



Measles IgG ELISA

For In-Vitro and professional use only
Store at 2° to 8° C

INTRODUCTION

measles or morbilli virus belongs to the RNA viruses of the family Paramyxoviridae. The virions are spherical particles of 150-250 nm in diameter consisting of the ribonucleoprotein with helical symmetry and an envelope with spikes containing the strain-specific and hemagglutinating antigens. Morbilli viruses have no neuraminidase activity. Measles is a classic childhood disease. The virus is endemic: at the age of 20 about 90% of the population has had immunological experience with it. Newborns are protected by maternal antibodies for the first 3-4 months of life; the active disease leaves lifelong immunity. The measles virus has a contagiousness index of about 96%, is worldwide distributed, and can be serious. Bacterial superinfection was a serious threat in the preantibiotic era, but the prognosis of uncomplicated measles is now good. CNS complications such as encephalomyelitis (0.1%) which may occur after the acute phase of measles infection subsides, however still have a high mortality (10%). Prognosis of recovery in these patients is poor. Between 10-30% of all cases are fatal; 20-50% develop significant damages. Subacute sclerosing panencephalitis (SSPE) is a rare (1:1000) degenerative disease of the CNS which is thought to be a slow virus infection.

Species	Disease	Symptoms	Mechanism of Infection
measles virus	Measles	Fever, catarrh, abdominal pain, typical lesions in the mouth (Koplik's spots), characteristic exanthema	Measles virus is transmitted by air or contact and invades the respiratory tract
	SSPE (Subacute sclerosing panencephalitis)	Complications: Encephalomyelitis, decrease in intellectual skills that progresses to an almost complete loss of brain function and death	The incubation period is 11 to 14 days

The presence of virus resp. infection may be identified by

- PCR
- Complement fixation, Hemagglutination-Inhibition, Neutrlization
- Detection of antibody production by ELISA

INTENDED USE

ATLAS Measles virus IgG-ELISA is intended for the qualitative determination of IgG class antibodies against Measles virus in human serum and plasma.

PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgG-class antibodies against Measles Virus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Measles antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample materials horseradish peroxidase (HRP) labeled anti-human IgG conjugate is added. This conjugate binds to the captured Measles Virus-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of Measles Virus-specific IgG antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450nm is read using an ELISA microwell plate reader.

MATERIALS

Reagents supplied

1. Measles Virus Coated Wells (IgG): 12 breakapart 8-well snap-off strips coated with Measlea Antigen; in resalable aluminum foil.
2. IgG Sample Diluent: 1 bottle containing 100ml of buffer for sample dilution; pH 7.2±0.2; coloured yellow; ready to use; white cap.
3. Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.2 mol/l; ready to use; red cap.
4. Washing Solution (20x conc.): 1 bottle containing 50ml of a 20-fold concentrated buffer (pH 7.2±0.2) for washing the wells; white cap.
5. Measles anti-IgG Conjugate**: 1 bottle containing 20ml of peroxidase labeled rabbit antibody to human IgG; coloured red, ready to use; black cap.
6. TMB Substrate Solution: 1 bottle containing 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use; yellow cap.

7. Measles IgG Positive Control***: 1 bottle containing 2 ml; coloured yellow; ready to use; red cap.

8. Measles IgG Cut-off Control***: 1 bottle containing 3 ml; coloured yellow; ready to use; green cap.

9. Measles IgG Negative Control***: 1 bottle containing 2ml; coloured yellow; ready to use; blue cap.

* Contains 0.01 % kathon after dilution

** Contains 0.2 % Bronidox L

*** Contains 0.1 % kathon

Materials supplied

* 1 strip holder

* 2 Cover foils

* 1 Test protocol

* 1 Distribution and identification plan

Materials and Equipment needed

* Elisa microwell plate reader, equipped for the measurement of absorbance at 450/620 nm

* Incubator 37°C

* Manual or automatic equipment for rinsing wells.

* Pipettes to deliver volumes between 10 and 1000 µl

* Vortex tube mixer

* Deionised or (freshly) distilled water

* Disposable tubes

* Timer

STABILITY AND STORAGE

The reagents are stable up to the expiry date stated on the label when stored at 2...8°C.

REAGENT PREPARATION

It is very important to bring all reagent, samples and controls to room temperature (20...25°C) before starting the test run!

1. Coated snap-off Strips

The ready to use breakapart snap-off strips are coated with Measles Antigen. Store at 2...8°C. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8°C. stability until expiry date.

2. Measles anti IgG Conjugate

The bottles contain 20 ml of a solution with anti human IgG horseradish peroxidase, buffer, stabilizer preservatives and inert red dye. The solution is ready to use. Store at 2...8°C. After first opening stability until expiry date when stored at 2...8°C.

3. Controls

The bottles labeled with Positive, Cut-off and Negative Control contain a ready to use control solution. It contains 0.1% kathon and has to be stored at 2...8°C. After first opening stability until expiry date when stored at 2...8°C.

4. IgG Sample Diluent

The bottle contains 100ml phosphate buffer, stabilizers, preservatives and inert yellow dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. After first opening stability until expiry date when stored at 2...8°C.

5. Washing Solution (20xconc.)

The bottle contains 50ml of a concentrated buffer, detergents and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution +190 ml fresh and germ free redistilled water. The diluted buffer is stable for 5 days at room temperature. Crystals in the solution disappear by warming up to 37°C in a water bath. After first opening the concentrate is stable until the expiry date.

6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue. It may have become contaminated and should be discharged. After first opening stability until expiry date when stored at 2...8°C.

7. Stop Solution

The bottle contains 15ml 0.2M sulphuric acid solution. This ready to use solution has to be stored at 2...8°C. After first opening stability until expiry date.

SPECIMEN COLLECTION AND PREPERATION

Use human serum or plasma (citrate) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.

Sample Dilution.

Before assaying, all samples should be diluted 1+100 with IgG Sample Diluent. Dispense 10µl sample and 1 ml IgG Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. Positive and negative controls are ready to use and must not be diluted.

ASSAY PRCEDURE

Test Preparation

Please read the package insert carefully **before** performing the assay. Result reliability depends on strict adherence to the package insert as described. The following test procedure is only

validated for manual procedures. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Please allocate at least:

- | | | |
|---------|--------------|-----------------------------|
| 1 well | (e.g. A1) | For the substrate blank |
| 1 well | (e.g. B1) | For the negative control, |
| 2 wells | (e.g. C1+D1) | For the cut-off control and |
| 1 well | (e.g. E1) | for the positive control. |

It is recommended to determine controls and patient samples in duplicate, if necessary.

Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each control and samples.

Adjust the incubator to $37^{\circ} \pm 1^{\circ}\text{C}$.

- 1- Dispense 100µl controls and diluted samples into their respective wells. Leave well A1 for substrate blank.
- 2- Cover wells with the foil supplied in the kit.
- 3- Incubate for 1 hour \pm 5 min at $37 \pm 1^{\circ}\text{C}$.
- 4- When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of Washing Solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5 sec. at the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!
Note:- Washing is critical ! Insufficient washing results in poor precision and falsely elevated absorbance values.
- 5- Dispense 100µl of Measles anti-IgG Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
- 6- Incubate for 30 min at room temperature. Do not expose to direct sunlight.
- 7- Repeat step 4.
- 8- Dispense 100µl of TMB Substrate Solution into all wells.
- 9- Incubate for exactly 15 min at room temperature in the dark.
- 10- Dispense 100µl of Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour developed during the incubation turns into yellow.
Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in ATLAS unit by 2.

- 11- Measure the absorbance of the specimen at 450/620 nm within 30 min after addition of the stop solution.

Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If – due to technical reasons – the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

RESULTS

Run Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- Substrate blank in A1: Absorbance value lower than 0.100.
- Negative control in B1: Absorbance Value lower than 0.200.
- Cut-off control in C1 and D1: Absorbance value between 0.250 and 0.900.
- Positive control in E1: Absorbance value equal to or greater than the cut-off value.

CALCULATION OF RESULTS

The cut-off is the mean absorbance value of the Cut-off control determinations.

Example: Absorbance value Cut-off control 0.39 + absorbance value Cut-off control 0.37 = $0.76 / 2 = 0.38$

Cut-off = 0.38

Interpretation of Results

Samples are considered POSITIVE if the absorbance value is higher than 10% over the cut-off.

Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → **grey zone**

It is recommended to repeat the test again 2-4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

RESULTS IN ATLAS Units

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{unit}} = \text{ATLAS unit}$$

Cut-off

Example: $\frac{1.786 \times 10}{0.38} = 47 \text{ ATLAS Unit}$

Cut-off:	10	ATLAS UNITS
Grey zone:	9-11	ATLAS UNITS
Negative:	<9	ATLAS UNITS
Positive:	>11	ATLAS UNITS

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

Interassay	n	Mean	Cv (%)
Pos. Serum	12	9.4	5.0
	12	76.9	1.5

Intrassay	n	Mean	Cv (%)
Pos Serum	18	0.36	9.4
	24	3.11	3.2

Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is >95%.

Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte it is >95%.

Interferences

Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5mg/ml triglycerides and 0.2 mg/ml bilirubin.

Note: The results refer to the groups of samples investigated; these are not guaranteed specifications.

LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the bases of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.

In immunocompromised patients and newborns serological data only have restricted value.

PRECAUTIONS AND WARNING

- Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the test kits with analyzers and similar equipment has to be validated.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
 - Do not interchange reagents or strips of different production lots.
 - No reagents of other manufacturers should be used along with reagents of the test kit.
 - Do not use reagents after expiry date stated on the label.
 - Use only clean pipette tips, dispensers, and lab ware.
 - Do not interchange screw caps of reagent vials to avoid cross-contamination.
 - After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
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 - To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	In the used concentration Bronidox L has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

12. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

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