TestLine
Clinical Diagnostics

Instruction for use

EIA Measles IgG

REF MeG096

Σ 96

Kit for professional use

IVD CE

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CONTENT

Enzyme immunoassay for the detection of IgG antibodies to Measles virus and IgG avidity in human serum or plasma

1 Úvod

Measles are a highly contagious viral infectious disease caused by the measles virus, genus Morbillivirus, family Paramyxoviridae. This disease was one of the most common causes of child mortality worldwide under the age of five, and the incidence of measles has decreased since the introduction of vaccination.

The only natural host of measles virus is human. The disease is transmitted via droplet infection or direct contact with the patient. The incubation period is approximately 10 days, with high fever, cough, conjunctivitis and rhinitis being the first characteristic features of measles. A typical deep red rash appears around the ear and gradually spreads across the face to the entire body after 3-5 days. Characteristic white spots (so-called Koplik spots) may appear on the inside of the cheeks. The rash fades after a few days, and gradually subsides. The most difficult complications of measles include brain inflammation (encephalitis), pneumonia and otitis. After recovery, the patient is usually immune to measles for life.

The main prevention of measles is the general vaccination of children with MMR vaccine, which contains weakened measles, mumps and rubella viruses.

Diagnosis of measles is based on clinical picture and laboratory tests. Serological methods testing for specific antibodies using ELISA are often used in laboratory diagnostics of measles. Specific antibodies are produced within 3-10 days after the onset of clinical signs in measles infection and are typical of IgM and IgG specific antibodies. While IgM antibodies disappear after a few weeks, IgG antibodies persist for a long time, usually for life. Significant increases in IgG antibody levels occur after vaccination, although titres of these antibodies are generally lower than after natural infection and may not persist for life. The determination of IgG antibody levels also serves as a control of vaccination effect.

2 Test Principle

The kit is intended for detection of specific IgG antibodies in a sample by means of a sandwich type of the EIA method (i.e. a solid phase coated with specific antigen – antibody from the analysed sample – labelled antibody). The labelled antibody (conjugate) is an animal immunoglobulin fraction to human IgG conjugated with horseradish peroxidase. Peroxidase activity is determined in the test by a substrate containing TMB. Positivity is indicated when blue colour appears; after stopping solution has been added, blue changes to yellow. The yellow colour intensity is measured by a photometer at 450 nm, and it is proportional to the concentration of specific IgG antibodies in the sample.

Antigen used

Purified and inactivated native antigen with high content of specific immunodominant epitopes.

3 Materials Provided

MICROPLATE	**Microtitre Plate** coated with antigen, 12 x 8 wells in bag with desiccant	1 pc
CONTROL - CAL1	**Negative Control (Calibrator 1) 25 IU/l** Solution containing no specific human antibodies, ready to use	1 × 2 ml
CUTOFF CAL2	**CUT-OFF (Calibrator 2) 250 IU/l** Solution containing specific human antibodies in cut-off concentration, ready to use	1 × 3 ml
CONTROL + CAL3	**Positive Control (Calibrator 3) 1000 IU/l** Solution containing specific human antibodies, ready to use	1 × 2 ml
CAL4	**Calibrator 4 (5000 IU/l)** Solution containing specific human antibodies, ready to use	1 × 2 ml
CONJUGATE	**Conjugate** Solution containing peroxidase labelled animal immunoglobulin to human IgG, ready to use	1 × 15 ml
DILUENT 2	**Sample Diluent 2** Buffer with protein stabilisers, ready to use	1 × 105 ml
SUBSTRATE 2	**TMB-Complete 2** Chromogenic substrate solution containing TMB/H_2O_2, ready to use	1 × 15 ml
WASH 20x	**Wash Solution** 20× concentrated buffer	1 × 75 ml
STOP	**Stop Solution** 1M sulphuric acid solution, ready to use	1 × 15 ml
AVIDITY 1	**Avidity Solution 1** Stabilised urea solution	1 × 7 ml
	Instructions for use	1 pc

4 Other Material Required for Manual Test Performance

Single and multichannel pipettes

Disposable tips

Microplate washer

Timer

Incubator (37°C)

Microplate reader

5 Storage and Stability

Store the kit at +2°C to +8°C. Do not freeze. If the kit is stored as described, the labelled expiration date is valid. The expiration date is indicated on the package. The opened kit should be used within three months.

Sample Preparation and Storage

The following human body liquids can be used for testing: serum and citrate plasma. Anticoagulants in the plasma (except for citrate) as well as bacterially contaminated, haemolytic or chylous samples can affect the test results.

Samples can be stored at +2°C to +8°C for one week. For a longer period, store samples at -20°C. Diluted samples should be used as soon as possible.

6 Preparation of Reagents

Dilute the Wash Solution 1:20 (1 part of solution and 19 parts of distilled water); e.g. 75 ml of the concentrated Wash Solution + 1425 ml of distilled water.

Salt crystals might develop in the bottle with the concentrated Wash Solution. Prior to use, it is necessary to dissolve the crystals by warming the bottle in a water bath. The diluted Wash Solution is stable at +2°C to +8°C for one week.

The Controls and the Calibrators are supplied ready to use, do not dilute further!

The Conjugate is supplied ready to use, do not dilute further!

TMB-Complete is a one-component chromogenic substrate solution ready to use, do not dilute further!

Interchangeability of reagents

The Sample Diluent, TMB-Complete and the Avidity Solution are interchangeable in EIA kits of TestLine Clinical Diagnostics s.r.o., provided they have the identical numeric marking (e.g. Sample Diluent 2, Sample Diluent 3, etc.). The Stop Solution and the Wash Solution are universal in all kits.

7 Preparation of Samples

Mix gently the Sample Diluent prior to use.

Dilution of sera and plasma samples

Dilute well mixed samples 1:101 with the Sample Diluent:

E.g.: 10 µl of sample + 1 ml of the Sample Diluent

Mix well.

8 Assay Procedure

Allow all reagents to come to room temperature and mix well. If you do not use a whole microplate, return unnecessary strips into the bag with desiccant. Seal the bag tightly and store at +2°C to +8°C. Keep dry!

1. Dispense the controls (calibrators) and the diluted samples according to the working schedule.

Semiquantitative evaluation in Index of Positivity (IP)

- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
- Pipette 100 µl of the diluted samples (see Chater Preparation of Samples) into the other wells.

Quantitative evaluation in Units IU/l

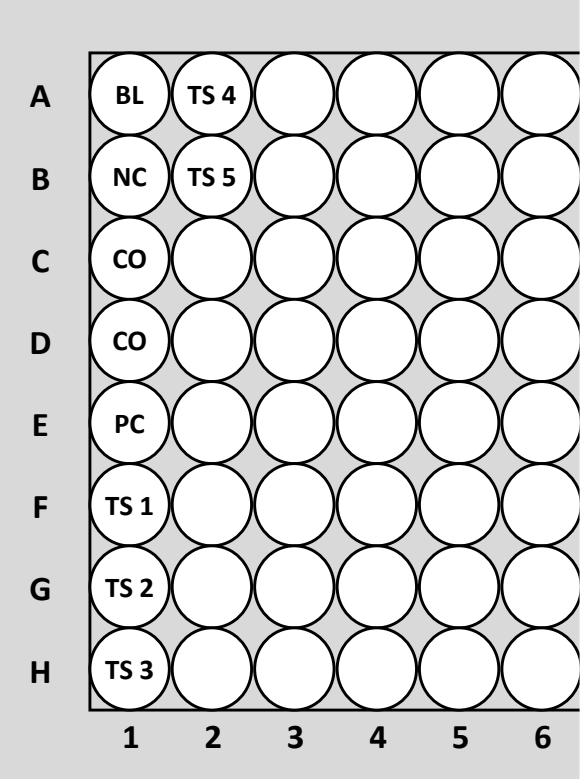
- Leave A1 well empty (blank).
- Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
- Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
- Pipette 100 µl of the Positive Control (Calibrator 3) into 2 wells.
- Pipette 100 µl of the Calibrator 4 into 2 wells.
- Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into the other wells.

2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Conjugate into all wells except A1 well.
5. Cover the microplate with the lid and incubate it at 37°C for 30 minutes.

6. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
7. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
8. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
9. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
10. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

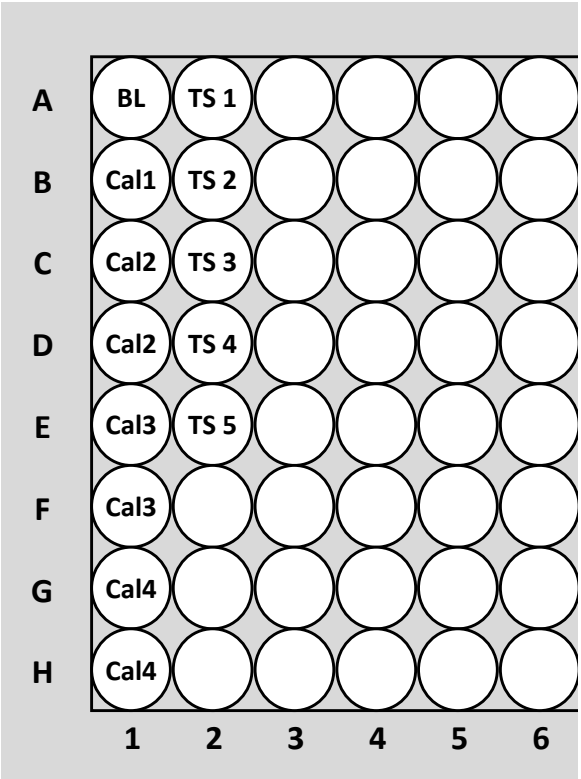
9 Working Schedule

Semiquantitative evaluation
Index of Positivity (IP)



BL	Blank (empty well)	
NC	100 µl	CONTROL - CAL1
CO	100 µl	CUTOFF CAL2
PC	100 µl	CONTROL + CAL3
TS 1-x	100 µl	diluted tested sample

Quantitative evaluation
Units IU/l



BL	Blank (empty well)	
Cal1	100 µl	CONTROL - CAL1
Cal2	100 µl	CUTOFF CAL2
Cal3	100 µl	CONTROL + CAL3
Cal4	100 µl	CAL4
TS 1-x	100 µl	diluted tested sample

10 Quality Control

The test is valid if:

The absorbance of blank is lower than 0.150.

BLANK < 0.150

The absorbance of the Negative Control (Calibrator 1) is lower than half of the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL - CAL1 < 0.5 × CUTOFF CAL2

The mean absorbance of CUT-OFF (Calibrator 2) is within a range of 0.150 – 0.900.

0.150 < CUTOFF CAL2 < 0.900

The absorbance of the Positive Control (Calibrator 3) is 1.5-fold higher than the mean absorbance of CUT–OFF (Calibrator 2).

CONTROL + CAL3 > 1.5 × CUTOFF CAL2

The absorbance of the Calibrator 4 is higher than the absorbance of the Positive Control (Calibrator 3).

CAL4 > CONTROL + CAL3

11 Results Interpretation

Calculation of Index of Positivity (IP)

Divide the absorbance of a tested sample by the mean absorbance of CUT-OFF measured in the same test run:

$$IP = \frac{\text{Absorbance of sample}}{\text{Mean absorbance of CUT-OFF}}$$

Interpretation of the test results is described in Table 1.

Table 1 Interpretation of test results

Index of Positivity (IP)	Evaluation
lower than 0.9	negative
0.9 to 1.1	borderline
higher than 1.1	positive

Examination of borderline samples, i.e. samples with Index of Positivity from 0.9 to 1.1, should be repeated from a new sample collected after certain time regarding to the disease specifics.

Quantitative evaluation in Units (IU/l)

Construct a calibration curve by plotting the concentration (X) of the calibrators in IU/l against the corresponding absorbance (Y). Construct the calibration curve by single point cross connection. Read the values of antibody level (IU/l) in samples from the calibration curve. Interpretation of the quantitative test results is described in Table 2.

Table 2 Quantitative interpretation in Units (IU/l)

Antibody level	Evaluation
lower than 225	negative
225 to 275	borderline
higher than 275	positive

Examination of borderline samples should be repeated from a new sample collected after 2 to 6 weeks regarding to the disease specifics.

Note

Quantitative evaluation in International Units was derived from the WHO international standard (3rd IS 97/648).

Serological finding can be interpreted only in the context of results of other laboratory tests and patient clinical picture.

12 Safety Precautions

The kit is intended for in vitro diagnostic use only.

The sera used for controls were tested and found to be negative for HIV 1 and HIV 2, HBsAg, HCV, TPHA. In spite of this fact, they still need to be handled as potentially infectious materials.

Some reagents contain sodium azide, which is a toxic compound. Avoid contact with skin.

The Stop Solution contains diluted acid solution. Avoid contact with eyes and skin.

It is necessary to observe the local safety rules and regulations.

First aid

In case of contact with eyes, flush with copious amount of water and seek medical assistance. In case of contact with skin and clothing, remove all the contaminated clothes. Wash the skin with soap and plenty of running water. In case of contact with solutions containing plasma or clinical samples, disinfect the skin. In case of accidental ingestion, flush the mouth with drinking water and seek medical assistance.

Remnants disposal

All the materials used for performing the test must be treated as potentially infectious due to the contact with biological materials. Therefore they need to be disposed together with biological waste.

Expired kit disposal

Disassemble the kit and dispose the components as biological material. Discard the packaging material as required by local regulations.

13 Procedural Notes

In order to obtain reliable results, it is necessary to **strictly follow the Instructions for Use**. Always use clean preferably disposable tips and glassware.

Microtitre Plate – in order to prevent water condensation on the surface of the microplate, always allow the bag with the microplate to warm up to room temperature before opening.

Wash Solution – use high quality distilled water for preparing the working strength Wash Solution.

Washing procedure – keep to the prescribed number of wash cycles and fill the wells to the upper edge. The soak time (i.e. interval between two different wash cycles during which the wells stay filled up with the Wash Solution) should be approx. 30-60 seconds.

TMB-Complete – the vessel used for multichannel pipetting should not be used for other reagents. Do not return the surplus TMB-Complete from the pipetting vessel into the vial.

Non-reproducible results might be caused by improper methodology as following:

- insufficient mixing of reagents and samples before use
- improper replacement of vial caps
- using the same tip for pipetting different reagents
- reagent exposure to excessive temperature; bacterial or chemical contamination
- insufficient washing or filling of the wells (the wells should be filled to the upper edge), improper aspiration of Wash Solution remnants
- contamination of the well edges with Conjugate or samples
- using reagents from different kit lots
- contact of reagents with oxidants, heavy metals and their salts

The kit might be used for sequential examinations. When preparing working strength solutions, use only the amount of reagents needed for the analysis.

The kit might be used in all types of automatic EIA analysers.

If necessary, TestLine Clinical Diagnostics s.r.o. can offer a certified modification of the Instructions for Use for the specific type of analyser.

The producer cannot guarantee that the kit will function properly if the assay procedure instructions are not strictly adhered to.

14 Index of Avidity

14.1 Introduction

Antibody avidity expresses the strength of bond between antigen and antibody. Low avidity antibodies are produced in the early stages of a primary infection. As the infection progresses, immune response of organism matures and avidity of antibodies increases. Antibodies show high avidity in the latent phase of the disease. High avidity IgG antibodies are produced by memory B-cells from the beginning of a secondary infection or reactivation.

Determination of IgG antibodies avidity enables differentiation of various stages of infection and is a useful addition to serological diagnostics.

14.2 Test Principle

Avidity determination is based on dissociation of antigen-antibody bond by means of Avidity Solution (urea solution). After Avidity Solution treatment, low avidity antibodies are released and washed out while high avidity antibodies remain bound to the antigen. The binding strength is expressed by the Index of Avidity (IAv). IAv determines the portion of IgG antibodies that remains bound to antigen after incubation with Avidity Solution. IAv assay procedure is a modification of the standard ELISA procedure using Avidity Solution.

The IgG avidity is determined only in IgG positive samples.

14.3 Dilution of Samples

Dilution of sera and plasma samples (see Chapter Preparation of Samples).

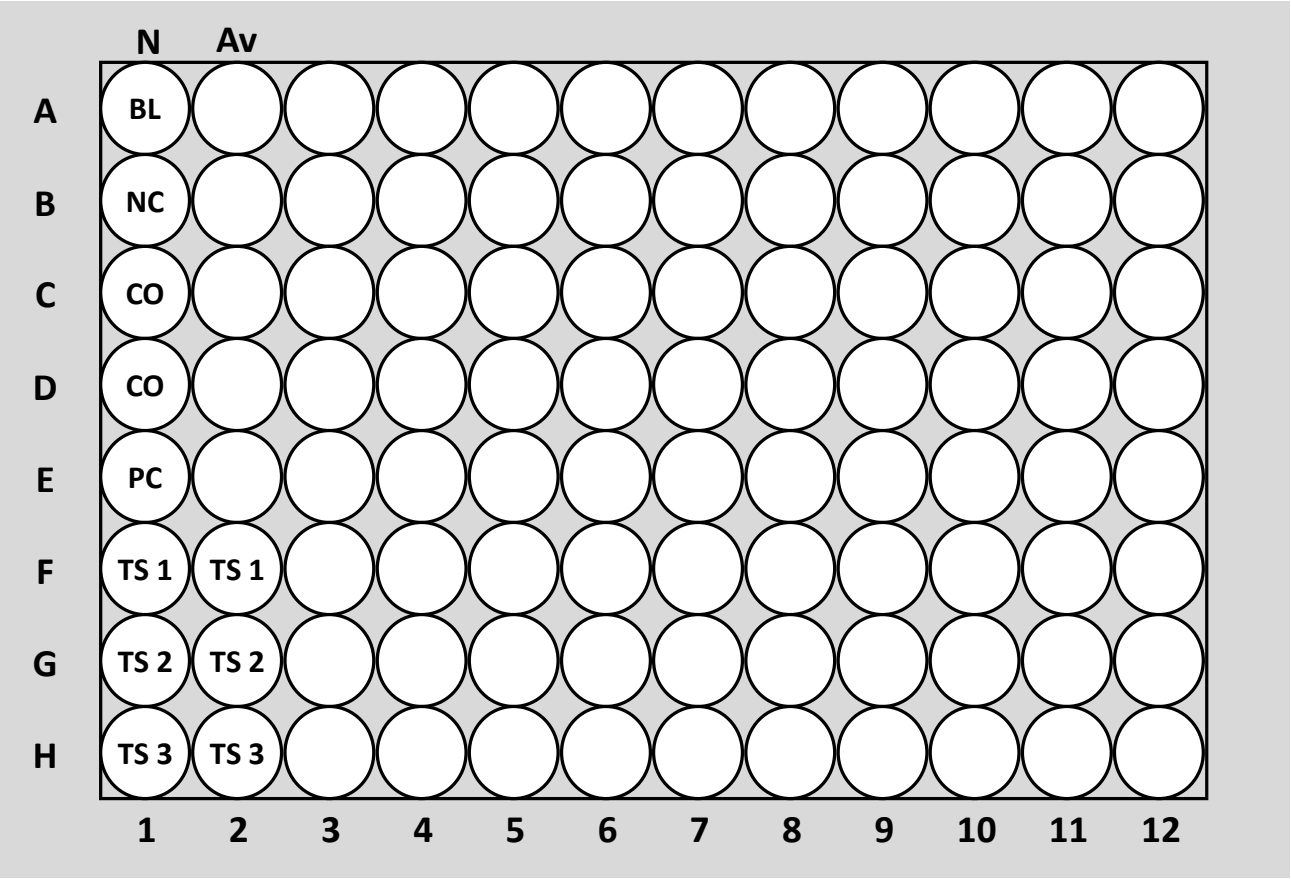
14.4 Assay Procedure

Allow all reagents including the Avidity Solution to come to room temperature and mix well. Crystals might develop in the vial with the Avidity Solution. Prior to use, it is necessary to dissolve the crystals by short-time warming up. The functionality of the Avidity Solution is indicated by yellow colour. The solution is thermolabile. The solution is deteriorated, if it changes its colour from yellow to red. A red-coloured Avidity Solution cannot be used further!

1. Dispense the controls and the diluted samples according to the working schedule.
   - Leave A1 well empty (blank).
   - Pipette 100 µl of the Negative Control (Calibrator 1) into 1 well.
   - Pipette 100 µl of CUT-OFF (Calibrator 2) into 2 wells.
   - Pipette 100 µl of the Positive Control (Calibrator 3) into 1 well.
   - Pipette 100 µl of the diluted samples (see Chapter Preparation of Samples) into two adjacent wells of N and Av strips.
2. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
3. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
4. Pipette 100 µl of the Avidity Solution into all wells of Av strips.
5. Pipette 100 µl of the working strength Wash Solution into all wells of N strip.
6. Cover the microplate with the lid and incubate at room temperature for 5 minutes.
7. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.

8. Pipette 100 µl of the Conjugate into all wells except A1 well.
9. Cover the microplate with the lid and incubate at 37°C for 30 minutes.
10. Aspirate the content of the wells and wash 5× with the working strength Wash Solution. Fill the wells up to the edge. Finally, tap the inverted microplate thoroughly on an absorbent paper to remove solution remnants.
11. Pipette 100 µl of TMB-Complete into all wells. Avoid contamination – see Chapter Procedural Notes.
12. Cover the microplate with the lid and incubate at 37°C for 30 minutes. Keep out of light.
13. Stop the reaction by adding 100 µl of the Stop Solution in the same order and intervals as the substrate was added.
14. Read the colour intensity in wells against blank (A1 well) using photometer set to 450 nm. The absorbance should be read within 30 minutes after stopping the reaction.

14.5 Working Schedule



N	strip for ELISA (without Avidity Solution)	
Av	strip for avidity test (incubation with Avidity Solution)	
BL	Blank (empty well)	
NC	100 µl	CONTROL - CAL1
CO	100 µl	CUTOFF CAL2
PC	100 µl	CONTROL + CAL3
TS 1-x	100 µl	diluted tested sample

14.6 Quality Control

Quality control of the test is performed in N strips (see Chapter Quality Control).

14.7 Results Interpretation

Calculation of Index of Avidity (IAv)

Divide the absorbance of a tested sample in Av strip by the absorbance of the tested sample in N strip measured in the same test run:

$$IAv = \frac{\text{Absorbance of sample in Av strip}}{\text{Absorbance of sample in N strip}} \times 100\ [\%]$$

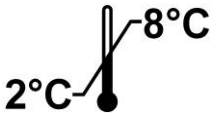
Interpretation of the test results is described in Table 3.

Table 3 Interpretation of test results

Index of Avidity (IAv) in %	Evaluation of avidity	Interpretation of results
≤ 40	low	primary infection
40 – 45	borderline	ambiguous, repeat examination
> 45	high	convalescence, seropositivity

Examination should be repeated in case of borderline results. Collect a second sample after more than seven days after the first collection and examine both samples in the same test run.

15 IFU Symbols

	Temperature limitation
	Keep dry
	Expiry date
	Lot number
	Manufactured by
	Consult instructions
	Catalogue number
	Number of tests
	In vitro diagnostic medical device

Notes

Summary of EIA Measles IgG Protocol

Step No.	Symbol	Test steps
1		Dilute samples serum/plasma 1:101 (10 µl + 1 ml)
2		Pipette Controls (Calibrators) and diluted samples – 100 µl Blank = empty well
3		Incubate at 37°C for 30 min
4		Aspirate and wash the wells 5×
5		Pipette Conjugate – 100 µl Blank = empty well
6		Incubate at 37°C for 30 min
7		Aspirate and wash the wells 5×
8		Pipette Substrate (TMB-Complete) – 100 µl Including blank
9		Incubate at 37°C for 30 min
10		Pipette Stop Solution – 100 µl Including blank
11		Read colour intensity at 450 nm