Anti-Measles Viruses NP ELISA (IgM)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EI 2610-9601-4 M	Measles viruses (nucleoprotein)	IgM	Ag-coated microplate wells	96 x 01 (96)

Principles of the test: The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgM class against measles virus nucleoprotein (measles virus NP) in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with recombinant measles virus nucleoprotein. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgM antibodies (also IgA and IgG) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgM (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 (IgM, human), ready for use	dark red	1 x 2.0 ml	CAL
3. Positive control (IgM, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
4. Negative control (IgM, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
5. Enzyme conjugate peroxidase-labelled anti-human IgM (goat), ready for use	red	1 x 12 ml	CONJUGATE
6. Sample buffer containing IgG/RF-Absorbent (Anti-human IgG antibody preparation obtained from goat), ready for use	green	1 x 100 ml	SAMPLE BUFFER
7. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
8. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
9. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
10. Test instruction	---	1 booklet	
11. Quality control certificate	---	1 protocol	
LOT Lot description	CE	Storage temperature	
IVD In vitro diagnostics		Unopened usable until	

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.

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.

- **Calibrator 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. The green coloured sample buffer contains IgG/RF absorbent. Serum or plasma samples diluted with this sample buffer are only to be used for the determination of IgM antibodies.

- **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.

Warning: The controls and calibrators used have been negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Preparation and stability of the patient samples

Sample material: 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. Provided that the cold storage is not interrupted, it is possible to store samples for up to 28 days. In these cases, the sample should be checked visually and should also be smelled (the development of a smell indicates a bacterial contamination). Diluted samples must be incubated within one working day.

Introduction: Before the determination of specific antibodies of class IgM, antibodies of class IgG should be removed from the patient sample. This procedure must be carried out in order to prevent any rheumatoid factors from reacting with specifically bound IgG, which would lead to false positive IgM test results, and to prevent specific IgG displacing IgM from the antigen, which would lead to false IgM negative test results.

Functional principle: The sample buffer (green coloured!) contains an anti-human antibody preparation from goat. IgG from a serum sample is bound with high specificity by these antibodies and precipitated. If the sample also contains rheumatoid factors, these will be absorbed by the IgG/anti-human IgG complex.

Separation properties:
- All IgG subclasses are bound and precipitated by the anti-human IgG antibodies.
- Human serum IgG in concentrations of up to 15 mg per ml are removed (average serum IgG concentration in adults: 12 mg per ml).
- Rheumatoid factors are also removed.
- The recovery rate of the IgM fraction is almost 100%.

Performance: The **patient samples** for analysis are diluted **1:101** with sample buffer. For example, add 10 µl sample to 1.0 ml sample buffer and mix well. Incubate the mixture for at least **10 minutes** at room temperature. Subsequently, it can be pipetted into the microplate wells according to the pipetting protocol.

Notes:
- Antibodies of the class IgG should not be analyzed with this mixture.
- It is possible to check the efficacy of the IgG/RF absorbent for an individual patient sample by performing an IgG test in parallel to the IgM test using the mixture. If the IgG test is negative, the IgM result can be considered as reliable.
- The calibrator and controls are ready for use, do not dilute them.

Incubation

(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 working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

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) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. 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 values.
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 IgM) 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).

Stop:
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 the 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, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on inquiry.
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**	**P 6**	**P 14**	**P 22**								
B	**pos.**	**P 7**	**P 15**	**P 23**								
C	**neg.**	**P 8**	**P 16**	**P 24**								
D	**P 1**	**P 9**	**P 17**									
E	**P 2**	**P 10**	**P 18**									
F	**P 3**	**P 11**	**P 19**									
G	**P 4**	**P 12**	**P 20**									
H	**P 5**	**P 13**	**P 21**									

The above pipetting protocol is an example of the **semiquantitative analysis** of antibodies in 24 patient samples (P 1 to P 24).

Calibrator (C), positive (pos.) and negative (neg.) control as well as the patient samples have been incubated in one well each. The reliability of the ELISA test can be improved by duplicate determinations of each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimizes 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

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (**cut-off**) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

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

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.

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 diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Test characteristics

Calibration: As no international reference serum exists for antibodies of the IgM class against measles viruses, results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the ratios of 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 activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. 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: Recombinant nucleoprotein from measles virus, strain HNT-PI, expressed in eukaryotes.

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 antibody titer. The lower detection limit of the Anti-Measles Viruses NP ELISA (IgM) is ratio 0.03.

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 Anti-Measles Viruses NP ELISA (IgM):

Antibodies against	n	Anti-measles virus NP positive
B. burgdorferi	10	0%
CMV	9	0%
EBV-CA	17	29.4%
Mumps virus	8	0%
Parvovirus B19	9	0%
Rubella virus	10	0%
HSV-1/2	2	0%
VZV	4	0%
Toxo. gondii	10	0%

Interference: Haemolytic, lipaemic and icteric samples showed no influence on the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for 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 sera. 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		
Serum	**Mean value (Ratio)**	**CV (%)**
1	0.9	2.9
2	1.8	3.2
3	3.7	2.4

Inter-assay variation, n = 4 x 6		
Serum	**Mean value (Ratio)**	**CV (%)**
1	0.9	4.2
2	2.0	5.2
3	3.7	2.7

Specificity and sensitivity: 50 clinically characterized patient samples (interlaboratory test samples fromf INSTAND, Germany) were examined with the EUROIMMUN Anti-Measles Viruses NP ELISA (IgM). The ELISA test showed a specificity of 100% and a sensitivity of 100%.

n = 50		INSTAND / LABQUALITY		
		positive	borderline	negative
EUROIMMUN ELISA	positive	16	0	0
	borderline	1	0	0
	negative	0	0	33

Reference range: The levels of the anti-measles-virus-NP antibodies (IgM) were analysed with this EUROIMMUN ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 0.2% of the blood donors were anti-measles virus NP positive (IgM).

Clinical significance

The measles virus (MV) is the most instantly recognizable 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, flue-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 aetiologic factor in otosclerosis. Anti-measles IgG for the serological diagnosis of otoslcerotic 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 CFS 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 concentrations CSQpath.-spec. (IgG) is put into relation to the CSF/serum quotient of the total IgG concentrations 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.

Life-long immunity is generally developed. However, antibody levels are 8 to 10 times lower in post-vaccination sera than in convalescent sera . A passive immunisation with specific immunoglobulin concentrates is usually given to immunosuppressed seronegative individuals, such as tumour patients and recipients of transplants, as well as to seronegative pregnant women after exposure to the virus.

The European Regional Office of the WHO aims at eliminating measles from the region in the following years by area-wide vaccination campaigns. This is expected to limit the number of apparent infections and especially of severe courses of the disease. For the diagnosis of the remaining cases of measles infection and of infections acquired outside Europe as well as for the clarification of atypical courses of the disease in partly immunised patients the antibody determination in serum and CSF will be of growing importance.

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