



Measles IgG capture EIA
An enzyme immunoassay for the detection of human IgG antibodies to
measles virus in human serum / plasma samples

Cat. No: MeVG011

For *in-vitro* diagnostic use.

IVD

Distributed by



Microimmune Ltd

104A High Street, Brentford, Middlesex, TW8 8AT, U.K.

Phone: 1-44-020 8230 8777

Fax: 1-44-020 8230 8778

Email: customerservice@microimmune.co.uk

orders@microimmune.co.uk

Distributed in the U.S.A. for Microimmune by:

BluePoint Bioscience

10075 Tyler Place, #9

Ijamsville, MD U.S.A 21754

Tel: 00 1 240-246-4912

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INTENDED USE AND APPLICATION

Enzyme immunoassay (EIA) for the detection of human IgG antibodies to measles virus in serum / plasma is intended to be used as an aid to diagnosis of measles infection and for the epidemiological surveillance of measles antibodies in population studies. 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 effectiveness of measles vaccination programs, to identify individual and population susceptibility and verify disease diagnosis.

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 IgG capture EIA, diluted serum / plasma is added to anti-human IgG coated microtitre wells. IgG in the specimen binds to the wells and after washing, recombinant measles nucleoprotein (rMVN) antigen is added. Measles specific IgG in the sample, if present, 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 IgG. The presence of measles specific IgG results in a color change in the TMB from colorless to blue and then yellow on termination of the enzymatic reaction. The color change and intensity are monitored using a spectrophotometric plate reader set at 450nm with a reference filter between 620 and 650nm. The presence of measles specific IgG 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 label fixed to the box containing the kit.

1. **ANTI-HUMAN IgG PLATE PN 2109** : 8 x 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.
3. **WASH BUFFER, 10x (100 mL) PN 2024**: One bottle containing 10x phosphate buffer saline, detergent and preservative. Dilute 1 in 10 with good quality deionized or distilled water.
4. **POSITIVE CONTROL (0.6 mL measles IgG EIA only) PN 2120**: One vial containing pre-diluted serum positive for measles IgG antibody, in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **Ready to use. DO NOT DILUTE.**
5. **NEGATIVE CONTROL (1.5 mL measles IgG) PN 2119**: One vial containing pre-diluted serum negative for measles IgG antibody, **Ready to use** in phosphate buffered saline containing detergent, protein stabilizer and antimicrobial agent. **DO NOT DILUTE.**
6. **rMVN ANTIGEN (10 mL Measles IgG EIA only) PN 2115**: One vial containing recombinant measles nucleoprotein antigen. in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and brown dye. **Ready to use.**
7. **CONJUGATE (10 mL Measles IgG EIA only) PN 2118**: One vial containing anti-MVN IgG antibody conjugated to horseradish peroxidase (HRP) in a buffered solution containing protein stabilizers, detergent, antimicrobial agent and orange / pink dye. **Ready to use.**
8. **TMB SUBSTRATE (10mL) PN 2030a**: One vial containing substrate. **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 / cover.
- 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. Sufficient WASH BUFFER, 10x is 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 and for Hepatitis B surface antigen. The controls should be handled and disposed of as though potentially infectious.
- The 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 handling this reagent. If TMB SUBSTRATE solution comes into contact with the skin, 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.

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 anti-human IgG microwell strips (PN 2109; reagent 1) 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.
4. Pipette 100 µL/well of the POSITIVE CONTROL (PN 2120; Reagent 4 - red color) and NEGATIVE CONTROL (PN 2119; 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 the diluted serum specimens to assigned wells. Only test the number of samples, in a single test run, that can be dispensed into assigned wells within ten minutes. Alternatively use a low binding microtitre plate to pre-dispense the samples and then transfer to a test plate using a multichannel pipette. Cover microtitre plate with lid or sealing tape and incubate at $37 \pm 2^\circ\text{C}$ 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 absorbent paper.
7. Pipette 100 µL/well of the rMVN ANTIGEN solution (PN 2115; Reagent 6 - brown color) 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 $30 \pm$ minutes.
8. Wash the well four times with wash buffer as in step 6.
9. Pipette 100 µL/well of the CONJUGATE solution (PN 2118; Reagent 7 - orange / pink color) to the wells. This is best performed with a multichannel pipette. Cover plate, cover 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 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 620 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 $OD_{450/620\text{nm}}$ of the POSITIVE CONTROL should be > 0.6 . The $OD_{450/620\text{nm}}$ value for each of the three NEGATIVE CONTROL (NC) wells should be >0.025 and ≤ 0.15

CALCULATIONS

Calculate the mean $OD_{450/620\text{nm}}$ of the three NEGATIVE CONTROL wells ($\bar{x}\text{NC}$). The $OD_{450/620\text{nm}}$ values of the individual wells should be >0.025 and not differ by more than 25% from the $\bar{x}\text{NC}$. If one of the three $OD_{450/620\text{nm}}$ values differs by more than 25% or is less than 0.025, it should be omitted and the mean value re-calculated.

INTERPRETATION OF RESULTS

The **Microimmune Measles IgG capture EIA kit** is intended to be used in conjunction with the Microimmune measles IgM capture EIA as an aid to **diagnosis** of acute measles infection. It can also be used to monitor prevalence of measles specific IgG antibodies in population studies.

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

Measles specific IgG **Positive:** OD_{450/620nm} of specimen is $\geq xNC \times 1.25$

Measles specific IgG **Negative:** OD_{450/620nm} of specimen is $\leq xNC \times 1.1$

Measles specific IgG **Equivocal:** OD_{450/620nm} of specimen is $> xNC \times 1.1$ and $< xNC \times 1.25$

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

A **negative** measles specific IgG result indicates that measles specific IgG was not detected and that the patient has not been exposed to measles virus either as a vaccine or to circulating measles virus. However, in cases of acute measles infection or recent vaccination measles specific IgG antibodies to the measles nucleoprotein may not have developed, giving a negative result in the Microimmune measles IgG capture EIA (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 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 Measles IgG negative.

LIMITATIONS OF THE TEST

Patient's profile, vaccination history, epidemiological data and other available test results should be considered in interpreting the diagnostic significance of the test results.

The measles IgG capture EIA detects antibodies specifically to MVN. Antibodies to other measles virus proteins are not detected in this assay.

Some specimens with high 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.

Microbiological contamination of the specimens may lead to erroneous results.

TEST PERFORMANCE

The Microimmune Measles IgG capture EIA and an indirect enzyme immunoassay employing tissue culture grown antigen was used to evaluate results on 116 sera collected as part of a sero-epidemiological survey in the U.K. The results are shown in Table 1

Table 1: Comparison of Microimmune Measles IgG capture EIA and competitor measles IgG assay on serum samples. For the serum samples, a positive cut-off of OD_{450/620nm} $\geq xNC \times 1.25$ was used.

Competitor EIA Serum Result	Microimmune EIA Serum Result		
	POS	NEG	Total
POS	69	3	72
NEG	1	39	40
EQV	0	4	4
Total	70	46	116

In the competitor test, 4 sera were equivocal, 40 were negative and 72 were positive for measles IgG.

The four samples giving equivocal results in the competitor indirect EIA were negative in the Microimmune Measles IgG capture EIA. Two of the three specimens positive by the Competitor EIA and negative in the Microimmune Measles IgG capture EIA were just above the positive cut-off of 0.2 in the competitor EIA (OD values 0.201 and 0.209). The sensitivity, specificity, positive and negative predictive value of the Microimmune Measles IgG capture EIA compared to the competitor EIA, in the limited number of samples tested, was 95.8% (95% CI 88.3% and 99.1%), 97.5% (95% CI 86.8% and 99.9%) 98.6% (95% CI 92.3% and 100.0%) and 92.9% (95% CI 80.5% and 98.5%) respectively.

REFERENCES

1. CDC Measles Laboratory Network website http://www.cdc.gov/Ncidod/dvrd/revb/measles/measles_general_info.htm
2. WHO (1998). Progress toward global measles control and regional elimination, 1990-1997. MMWR, 47/48, 1049-1054.
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.