

**VIROTECH Masern/Measles IgM ELISA
(Masern/Measles IgM ELISA)**

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FOR IN VITRO DIAGNOSIS ONLY

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1. Intended Use

The Measles ELISA is intended for the detection of an acute or recent infection with Measles respectively for the detection of vaccination antibodies.

2. Diagnostic Relevance

The disease is caused by an RNA virus which is an exclusively human pathogen. It is a member of the Morbillivirus genus, Paramyxovirus family.

Measles is transmitted by direct contact, by inhalation of infectious droplets of expired air, or by infectious secretions from the nose or throat. The measles virus produces an infection even on brief exposure, with an index of contagion close to 100%, and provokes clinical symptoms in over 95% of unprotected infected individuals. The incubation period is 8-10 days to the start of the catarrhal stage and 14 days to the appearance of the rash (1).

The prevalence of IgG antibodies in the German population varies with age, lying between 77% at 2-4 years and 99.5% at ≥40 years (2).

The initial symptoms of measles are fever, conjunctivitis, coryza, coughing, and a rash on the palate. A pathognomonic feature is Koplik's spots, which can often be demonstrated. The characteristic maculopapular rash of measles develops on the 3rd to 7th day after onset of the initial symptoms. On day 5 to 7 of the illness, the temperature falls. Infection with measles confers lifelong immunity. Subacute sclerosing panencephalitis (SSPE) is a very rare late complication, occurring in 1-5 cases per million patients; it develops after a mean of 6-8 years following infection. It starts with psychological and intellectual changes, and runs a progressive course with neurological disturbances and failure of function, culminating in the loss of cerebral function. The prognosis is always poor.

In immunosuppressed individuals or those with cellular immune deficiency, measles infections may superficially appear mild - the measles rash may not develop, or may have an atypical appearance; however, severe organ complications may occur in the form of progressive giant-cell pneumonia or measles inclusion-body encephalitis, both of which have a mortality of around 30%.

The measles vaccine is a live virus vaccine prepared from attenuated measles virus. The vaccine of choice is MMR (a combined vaccine containing measles, mumps and rubella inocula).

The primary vaccination should be given at the age of 11-14 months, i.e. after the disappearance of maternal antibodies. The vaccines licensed in Germany produce seroconversion in about 90% of individuals after primary vaccination. Up to 5% of vaccinated children show what is known as vaccine-strain measles, with moderate fever, a fleeting rash and respiratory symptoms, mostly occurring in the second week after vaccination. The immune response produced by vaccination is detectable after 4-6 weeks.

Measles shows a fairly typical clinical picture, so laboratory tests to confirm the clinical diagnosis have in the past been the exception. With the introduction of protective vaccination, clinical measles has become very much rarer here, with the result that the diagnosis is uncertain in some cases, and laboratory diagnostic techniques have become increasingly important.

A wide range of methods are available for laboratory diagnosis, involving the detection of specific antibodies or of the virus itself. The detection of virus-specific IgM antibodies, as a marker of an ongoing disease process, is currently the quickest and safest method; as a rule it becomes positive at the time of onset of the rash, but it may remain negative up to day 3 of the rash in up to 30% of patients. IgM antibodies may persist for up to 6 weeks or even longer, making it possible to diagnose an exanthematous disease retrospectively.

In vaccinated individuals experiencing a reinfection, in whom no clear IgM response is seen, a negative finding does not rule out the diagnosis of measles. In such cases, a further serum sample should be investigated after an interval of 7-10 days. If the patient has measles, ELISA (IgG) or a complement fixation test can then demonstrate a significant rise in antibody titres and IgM ELISA can then have a positive result in the paired sera.

Viral cultures are extremely expensive and only justifiable in exceptional cases. A positive demonstration of measles virus RNA by RT-PCR in patient samples taken shortly before the onset of the rash confirms acute infection in the same way as the demonstration of IgM antibodies. However, a negative finding in the viral genome test does not rule out the disease (1).

Where the clinical picture suggests measles, virological and serological studies for differential diagnosis should be aimed primarily at ruling out rubella, erythema infectiosum (fifth disease), adenoviruses, enteroviruses and HHV-6 (3).

3. Test Principle

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. Package Contents (IgM Testkit)

1. **1 Microtiter-Plate** consisting of 96 with antigen coated, breakable single wells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7,2, with preservative and Tween 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7,2, with preservative and Tween 20
4. **IgM negative Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
5. **IgM cut-off Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
6. **IgM positive Control, 2000µl**, human serum with protein-stabilizer and preservative, ready to use
7. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

5. Storage and Shelflife of the Testkit and the ready to use reagents

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not useable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

Material	Status	Storage	Shelflife
Test Samples	Diluted	+2 to +8°C	max. 6h
	Undiluted	+2 to +8°C	1 week
Controls	After Opening	+2 to +8°C	3 months
Microtitreplate	After Opening	+2 to +8° (storage in the provided bag with desiccant bag)	3 months
Rheumatoid factor - Absorbent	Undiluted, After Opening	+2 to +8°C	3 months
	Diluted	+2 to +8°C	1 week
Conjugate	After Opening	+2 to +8°C (protect from light)	3 months
Tetramethylbenzidine	After Opening	+2 to +8°C (protect from light)	3 months
Stop Solution	After Opening	+2 to +8°C	3 months
Washing Solution	After Opening	+2 to +8°C	3 months
	Final Dilution (ready-to-use)	+2 to +25°C	4 weeks

6. Precautions and Warnings

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. Material required but not supplied

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA handwasher or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. Test Procedure

Working exactly referring to the VIROTECH Diagnostics user manual is the prerequisite for obtaining correct results.

8.1 Examination Material

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.

Always prepare patient-dilution freshly.

For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbially contaminated and turbid sera should not to be used (false positive/negative results).

8.2 Preparation of Reagents

The VIROTECH Diagnostics System offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are **parameter specific** and **only to use** with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. **For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech** (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 VIROTECH ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgM-positive, negative and cut-off control as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (with cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (with cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (with cover, keep in dark).

9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All VIROTECH Diagnostics ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

VIROTECH Diagnostics recommends the following procedure:

1. VIROTECH Diagnostics recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.

9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgM-antibodies. Their concentration can be expressed in VIROTECH units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values

The OD of the blank should be < 0.15.

The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) VIROTECH Units (VE)

The VIROTECH Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the VIROTECH Units (VE)

The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

$$\text{VE (positive control)} = \frac{\text{OD (positive control)}}{\text{OD (cut - off control)}} \times 10$$

$$\text{VE (patient serum)} = \frac{\text{OD (patient serum)}}{\text{OD (cut - off control)}} \times 10$$

9.3 Interpretation Scheme IgM

Result (VE)	Evaluation
< 9,0	negative
9,0 - 11,0	borderline
> 11,0	positive

1. If the measured values are above the defined borderline range, they are considered to be positive (please take notice of vaccination management!).
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

9.4 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. Anti-doublestrand DNA (α -dsDNA) sera (ANA, systemic lupus erithematodes) may show cross reactivities to the VIROTECH Diagnostics Measles ELISA IgM.
3. A possible additional cross-reaction cannot be completely ruled out between IgM antibodies, which are originally directed against other viruses, and an epitope of the measles antigen. Accordingly, as is the case with polyclonal stimulation, the measles IgM test may result in a false-positive result in individual cases. (5)
4. In rare cases, the IgM diagnostic may give a false positive result for the sera of pregnant women.

10. Performance Data

10.1 Diagnostical Sensitivity

A vaccination serum which had showed a clear negative result before vaccination was tested. 14 serum withdrawals (taken in a period of up to 55 days after vaccination) show a continuous increase of IgM antibodies until the fifth withdrawal (20th day after vaccination) and then a continuous decrease of the IgM antibodies in the following withdrawals.

10.2 Sensitivity and Specificity

A sera collective (37 sera obtained from routine tests, 120 blood bank sera, 65 sera from pregnant women, 12 children's sera, 29 sera from proficiency tests, 15 vaccination sera) was tested and showed in regard to the finding a sensitivity and specificity of >99,8% each.

Sera collective (n=278)		Measles ELISA IgM		
Finding		Negative	Borderline	Positive
	Negative	228	2	-
	Borderline	5	3	-
	Positive	-	2	38

Borderline results have not been considered when calculating sensitivity and specificity.

10.3 Prevalence (Expected Values)

The following table shows the results of the examination of 78 blood bank sera:

	IgM (n=78)
Negative	77
Borderline	1
Positive	0

10.4 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested in a chessboard pattern with two sera. The obtained coefficients of variation are < 9%.

10.5 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs on three different testdays. The obtained variation coefficient values are <15%.

11. Literature

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4. Evans, A.S., Kaslow, R.A., Viral Infections of Human, fourth edition, Plenum Medicals Book Company, 1997, 508-509.
5. Labordiagnostik schwangerschaftsrelevanter Virusinfektionen, S2k-Leitlinie, AWMF Registernummer 0093/001

Preparation of Patient Samples and Washing Solution

▼ **Washing Solution:** Fill up concentrate to 1 liter with aqua dest./demin.

▼ **IgM-Samples – Dilution**
1:101

Rheumafactor-absorption with RF-SorboTech

e.g.:

5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

