from the xNC. If one of the three OD_{450/620nm} values 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 measles specific IgM Positive, Negative or Equivocal.

Measles specific IgM Positive: OD_{450/620nm} of specimen is ≥ (xNC + 0.15) x 1.1

Measles specific IgM Negative: OD_{450/620nm} of specimen is ≤ (xNC + 0.15) x 0.9

Measles specific IgM Equivocal: OD_{450/620nm} of specimen is > (xNC + 0.15) x 0.9 and < (xNC + 0.15) x 1.1

Interpretation of a measles specific IgM positive result is that the patient is likely to have had recent exposure to measles virus either through vaccination (for e.g. with MMR) or through contact with circulating wild type measles virus. In areas of low measles prevalence, an acute case of measles should always be confirmed by other tests. For example the use of the Microimmune Measles IgG capture EIA to monitor seroconversion, PCR on clinical specimens such as oral fluid or throat swabs and virus isolation may aid diagnosis of acute measles (See also LIMITATIONS OF THE TEST).

A negative result indicates that the patient is not acutely infected with measles. However, IgM antibodies may not have developed in samples taken very soon after onset of rash symptoms.

A sample giving an equivocal result should be re-tested. If the equivocal status cannot be resolved on re-testing, follow up sample 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 testing the follow up sample it should be reported as measles specific IgM negative.

LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

Some serum specimens with rheumatoid factor (RF) can give false positive results in the test. If RF is suspected, repeat the test and include a control well to which antigen is omitted (and replaced with diluted wash buffer). RF is indicated if the control well gives OD value above 0.1.

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

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

TEST PERFORMANCE

The performance of the Microimmune Measles IgM capture EIA was evaluated on serum samples that had been previously tested by MACRIA.

A. Serum Samples: Included 10 sera from the measles AccuPanel (Quest Biomedical, U.K), 30 sera received for routine testing in a measles reference laboratory and 40 sera from a WHO panel for testing. In addition 37 parvovirus B19 IgM positive specimens were tested.

B. Serum clinic based study: Included 33 sera from 26 patients enrolled in a clinic-based study of measles in Niteroi, Brazil in 1998. Most of these cases were in adults between 17 and 30 years of age. Two cases were from infants 6 months and 9 months of age.

Results:

A: Serum Panel

Table 1: Detection of measles specific IgM by MACRIA and by IgM capture EIA on serum samples

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

*One sample was a rubella IgM positive specimen and the other a RF positive specimen: ** These specimens were negative in a commercial indirect measles EIA.

Thirty-six of the 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 EIA and also gave a high reading in the Microimmune measles IgM capture EIA in the absence of added rMVN 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 from another disease. Compared to MACRIA, the specificity of the test was 96.1% (49/51) and 96.6% (85/88) including the parvovirus B19 specimens.

B: Serum specimens from subjects enrolled in a clinic based study of measles.

IgM was not detected in serum, from one subject, taken two days after rash by both MACRIA and Microimmune IgM capture EIA. An oral fluid from this subject taken at the same time was positive by RT-PCR for measles. The remaining 32 sera from the 26 subjects were positive for measles specific IgM by both assays. This included eight sera from subjects taken on the first day of rash, and 7 others taken

within 3 days of rash. In the limited number of serum specimens investigated in this study, the sensitivity of the Microimmune Measles IgM capture EIA compared to MACRIA was 100%.

REFERENCES

1. CDC Measles Laboratory Network website http://www.cdc.gov/Ncidod/dvrd/revb/measles/measles_general_info.htm
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3. Ramsay M, Brugh R and Brown D (1997) Surveillance of measles in England and Wales: implications of a national saliva testing program. Bulletin of the World Health Organization, 75, 515-521.

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.