NovaTec Immundiagnostica GmbH

NovaLisa®

Measles Virus IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MEAM0330 (96 Determinations)

ENGLISH

1. 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 contagiousity 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 (e.g.)	Transmission route
Measles Virus	Measles	Fever, malaise, productive cough and runny nose, headache, abdominal pain, typical lesions in the mouth (Koplik spots), characteristic exanthema	By air or contact with saliva or nasal secretions
	SSPE (Subacute sclerosing panencephalitis)	Complications: Encephalomyelitis, decrease in intellectual skills that progresses to an almost complete loss of brain function and death	

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Measles Virus IgM ELISA is intended for the qualitative determination of IgM class antibodies against Measles Virus in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate (IgM):** 12 break-apart 8-well snap-off strips coated with Measles Virus antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Measles Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

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 thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration 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 important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

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

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis 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.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.417	6.68
#2	24	0.995	2.99
#3	24	1.987	3.20
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	24.29	4.31
#2	12	52.75	6.65
#3	12	4.70	9.87

10.2. 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 100% (95% confidence interval: 98.71% - 100%).

10.3. 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 100% (95% confidence interval: 91.19% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this 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.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. 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.

13. ORDERING INFORMATION

Prod. No.: MEAM0330 Measles Virus IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Das Masern- oder Morbillivirus ist ein RNA-Virus der Familie der Paramyxoviridae. Masernviren sind antigenisch stabil und bilden einen Serotyp. Bei Untersuchungen mit Hilfe molekularbiologischer Methoden werden mehrere Genotypen unterschieden (in Mitteleuropa kommen gegenwärtig C2 und D6 vor). Das Masernvirus ist sehr empfindlich gegenüber äußeren Einflüssen wie erhöhten Temperaturen, Licht, UV-Strahlen, Fettlösungs- und Desinfektionsmitteln. Masernvirusinfektionen treten typischerweise in der Kindheit auf. Der einzige Wirt für das hochkontagiöse Virus ist der Mensch. Die Übertragung erfolgt aerogen durch Tröpfcheninfektion und führt bereits bei kurzer Exposition nicht-immuner Personen zur Erkrankung (Kontagionsindex nahe 100%).

Das Virus ist sehr lymphotrop und verursacht nach Replikation in den lymphatischen Geweben eine transiente Lymphopenie mit begleitender Immunsuppression. Nach hämatogener Aussaat erreicht das Virus die Haut und die oberen Atemwege. Die einsetzende zelluläre Immunantwort führt schließlich zur Ausbildung des typischen Exanthems. Nach einer Inkubationszeit von etwa 2 Wochen entwickelt sich eine unspezifische katarrhalische Symptomatik. Es entwickelt sich eine Entzündung der oberen Atemwege (Rhinitis, Pharyngitis, Laryngitis, Tracheitis, Bronchitis), sowie häufig auch eine Konjunktivitis. Pneumonien sind möglich, jedoch selten. Unter Fieberanstieg bis 41°C entsteht schließlich das makulopapulöse Masernexanthem (beginnend am Kopf mit kraniokaudaler Ausbreitung). Besonders komplikationsreich sind die Masernenzephalitiden, die nach einer akuten Infektion auftreten können. Man unterscheidet drei Verlaufsformen:

die akute postinfektiöse Form (bei etwa 10-20 % der Betroffenen endet sie tödlich, bei etwa 20-30 % muss mit Residualschäden am ZNS gerechnet werden)

die akute, progressive Form (Auftreten gilt als infaust; Komplikation bei Patienten mit eingeschränkter Immunkompetenz) und

die subakute sklerosierende Panenzephalitis (SSPE) als sehr seltene Spätkomplikation (1-5 Fälle pro 1 Mio. Erkr.), die sich nach durchschnittlich 6-8 Jahren manifestiert. Beginnend mit psychischen und intellektuellen Veränderungen entwickelt sich ein progredienter Verlauf mit neurologischen Störungen und Ausfällen bis zum Verlust zerebraler Funktionen. Die Prognose ist stets infaust.

Eine Masernerkrankung kann vorübergehend die zelluläre Immunität so unterdrücken, dass eine Tuberkulose exazerbiert.

Zur Prophylaxe steht ein Lebendimpfstoff, sowohl als Monopräparat als auch als Kombinationsimpfstoff, zur Verfügung.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Masernvirus (Morbillivirus) **(auf English Measles Virus)**	Masern	Fieber, Malaise, produktive Husten und laufende Nase, Kopfschmerzen, Bauchschmerzen, Koplik-Flecken (kalkspritzerartige, weiße Flecken in der Wangenschleimhaut), charakteristisches Exanthem	aerogen durch Tröpfchen oder Kontakt mit Speichel und Nasensekret
	Subakute sklerosierende Panenzephalitis	Komplikationen: Enzephalomyelitis, Abnahme der intellektuellen Fähigkeiten bis zu einem fast vollständigen Verlust der Hirnfunktion und Tod	

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Measles Virus IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Measles Virus **(Masernviren)** in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Measles Virus IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MEAG0330 (96 Determinations)

ENGLISH

1. 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 contagiousity 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 (e.g.)	Transmission route
Measles Virus	Measles	Fever, malaise, productive cough and runny nose, headache, abdominal pain, typical lesions in the mouth (Koplik spots),characteristic exanthema	By air or contact with saliva or nasal secretions
	SSPE (Subacute sclerosing panencephalitis)	Complications: Encephalomyelitis, decrease in intellectual skills that progresses to an almost complete loss of brain function and death	

Infection or presence of pathogen may be identified by:

- PCR
- Serology: e.g. by ELISA

2. INTENDED USE

The Measles Virus IgG ELISA is intended for the qualitative determination of IgG class antibodies against Measles Virus in human serum or plasma (citrate, heparin).
Measles Virus IgG avidity can be determined with assay Avidity Measles Virus IgG (Product code: AMEA7330).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Measles Virus antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Measles Virus antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

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 thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration 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 important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

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

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

	NTU	**mIU/mL** (3rd International Standard)	
Cut-off	10	-	-
Positive	> 11	> 220	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11	120 – 220	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9	< 120	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis 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.			

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

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

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.314	6.93
#2	24	0.931	3.70
#3	24	0.718	9.98
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	33.93	2.76
#2	12	28.09	7.46
#3	12	3.85	12.11

10.2. 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 100% (95% confidence interval: 90.0% - 100%).

10.3. 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 97.01% (95% confidence interval: 93.93% - 98.79%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this 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.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. 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.

13. ORDERING INFORMATION

Prod. No.:	MEAG0330	Measles Virus IgG ELISA (96 Determinations)

For avidity testing:

AMEA7330	Avidity Measles Virus IgG ELISA (48 Determinations)

DEUTSCH

1. EINLEITUNG

Das Masern- oder Morbillivirus ist ein RNA-Virus der Familie der Paramyxoviridae. Masernviren sind antigenisch stabil und bilden einen Serotyp. Bei Untersuchungen mit Hilfe molekularbiologischer Methoden werden mehrere Genotypen unterschieden (in Mitteleuropa kommen gegenwärtig C2 und D6 vor). Das Masernvirus ist sehr empfindlich gegenüber äußeren Einflüssen wie erhöhten Temperaturen, Licht, UV-Strahlen, Fettlösungs- und Desinfektionsmitteln. Masernvirusinfektionen treten typischerweise in der Kindheit auf. Der einzige Wirt für das hochkontagiöse Virus ist der Mensch. Die Übertragung erfolgt aerogen durch Tröpfcheninfektion und führt bereits bei kurzer Exposition nicht-immuner Personen zur Erkrankung (Kontagionsindex nahe 100%).

Das Virus ist sehr lymphotrop und verursacht nach Replikation in den lymphatischen Geweben eine transiente Lymphopenie mit begleitender Immunsuppression. Nach hämatogener Aussaat erreicht das Virus die Haut und die oberen Atemwege. Die einsetzende zelluläre Immunantwort führt schließlich zur Ausbildung des typischen Exanthems. Nach einer Inkubationszeit von etwa 2 Wochen entwickelt sich eine unspezifische katarrhalische Symptomatik. Dadurch kommt es zu einer Entzündung der oberen Atemwege (Rhinitis, Pharyngitis, Laryngitis, Tracheitis, Bronchitis), sowie häufig auch eine Konjunktivitis. Pneumonien sind möglich, jedoch selten. Unter Fieberanstieg bis 41°C entsteht schließlich das makulopapulöse Masernexanthem (beginnend am Kopf mit kraniokaudaler Ausbreitung). Besonders komplikationsreich sind die Masernenzephalitiden, die nach einer akuten Infektion auftreten können. Man unterscheidet drei Verlaufsformen:

- die akute postinfektiöse Form (bei etwa 10-20 % der Betroffenen endet sie tödlich, bei etwa 20-30 % muss mit Residualschäden am ZNS gerechnet werden)
- die akute, progressive Form (Auftreten gilt als infaust; Komplikation bei Patienten mit eingeschränkter Immunkompetenz) und
- die subakute sklerosierende Panenzephalitis (SSPE) als sehr seltene Spätkomplikation (1-5 Fälle pro 1 Mio. Erkr.), die sich nach durchschnittlich 6-8 Jahren manifestiert. Beginnend mit psychischen und intellektuellen Veränderungen entwickelt sich ein progredienter Verlauf mit neurologischen Störungen und Ausfällen bis zum Verlust zerebraler Funktionen. Die Prognose ist stets infaust.

Eine Masernerkrankung kann vorübergehend die zelluläre Immunität so unterdrücken, dass eine Tuberkulose exazerbiert.

Zur Prophylaxe steht ein Lebendimpfstoff, sowohl als Monopräparat als auch als Kombinationsimpfstoff, zur Verfügung.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Masernvirus (Morbillivirus) **(auf English Measles Virus)**	Masern Subakute sklerosierende Panenzephalitis	Fieber, Malaise, produktiver Husten und laufende Nase, Kopfschmerzen, Bauchschmerzen, Koplik-Flecken (kalkspritzerartige, weiße Flecken in der Wangenschleimhaut), charakteristisches Exanthem Komplikationen: Enzephalomyelitis, Abnahme der intellektuellen Fähigkeiten bis zu einem fast vollständigen Verlust der Hirnfunktion und Tod	aerogen durch Tröpfchen oder Kontakt mit Speichel und Nasensekret

Nachweis des Erregers bzw. der Infektion durch:

- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Measles Virus IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Measles Virus **(Masernviren)** in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.
Die Masernvirus IgG Avidität kann bestimmt werden mit dem Test: Avidity Measles Virus IgG (Produktnummer: AMEA7330).

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

NovaTec Immundiagnostica GmbH

NovaLisa®

Avidity Measles Virus IgG

ELISA Supplementary Instruction

CE

Only for in-vitro diagnostic use

Product Number: AMEA7330 (48 Determinations)

ENGLISH

1. INTRODUCTION

The presence of IgG antibodies to Measles virus indicates the occurrence of the infection but does not distinguish between recent and past infection. Specific IgM antibodies are first detected approximately in ten days and peak at about four weeks post infection. They may persist for several months after acute infections. Based on the evidence that antibody avidity gradually increases after exposure to an immunogen, avidity of IgG antibodies can be used as a marker for distinguishing recent primary from long-term infections. Avidity describes the binding strength of a specific antibody to its antigen. Low-avidity IgG antibodies indicate a primary infection, whereas the presence of IgG antibodies with high avidity points to persistency or reactivation of infection.

2. INTENDED USE

The Avidity Measles Virus IgG ELISA is intended to indicate the Measles-specific IgG avidity in human serum or plasma (citrate, heparin) to differentiate between acute and past infection.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microplates are coated with specific antigens to bind corresponding antibodies of the sample (dual pipetting). After washing the wells to remove all unbound sample material, one well is incubated with avidity reagent and the corresponding well with washing buffer. The avidity reagent removes the low-avidity antibodies from the antigens whereas the high-avidity ones are still bound to the specific antigens. After second washing step to remove the rest of avidity reagent and low-avidity antibodies, a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a third washing step unbound conjugate is removed. 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 specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Avidity Reagent:** 1 bottle containing 15 mL of an urea solution, coloured blue, ready to use; black cap.
- **Control Low:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.02% (v/v) MIT.
- **Control High:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 instruction for use Avidity Measles Virus IgG ELISA (Product Number: AMEA7330)
- 1 Instruction for use Measles Virus IgG ELISA (Product Number: MEAG0330)
- 1 empty labelled bottle (white with white cap) for ready to use Washing Buffer

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Avidity Reagent

If crystals have formed in the reagent warm up to 37 °C e.g. in a water bath and mix gently until they disappear.

6.2. Washing Buffer

It is recommended to fill 15 mL ready to use Washing Buffer into supplied bottle (s. 4.2) to use it in step Step 5 of the test preparation.
Note: Ready to use Washing Buffer is stable for 5 days at room temperature (20…25 °C).

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) **Measles Virus IgG positive** samples with this assay.
Note: For samples with high absorbance values (OD > 2.000) appropriate higher dilutions should be used.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

For avidity determination dual pipetting of standards/controls and diluted samples is needed.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave wells A1/A2 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration 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 important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL of Avidity Reagent in wells B1, C1, D1, E1 etc, except for the Substrate Blank well A1.

   Dispense 100 µL of Washing Buffer in wells B2, C2, D2, E2 etc, except for the Substrate Blank well A2.
6. **Incubate for exactly 10 min at 37 ± 1 °C.**
7. Repeat step 4.
8. Dispense 100 µL Conjugate into all wells except in the blank wells (A1/A2).
9. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
10. Repeat step 4.
11. Dispense 100 µL TMB Substrate Solution into all wells.
12. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
13. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate, thereby a colour change from blue to yellow occurs.
14. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value 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 standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Control Low**: Avidity (%): **< 45 %**
- **Control High**: Avidity (%): **> 55 %**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

For each patient sample or control calculate the ratio between the absorbance of the well dispensed with Avidity Reagent and the absorbance of the well dispensed with Washing Buffer multiplied by 100:

$$\frac{\text{Absorbance (sample or control) Avidity Reagent}}{\text{Absorbance (sample or control) Washing Buffer (diluted 1+19)}} \times 100 = \text{Avidity (\%)}$$

Note: For samples with high absorbance values (OD > 2.000) appropriate higher dilutions should be used.

9.3. Interpretation of Results

Result	Avidity	Interpretation
Low-avidity IgG	< 45 %	An avidity index of less than 45 % indicates a primary infection acquired within the past 2 months.
Equivocal	45 – 55 %	No clinical interpretation can be deduced from an equivocal result. It is recommended to take a second sample within an appropriate period of time (e.g. 2 weeks) and repeat testing. If the result of the repeated test is still equivocal, precise statements regarding the time of infection cannot be made.
High-avidity IgG	> 55 %	The presence of high-avidity IgG indicates a past infection or reinfection.
Diagnosis of an infectious disease should not be established on the basis 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. A result of high avidity can not exclude the possibility of a recent infection.		

9.3.1. Antibody Isotypes and State of Infection

IgG	IgM	IgG-Avidity	Probable result
+	-	low	Vague, further investigation necessary
+	-	high	Indicatives of a past infection
+	+	low	Suggests a current or very recent infection
+	+	high	Suggests a past infection with persisting IgM or reactivation of infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.
For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Diagnostic Performance

The evaluation of the diagnostic performance of the Avidity Measles Virus IgG test was performed in comparison to well defined samples. The resulting relative agreement was 100 %.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- 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.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this 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.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray.
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. 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.

13. ORDERING INFORMATION

Prod. No.: AMEA7330 Avidity Measles Virus IgG (48 Determinations)

DEUTSCH

1. EINLEITUNG

Obwohl die Anwesenheit von IgG-Antikörpern gegen das Masernvirus auf das Vorliegen einer Infektion hindeutet, kann man nicht zwischen einer akuten und einer bereits abgelaufenen Infektion unterscheiden. Virus-spezifische IgM-Antikörper sind ca. zehn Tage nach der Infektion erstmals nachweisbar und erreichen nach ungefähr vier Wochen ihre höchste Konzentration. Sie können für mehrere Monate nach der akuten Infektion im Blut persistent sein. Aufgrund der Tatsache, dass die Antikörper-Avidität nach der Exposition zum Antigen allmählich zunimmt, kann die Avidität von IgG Antikörpern als Marker benutzt werden, um zwischen einer akuten oder einer bereits länger zurückliegenden Infektion zu unterscheiden. Avidität beschreibt die Bindungsstärke der spezifischen Antikörper an das Antigen. Niedrig-avide IgG-Antikörper deuten auf eine frische Infektion hin, während hoch-avide IgG Antikörper ein Hinweis auf eine länger zurückliegende Infektion oder eine Reaktivierung sind.

2. VERWENDUNGSZWECK

Der Avidity Measles Virus IgG ELISA ist für die Aviditätsbestimmung der spezifischen IgG-Antikörper gegen das Masernvirus in humanem Serum oder Plasma (Citrat, Heparin), zur Differenzierung zwischen akuten und zurückliegenden Infektionen bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe (zweifaches Pipettieren) binden. Nach dem Waschen, wodurch das ungebundene Probenmaterial entfernt wird, wird eine Vertiefung mit dem Aviditätsreagenz und die andere dazugehörige Vertiefung mit dem Waschpuffer inkubiert. Durch das Aviditätsreagenz wird die Bindung zwischen den niedrig-aviden Antikörpern und den Antigenen gelöst, während die hoch-aviden Antikörper noch an den spezifischen Antigenen gebunden bleiben. Nach dem zweiten Waschschritt werden die Reste des Aviditätsreagenzes sowie niedrig-avide Antikörper entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem dritten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Aviditätsreagenz:** 1 Flasche mit 15 mL Harnstofflösung; blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **Kontrolle Niedrig:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; gebrauchsfertig; blaue Verschlusskappe; ≤ 0,02% (v/v) MIT.
- **Kontrolle Hoch:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; gebrauchsfertig; rote Verschlusskappe; ≤ 0,02% (v/v) MIT.

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 Arbeitsanleitung Avidity Measles Virus IgG ELISA (Produktnummer: AMEA7330)
- 1 Arbeitsanleitung Measles Virus IgG ELISA (Produktnummer: MEAG0330)
- 1 leere, etikettierte Flasche (weiß mit weißem Deckel) für den gebrauchsfertigen Waschpuffer

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Aviditätsreagenz

Sollte eine Kristallisation im Aviditätsreagenz auftreten, das Reagenz z. B. in einem Wasserbad auf 37 °C erwärmen und vor der Verwendung gut mischen.

6.2. Waschpuffer

Es wird empfohlen 15 mL des gebrauchsfertigen Waschpuffers in die mitgelieferte Flasche (s. 4.2) zu überführen, um diese in dem Schritt 5 der Testvorbereitung zu verwenden.
Beachte: Der gebrauchsfertige Waschpuffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar.