



Anti-Measles Virus ELISA (IgG) Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2610-9601 G	Measles virus	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: The enzyme immunoassay (ELISA) test kit provides a quantitative or semiquantitative *in vitro* assay for human antibodies of the immunoglobulin class IgG against measles virus in serum or plasma to support the diagnosis of infections with measles virus. The product is designed for use as **IVD**.

Application: The detection of specific antibodies against measles virus is used for the diagnosis of measles virus infections and the determination of the immune status. A positive result for measles virus IgG indicates a past infection or vaccination if the IgM determination is negative at the same time. A positive IgM test and/or a serum conversion or a significant increase of the IgG activity in two successive samples indicates an acute infection with measles virus. The serological diagnosis of acute infections with measles virus can be complemented by molecular diagnostic methods (PCR). For a reliable evaluation of measles-virus specific antibody tests the vaccination history of the patient should always be taken into account.

Principles of the test: The test kit contains microplate strips each with 8 break-off reagent wells coated with measles virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens: 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	—	12 x 8	STRIPS
2. Calibrator 1 5000 IU/l (IgG, human), ready for use	red coloured in decreasing intensity	1 x 2.0 ml	CAL 1
3. Calibrator 2 1000 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 2
4. Calibrator 3 250 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 3
5. Calibrator 4 50 IU/l (IgG, human), ready for use		1 x 2.0 ml	CAL 4
6. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
7. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
8. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
10. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
11. Chromogen/substrate solution TMB/H ₂ O ₂ , ready for use	colourless	1 x 12 ml	SUBSTRATE
12. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
13. Test instruction	—	1 booklet	
14. Quality control certificate	—	1 protocol	
LOT Lot description	CE Storage temperature		
IVD In vitro diagnostic medical device			
			Unopened usable until

Updates with respect to the previous version are marked in grey.



Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag). Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

Calibrators and controls: Ready for use. The reagents must be mixed thoroughly before use.

Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.

Sample buffer: Ready for use.

Wash buffer: The wash buffer is a 10x concentrate. If crystallization occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionized or distilled water (1 part reagent plus 9 parts distilled water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.

The working-strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The Chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

Stop solution: Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain sodium azide in a non-declared concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted 1:101 in sample buffer. For example: dilute 10 µl sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The Calibrators and controls are prediluted and ready for use, do not dilute them.



Incubation

For **semiquantative analysis** incubate **calibrator 3** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1 to 4** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)

Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol.

Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Manual: Empty the wells and subsequently wash 3 times using 300 µl of working-strength wash buffer for each wash.

Automatic: Wash reagent wells 3 times with 450 µl of working-strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.

Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells.

Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells.

Incubate for **15 minutes** at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping the reaction:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, EUROIMMUN Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry. Automated test performance using other fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.



Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	C 3	P 6	P 14	P 22			C 1	P 3	P 11	P 19		
B	pos.	P 7	P 15	P 23			C 2	P 4	P 12	P 20		
C	neg.	P 8	P 16	P 24			C 3	P 5	P 13	P 21		
D	P 1	P 9	P 17				C 4	P 6	P 14	P 22		
E	P 2	P 10	P 18				pos.	P 7	P 15	P 23		
F	P 3	P 11	P 19				neg.	P 8	P 16	P 24		
G	P 4	P 12	P 20				P 1	P 9	P 17			
H	P 5	P 13	P 21				P 2	P 10	P 18			

The pipetting protocol for microplate strips 1 to 4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).

The pipetting protocol for microplate strips 7 to 10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 4), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The reagent wells are break-off format. Therefore, the number of tests performed can be matched to the number of samples, minimizing reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the **extinction** of the control or patient sample over the **extinction** of calibrator 3. Calculate the ratio according to the following formula:

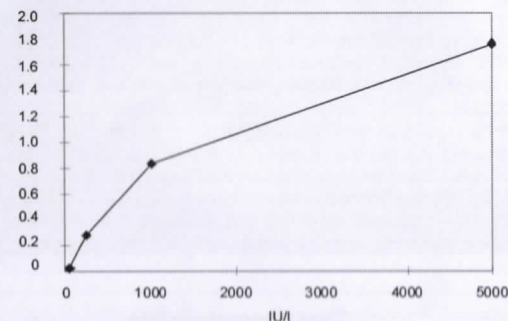
$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <0.8:	negative
Ratio ≥0.8 to <1.1:	borderline
Ratio ≥1.1:	positive

In cases of borderline test results, an additional patient sample should be taken 7 days later and re-tested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

Quantitative: The standard curve from which the concentration of antibodies in the patient samples can be taken is obtained by point-to-point plotting of the extinction readings measured for the 4 calibrators against the corresponding units (linear/linear). Use "point-to-point" plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



The extinction of a patient sample lies above the extinction of calibrator 1 (corresponding to 5000 IU/l), the result should be given as ">5000 IU/l". It is recommended that the sample be re-tested at a dilution of 1:400. The result in IU/l read from the calibration curve for this sample must then be multiplied by factor 4.

The upper limit of the reference range of non-infected persons (**cut-off value**) recommended by EUROIMMUN is **250 International Units (IU/l)**. EUROIMMUN recommends interpreting results as follows:

<200 IU/l:	negative
≥200 to <275 IU/l:	borderline
≥275 IU/l:	positive

Evaluation information: For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For the interpretation of borderline results an investigation using further tests (e.g. avidity determination of antibody class IgG) can be helpful.

Detection of changes in the antibody activity by parallel investigation of two serum samples taken at an interval of minimum 7 days can confirm the diagnosis.

To assess the immune status to measles virus, no general recommendations are yet available. In some publications, antibody concentrations against measles virus have been described in which immunity can be assumed starting from a threshold value of 200 IU/l^{1,2,3,4}. Corresponding to these publications, the following result interpretation can be recommended to determine the immune status:

< 150 IU/l:	negative
150 to < 200 IU/l:	borderline
≥ 200 IU/l:	positive

The cellular immune response must be taken into account alongside the humoral immune response.

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- World Health Organization. Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome. Immunization, Vaccines and Biologicals; Third edition, June 2018



A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. In case of a borderline result, a secure evaluation is not possible. If there is a clinical suspicion and a negative test result, we recommend clarification by means of other diagnostic methods and/or the serological investigation of a follow-up sample. A positive result indicates that there has been contact with the pathogen. In the determination of pathogen-specific IgM antibodies, polyclonal stimulation of the immune system or antibody persistence may affect the diagnostic relevance of positive findings. A significant increase in the specific IgG antibody activity (exceeding factor 2) and/or seroconversion in a follow-up sample taken after at least 7 to 10 days can indicate an acute infection. To investigate changes in the specific antibody activity, sample and follow-up sample should be incubated in adjacent wells of the ELISA microplate within the same test run. For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: The controls of the Anti-Measles Virus ELISA (IgG) were calibrated using the international standard serum NIBSC 97/648 (anti-measles and anti-polio virus serum, National Institute for Biological Standards and Control, Hertfordshire, England; approved as international reference preparation by the WHO Expert Committee on Biological Standardization). The NIBSC 97/648 serum contains 3 International Units (IU) per ampoule by definition and was resuspended in a concentration of 3 IU/ml.

For every group of tests performed, the extinction readings of the calibrators and the international units determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during incubation steps, the greater will be the extinction. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The antigen source is provided by inactivated cell lysates of Vero cells infected with the "Edmonston" strain of measles viruses.

Linearity: The linearity of the anti-Measles Virus ELISA (IgG) was determined by assaying at least 4 serial dilutions of different patient samples. The Anti-Measles Virus ELISA (IgG) is linear at least in the tested concentration range (52 IU/l to 4865 IU/l).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable relative antibody concentration. The lower detection limit of the Anti-Measles Virus ELISA (IgG) is 8 IU/l.

Cross-reactivity: The quality of the antigen used ensures a high specificity of the ELISA. Sera from patients with infections caused by various agents were investigated with the EUROIMMUN Anti-measles virus ELISA (IgG).



Antibodies against	n	Positive in Anti-Measles Virus ELISA (IgG)
Adenovirus	8	0%
CMV	6	0%
EBV-CA	11	0%
HSV-1	3	0%
Influenza virus type A	5	0%
Influenza virus type B	11	0%
Mumps virus	4	0%
Mycoplasma pneumoniae	4	0%
Parainfluenza virus types 1-4	11	0%
RSV	9	0%
Rubella virus	6	0%
Toxoplasma	3	0%
VZV	5	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to concentrations of 10 mg/ml haemoglobin, 20 mg/ml triglycerides and 0.4 mg/ml bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-assay variation, n = 20		
Sample	Mean value (IU/l)	CV (%)
1	830	8.0
2	3410	6.6
3	3725	5.6

Inter-assay variation, n = 4 x 6		
Sample	Mean value (IU/l)	CV (%)
1	796	11.6
2	3635	5.0
3	3946	6.8

Sensitivity and specificity: 112 clinically characterized patient samples (INSTAND, Labquality and NEQAS) were examined with the EUROIMMUN Anti-Measles Virus ELISA (IgG). The test showed a specificity and a sensitivity of 100% each.

n = 112		INSTAND/Labquality/NEQAS		
		positive	borderline	negative
EUROIMMUN Anti-Measles Virus ELISA (IgG)	positive	89	1	0
	borderline	0	0	0
	negative	0	0	22

Reference range: The levels of anti-measles virus antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off of 250 IU/l, 94% of the blood donors were anti-measles virus positive (IgG), which reflects the known percentage of infections in adults.

Clinical significance

The measles virus (MV) is the most instantly recognisable member of Morbilliviruses, a group of viruses belonging to the Paramyxoviridae family. No animal reservoir is known. The measles virus causes an acute feverish illness which occurs mainly in childhood and is very infectious. In 1999, measles still caused worldwide 873,000 deaths per year. Today they are less frequent because of vaccination, especially in the western hemisphere. However, measles epidemics are still observed in some countries. Individuals acutely infected with the virus exhibit a wide range of clinical symptoms ranging from a characteristic mild self-limiting infection to death.



MV infections are characterised by an incubation period of about 10 days, flu-like symptoms with fever, malaise, catarrh of the upper respiratory tract, cough, congestion and conjunctivitis. Soon afterwards the measles rash, a typical exanthema, appears first near the ears, then on the forehead, in the face and over the rest of the body.

Complications arising from MV infections include secondary bacterial pneumonia, otitis media (approx. 1%), encephalitis (approx. 1%), myocarditis, miscarriage and a condition called subacute sclerosing panencephalitis (SSPE). Persistent MV infection of the otic capsule is an aetiological factor in otosclerosis. Anti-measles IgG for the serological diagnosis of otosclerotic hearing loss has a high specificity and sensitivity. SSPE is a progressive, generally fatal brain disorder caused by chronic measles virus infection. It occurs about 7 to 10 years after the infection and generally kills within 3 years from the onset of the symptoms. The patients suffer from behavioural changes, cognitive deterioration, vision problems and eventually advanced neurological symptoms, such as severe spasms, and finally severe physical and mental impairment that leads to death. Males are more commonly affected than females. The risk of SSPE from measles was underestimated according to older data. Actual papers put it at closer to 6.5 to 11 cases of SSPE per 100,000 measles infections; that means 7 to 13 times higher than the earlier estimates.

Women with acute measles infection during pregnancy and a negative result for measles-specific antibodies were observed e.g. in Japan, India, Thailand, Kenya and Brazil: 3 of 4 pregnancies ended in preterm delivery, spontaneous abortion or stillbirth; 2 of 4 neonates were found to have congenital measles with a positive result for IgM antibodies.

Antibodies against MV can be found in the serum of almost all patients during and after a measles infection. IgM antibodies develop soon after the onset of symptoms and can be measured using ELISA or indirect immunofluorescence tests (IIFT). 50% of patients have IgM antibodies within three days, more than 90% within 10 days after occurrence of the rash. The Anti-Measles Virus IgM ELISA is more rapid and sensitive for the serological diagnosis of measles infections than other tests.

MV infections often cause an increase in heterologic antibodies. The statistically evaluated detection rate for antibodies is significantly higher for ELISA and IIFT in comparison with e.g. neutralisation tests. IgG and IgM antibodies against MV are reliable markers to confirm suspected measles infections.

Measles myelitis or encephalitis can be verified by detecting antibodies against measles in the cerebrospinal fluid (CSF). These specific antibodies are synthesised in the brain. The CSF-serum quotient (LSQ) allows to differentiate between a blood-derived and a pathological, brain-derived specific antibody fraction in CSF, taking into account individual changes in the blood/CSF barrier function. Therefore it is necessary to confirm the presence of antibodies against MV using ELISA both in CSF and in the serum. During measles myelitis or encephalitis an intrathecal synthesis of antibodies against MV in CSF takes place. Due to the fact that specific antibodies can pass the blood-cerebrospinal fluid barrier by diffusion from serum to CSF it is necessary to determine the relative CSF/serum quotient (CSQrel., synonym: antibody specificity index). The quotient is calculated from the amount of specific anti-measles virus IgG antibodies in total CSF IgG in proportion to the amount of specific IgG antibodies in total serum IgG. During conversion the CSF/serum quotient of the pathogen-specific IgG-antibody concentration CSQpath-spec. (IgG) is put into relation to the CSF/serum quotient of the total IgG concentration CSQtotal (IgG). A relative CSQ result above 1.5 indicates the production of specific antibodies in the central nervous system (CNS) and the involvement of the CNS in the disease.

With respect to the severe complications known from measles infections, the Robert Koch Institute in Germany recommends vaccinating small children, with a first shot between the age of 11 to 14 months and a second between 15 and 23 months. Neutralisation activity and persistence of antibodies are induced in response to the immunisation.

Usually, lifelong immunity develops which is not sufficient any more in people over 60 years to prevent reinfection. Moreover, antibody titers in post-vaccine sera are 8 to 10 times lower than in convalescent sera. Consequently, a booster injection is highly recommended at an older age to prevent severe, life-threatening disease courses, as occur frequently at that age. In immunosuppressed persons, such as tumour and transplant patients, as well as in seronegative pregnant women after exposition, passive immunisation with specific immunoglobulin is indicated.



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