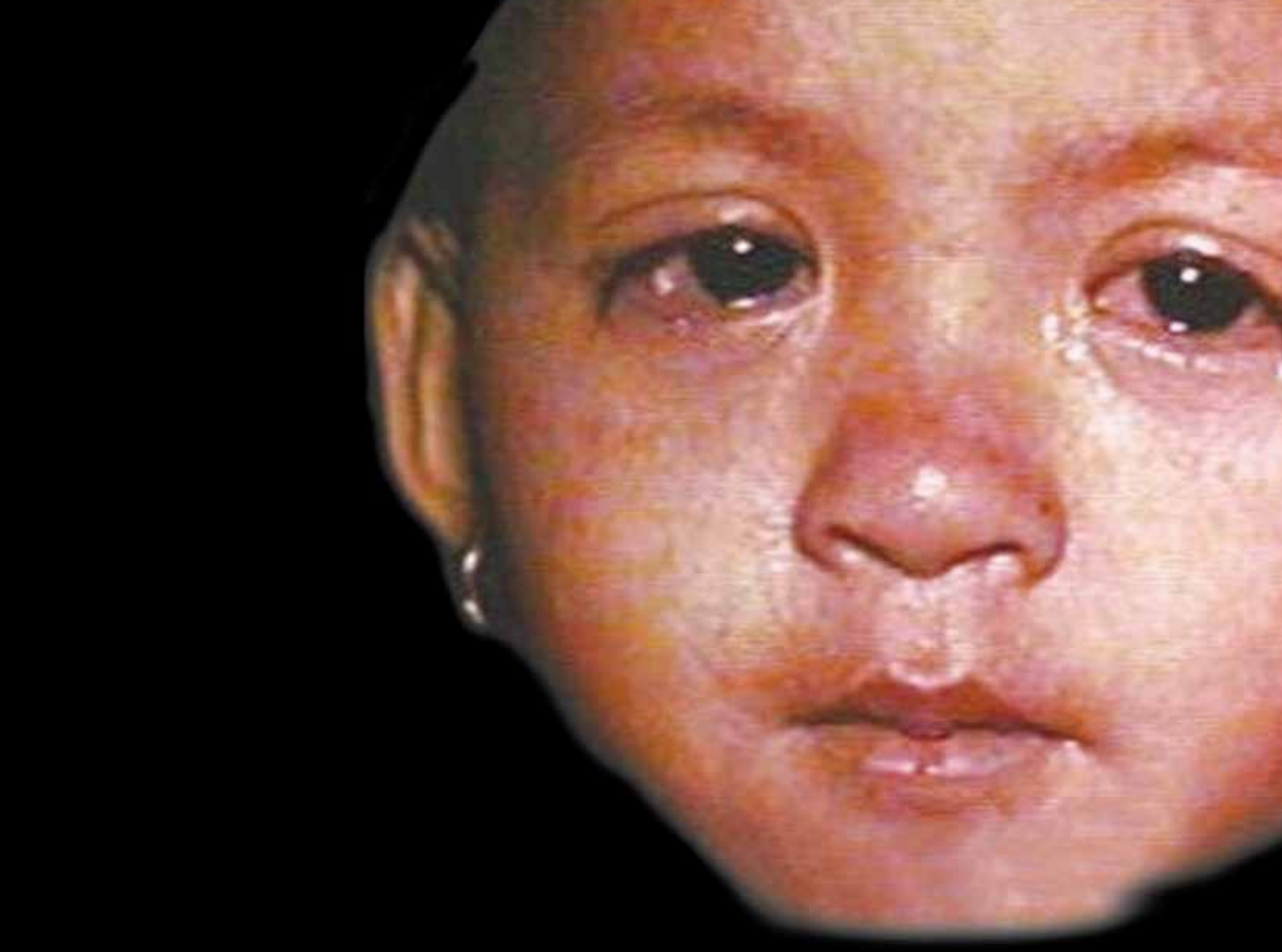

Measles Outbreak in Kapilvastu, Nepal: An Outbreak Investigation 2016

World Health Organization
Country Office for Nepal

NEPAL HEALTH RESEARCH COUNCIL
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Cover Photo source: Google Image

Few words

We are grateful and indebted to Dr. Khem Bahadur Karki (Executive Chief) of the Nepal Health Research Council for providing us an opportunity to conduct the epidemiological outbreak research. We would like to thank the entire study team and advisory committee for their continuous support to complete this study. Additionally, my special thanks goes to Ms. Namuna Shrestha, Ms. Jasmine Maskey, Ms. Arpana Pandit, Mr. Hari Datta Joshi, Mr. Anil Poudyal, and Ms. Trishna Acharya for their support especially to design the study, analysis the findings and drafting the report.

Similarly, we would like to express our sincere thanks to Kapilvastu District Public Health Officer for his kind support to collect data from measles outbreak areas. Likewise, we are thankful to Dr. Geeta Shakya, Ms. Sailaja Adhikari and her team of National Public Health Laboratory for investigating the laboratory finding.

We are also equally indebted to the community people who helped for obtaining the blood sample from their children and for giving details of the verbal autopsy without any hesitation. The research team would also like to thank the entire staffs of the Nepal Health Research Council for their administrative and financial support to undertake the outbreak investigation.

Principal investigator

Dr. Khem Bahadur Karki

Acronyms

CI	: Confidence Interval
CHD	: Child Health Division
DHO	: District Health Office
DPHO	: District Public Health Officer
EDCD	: Epidemiology and Disease Control Division
EPI	: Expanded Program on Immunization
IgG	: Immunoglobulin G
IgM	: Immunoglobulin M
MR	: Measles and Rubella
NHRC	: Nepal Health Research Council
NPHL	: National Public Health Laboratory
OR	: Odds Ratio
TU	: Tribhuvan University
VDC	: Village Development Committee
WHO	: World Health Organisation

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Background

Measles is a highly contagious viral disease and a leading cause of vaccine-preventable death among children, despite the availability of safe and effective vaccine for more than 50 years. It is transmitted via droplets from the nose, mouth or throat of infected persons. Initial symptoms usually appear 8–12 days after infection; include high fever, runny nose, bloodshot eyes, and tiny white spots inside of the mouth. In later symptoms, on around the 4th day of fever, rash develops, starting on the face and upper neck and gradually spreading downwards.

According to World Health Organization (WHO), deaths from measles have decreased by 75 percent in recent years globally while it is still common in many developing countries, particularly in parts of Africa and Asia. The overwhelming majority (more than 95%) of measles deaths occur in countries with low per capita income and weak health infrastructures.[1]

Measles is a highly endemic in Nepal for the last several years, affecting thousands of children and young adults. Nepal has been able to prevent its' spread and achieved outbreak free status in 2013 and 2014 due to the comprehensive programme for Measles and Rubella (MR) elimination. However, in the year of 2015, out of a total of seven suspected measles outbreak, the four outbreaks were lab-confirmed measles in (i) Gumba, Sindhupalchowk; (ii) Paraspur, Banke;(iii) Nepalgunj Municipality-7, Banke; and (iv) Guleria Sub-metropolitan-12, Bardia). The two recent ones were Nepalgunj Municipality-7 in Banke and Guleria Sub-metropolitan-12, Bardia, occurring almost at the same period (Banke on 7 June 2015 and in Bardiya 11 June 2015).[2]

Likewise, in the month of January 2016, six children died and 50 others suffered from the disease that resembled measles at Somdiha 7 and 8 in Kapilvastu district. Furthermore, major national daily newspaper reported that 95 percent of childrens were not immunized in the village which has increased the risk of contracting measles for other children.[3]

In this context, Nepal Health Research Council (NHRC) conducted an outbreak investigation to find out the determinants for epidemic, including the effectiveness of the measles vaccines by measuring the anti-measles IgG.

1 http://www.who.int/immunization/diseases/measles/en/
2 http://www.searo.who.int/nepal/areas/ipdnepalbulletin_july_2015.pdf
3 http://kathmandupost.ekantipur.com/printedition/news/2016-01-20/unidentified-disease-claims-lives-of-six-kids.html

1.1 Case definition

1.1.1 Clinical case definition

A child with fever; with or without conjunctivitis, coryza and cough; followed by a maculopapular rash at around the 4th day of illness.

1.2 Laboratory criteria for diagnosis in serum

Presence of measles-specific IgM antibodies

1.3 Case classifications

1.3.1 ***Clinically confirmed*:** A case that meets the clinical case definition

1.3.2 Laboratory-confirmed: A case that meets the clinical case definition with the presence of IgM.

1.4 Objectives

1. To identify the factors that lead to measles infection on Kapilvastu District
2. To find out the immunization status of the children of the affected area and explore their anti measles IgG (Immunity level of measles) in the community.

Methodology

2.1 Study design

The study design was community-based case control study. Cases were defined as any patient diagnosed with measles and meeting the above case definition. Controls were selected from same community, among those who didn't have sign & symptoms of measles at a time of field visit.

2.2 Study duration

The investigation period of the study was from February 2016 to May 2016. In January 2016, six children died and 50 others suffered from a disease that resembled measles at Somidiha -7 and 8 in Kapilvastu district. NHRC called upon a meeting on 28 January 2016 and decided to conduct an outbreak investigation by mobilizing study team on 1 February 2016.

2.3 Study population

The under five children with or without measles from 5 different VDCs of Kapilvastu district, namely Basantapur, Kopwa, Parshioya, Rangapur and Somadi were considered as the study population.

2.4 Sample size

A total of 127 children less than 5 years were taken as a sample size of the study. According to their clinical findings, 37 were cases and 90 were controls.

2.5 Data collection method

A meeting was conducted with District Public Health Officer (DPHO), EPI focal person and health staffs in Kapilvastu District Health Office for sharing the objectives and the process of an outbreak investigation. The meeting also requests them to support them to conduct the study before visiting field for data collection. The semi-structured questionnaire was used to collect information from all cases and controls. WHO verbal autopsy tools were used to validate the death cases where as reviews of records were done in the district hospital. Additionally in-depth interviews were taken with VDCs level health professionals and community leaders.

2.6 Blood sample collection

5 ml of blood was collected by venipuncture into a duly-labeled sterile tube from both case and control group. The samples were transported to the National Public Health Laboratory (NPHL) for analysing the anti measles IgM and anti measles IgG, maintain a standard protocol for shipping of the sample to the laboratory.

2.7 Details of Laboratory Procedure

The collected blood sample was centrifuged at 1000 x g for 10 minutes to separate the serum. The serum was stored at 4-8 °C until ready for shipment. For longer period, sera were frozen at -20 °C and

transported to the laboratory on frozen ice packs.

A laboratory request form was duly filled up at the time of specimen collection and accompanied with shipment.

Below listed procedure was followed.

- Specimens was placed in zipping lock;
- Styrofoam boxes were used;
- Specimen form was placed in plastic bag and taped in inner top of Styrofoam box;
- Sample was placed between ice packs.

Registered and stores date of the sample was recorded and laboratory analysis was carried out in National Public Health Laboratory (NPHL) following well-established process (details about laboratory process, method and standard are on ANNEX I)

2.8 Data management and analysis

Collected data were cleaned and checked for consistency. Analyses were done using descriptive and inferential statistics to determine the association of major risk factors to the disease occurrence. SPSS 20 version Statistics was used to analyze the collected data.

Another form included the following data: patient identification (unique ID, name, place of residence, age); basic clinical information (date of onset of rash, whether the patient fulfills case definition), and immunization history (number of doses of measles-containing vaccine, date of the last dose).

2.9 Ethical consideration

Ethical approval was obtained from the ethical review board of the NHRC. Written informed consent was taken from parents of all the participants after informing about the purpose of study.

Findings

Table 1: Socio-demographic characteristics of respondents according to laboratory findings

Variables	Positive	Negative	Equivocal	Total	
	Frequency	Frequency	Frequency	Total	Percentage
Gender					
Male	18	35	2	55	43.31
Female	26	43	3	72	56.69
Religion					
Hindu	32	66	4	102	80.31
Muslim	12	12	1	25	19.69
Age group					
<1 year	10	9	2	21	17.21
1-2 years	12	32	1	45	36.89
2-3 years	9	10	1	20	16.39
3- 4 years	6	6	0	12	9.84
4-5 years	5	18	1	24	19.67
Village Development Committee (VDC)					
Basntapur	1	37	0	38	29.92
Kopwa	0	1	0	1	0.79
Parshioya	0	1	0	1	0.79
Rangapur	19	26	3	48	37.80
Somadi	24	13	2	39	30.71

The study shows that more than half (56.67 percent) of the respondents were female. Almost one fourth (19.69 percent) of the respondents were from the Muslim community. According to National Muslim Commission, about 18.15 percent of the total Muslim lived in Kapilvastu district. An age group of 1-2 years (36.89 percent) were affected more than age group below one year (17.21 percent). About 19.67 percent of the respondents were in the age group of 4-5 years, followed by 16.39 percent of the respondents who were in the age group of 2-3 years and relatively lower percent (9.84 percent) of the respondents were in the age group of 3-4 years.

Respondents were taken from five different VDCs of Kapilvastu district, i.e. Basantapur, Kopwa, Parshioya, Rangapur, and Somdi. The majority (37.8) percent of the respondents were from Rangapur VDC followed by (30.71) percent were from Somadi and so on. (Table. 1)

Table 2: Clinical and laboratory diagnosis of measles case, according to socio-demographic variables

Variables	Clinically diagnosed (Positive)		Laboratory Diagnosis (Positive)	
	Frequency	Percentage	Frequency	Percentage
Gender				
Male	13	35.14	18	40.9
Female	24	64.86	26	59.09
Religion				
Hindu	27	72.97	32	72.72
Muslim	10	27.03	12	27.27
Age group*				
<1 year	10	27.78	10	23.8
1 - 2 years	9	25.00	12	28.57
2 - 3 years	7	19.44	9	21.42
3 - 4 years	5	13.89	6	14.28
4 -5 years	5	13.89	5	11.9
V.D.C				
Basntapur	0	0.00	1	2.3
Kopwa	0	0.00	0	0
Parshioya	0	0.00	0	0
Rangapur	18	48.65	19	43.2
Somadi	19	51.35	24	54.5
Total	37	100	44	100
* A respondent age was missing on clinically diagnosed (positive, IgM) *2 respondent age were missing on laboratory diagnosed (positive)				

During measles outbreak in Kapilvastu, respondents were diagnosed clinically through face to face interview (history taking) and laboratory blood sample collection and testing. Out of the total respondents (n=127), 64.86 percent of the clinically diagnosed and 59.09 percent of the laboratory diagnosed respondents were female. Almost similar 27.03 percent of the clinically diagnosed and 27.27 percent of the laboratory diagnosed respondents were from Muslim community.

About 27.78 percent of the respondents who were diagnosed clinically were below 1 year, followed by 25 percent of 1-2 years and so on. Likewise, majority (28.57 percent) of the respondents were diagnosed by laboratory are of the age group 1 -2 years, followed by 23.8 percent below 1 year and so on. (Table 2)

Among them, more than half (51.35 percent) of the measles positive cases were diagnosed clinically from Somadi VDC followed by 48.46 percent in Rangapur VDC. About 54.5 percent of the measles positive cases that were diagnosed from the laboratory were from Somadi VDC, followed by 43.2 percent of measles case from Rangapur. From laboratory, 2.3 percent of measles positive cases were found in Basantapur VDC.

Table 3: Measles vaccination status and the Laboratory confirmed cases

Measles vaccination	Laboratory confirmed cases						
	Positive IgM		Negative IgM		Equivocal		Total
	Number	%	Number	%	Number	%	Number
Yes	21	26.58	54	68.35	4	5.06	79
No	12	40	17	56.67	1	3.33	30
Unknown	11	61.11	7	38.88	0	0	18

Out of total 79 cases, 26.58 % of vaccinated respondents' blood sample had anti-Measles IgM positive indicating a recent measles infection. This could be probably because of failure on handling vaccines from national to local level (cold chain, vaccine administering staffs etc.)

11 out of 18 with unknown vaccinated status respondents' blood sample had an IgM positive. This finding indicates that the risk of measles in Kapilvastu district is high due to low measles vaccine coverage.

Table 4: Measles laboratory diagnosis and clinical diagnosis

Laboratory Diagnosis	Antibodies	Clinical Diagnosis											
		Positive (n=37)						Negative (n=90)					
		Positive		Negative		Equivocal		Positive		Negative		Equivocal	
		N	%	N	%	N	%	N	%	N	%	N	%
	Anti-measles-IgM	35	94.6	1	2.7	1	2.7	9	10	77	85.6	4	4.4
	Anti-measles-IgG	24	64.9	9	24.3	4	10.8	30	33.3	54	60	6	6.7

Out of total 37 clinically positive cases, 94.6% showed reactive with anti-measles IgM whereas 2.7% showed IgM negative. Similarly, among 90 clinically measles ruled out respondents, 9 (10%) of them showed IgM reactive which indicates presence of subclinical cases.

For anti-measles IgG test, out of 37 clinically diagnosed cases only 64.9% show IgG reactive, whereas 24.3% clinically diagnosed cases show non-reactive to IgG. Similarly, out total 90 clinically measles ruled out respondents, 33.3% respondents sample show IgG reactive.

Table 5: Finding of IgM and IgG result

Anti-measles IgM	Anti-measles IgG	Findings	Interpretation
Non-reactive	Nonreactive	52 (67%)	No evidence of current or previous measles. Non-immune and susceptible to measles virus infection
Nonreactive	Reactive	20 (26%)	Successful vaccination or previous natural measles virus infection
Reactive	Nonreactive	9 (21%)	Acute measles virus infection.

Out of the total, 67 % (52) of the respondents were unvaccinated or has no previous history record of vaccination; this indicates respondents are susceptible to measles infection. Low measles vaccine coverage in the whole district can intensify the vulnerability of measles infection in the coming years. Additionally, low literacy of parents, poor nutritional status of children and poor sanitation can further increase the risk of measles infection in future. Similarly, one of the most notable finding is that 2 cases were under 9 months' respondents, which defy the medical assumption that up to the 9 months of age maternal antibodies are protective. 54 out of 127 were reactive IgG positive, that means only 27% of the respondents were immune to measles virus either by vaccination or previous infection.

Table 6: Association of different factors with measles

Variables	Odds Ratio	95% C.I.		P-value
		Lower	Upper	
Gender				
Male	1*			
Female	1.049	0.451	2.436	0.912
Religion				
Hindu	1*			
Muslim	1.858	0.685	5.04	0.224
Age category				
<1 year	1*			
1-2 years	0.294	0.073	1.182	0.085
2 - 3 years	0.856	0.246	2.974	0.806
3- 4 years	0.373	0.092	1.506	0.166
4-5 years	0.260	0.055	1.222	0.088
Measles vaccination				
Yes	1*			

No	2.412	0.673	8.639	0.174
Unknown	3.546	1.116	11.26	0.032

Regression analyses were used to examine the relationship between disease status and indicators like; gender, religion, age and measles vaccination. Regression analysis showed that in age category, age below 1 year (OR, 0.065; 95% C.I., 0.010-0.418), 2 years (OR, 0.0147; 95% C.I., 0.024-0.900) and 5 years (OR, 0.111; 95% C.I., 0.015-0.823) were significantly associated with disease status. Gender, religion, and measles vaccination have no significant effect on the disease status of measles. Similarly, the age of 3 years and 4 years has also no significant effect on the disease status of measles.

3.1 Findings of observation and in-depth interview

An in-depth interview finding shows that large numbers of families across the border are connected through marital relations. Even a herd of cattle and wild animal walk freely across the border. There might be high chances of transmission of disease from both the country due to porous and open border.

According to local health workers, birth rate among Muslim community is comparatively higher than in the Hindu community. Many Muslim families have not immunized their children. This might be one of the causes of measles outbreak. The main challenge recorded during the epidemic outbreak investigation period in Kapilvastu was lower sanitation and hygiene status of the community. Although measles outbreak VDCs are declared as open defecation free VDCs, people are still defecating openly in these VDCs.

The government record shows that the coverage of measles vaccination status in Kapilvastu district is 66 percent, which is lower as compared to the national coverage of 88 percent. In addition, the measles outbreak VDCs has the lowest measles vaccination coverage status in the district. A gender bias is recorded while vaccinating the children as male children are more vaccinated compared to female children. Furthermore, the study report also showed that the literacy rate is very low in measles outbreak VDCs of Kapilvastu district, which has ultimately impact in lower utilization of health services.

Conclusion

Evidences from both clinical and laboratory findings showed the measles cases were recorded in Kapilvastu district. The measles outbreak was predominantly localized in the high-risk areas of Kapilvastu district reflecting the low vaccination coverage. This outbreak threatens the national measles elimination programs of the country. The vaccination coverage was found lower than expected, which needs to be increased in a holistic approach for which safe and effective vaccination drive is encouraged. The findings of the study showed that sanitation, open border and health system malfunction might be some of the reasons for outbreaks of measles in Kapilvastu district. Furthermore, this investigation emphasizes for the further studies to confirm the effectiveness of vaccine administered.

Catalogue of photographs

Translating the language by local people

Data collection and investigation of Measles case

Discussion about the impact of Measles

Verbal Autopsy

Grandparents showing their grand children

Doctor's investigation

Observing the rash of children

Local health workers, helping hands of our research team

Coughing by measles suspected case

Measles case

Measles investigation team with local health workers and stakeholders

ANNEX I

Enzygnost® Anti-Measles Virus/IgG

MEASLES/IgG

Revision bar indicates update to previous version.

Intended Use

Enzyme immunoassay for the qualitative detection and quantitative determination of specific IgG antibodies to Measles virus in human serum and plasma.

The enzyme immunoassay can be processed using the ELISA processors, BEP® III System, BEP® 2000 System or the BEP 2000 *Advance*® System. A non-automated processing of the test is also possible.

Summary and Explanation

Measles is a serious problem especially in developing countries and can only be combated by large-scale vaccination programs[1]. In the USA, systematic immunization has been carried out for a number of years and has led not only to a drastic decrease in the incidence of measles fatalities but also in severe measles-related diseases, such as subacute sclerosing panencephalitis (SSEP)[2].

Enzygnost® Anti-Measles Virus/IgG is used for the determination of the immune status[3,4] as well as for verifying the success of vaccination[5-7].

Principle of the Method

The specific IgG antibodies to measles virus contained in the test sample bind to the antigen in the reaction wells of the test plate. The Anti-Human IgG/POD Conjugate binds to these specific antibodies. The enzyme portion of the conjugate causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow.

IgG against cellular antigens is detected in the same way in the wells coated with control antigen. The difference between the color intensity in the well coated with antigen and in the well coated with control antigen is a measure of the immunochemical reactivity of the Measles virus-specific IgG antibodies in the sample.

Quantification in International Units is performed by calculation using the α -method.

Reagents

Symbols	Materials provided	
MEASLES/IgG	*Enzygnost® Anti-Measles Virus/IgG*	*2 x 48*
MTP	Enzygnost® Anti-Measles Virus/IgG test plate	2 pcs.
CONJUGATE ANTI-G	Anti-Human IgG/POD Conjugate	1 x 1 mL
MICROBIOL G	Conjugate Buffer Microbiol	4 x 12.5 mL
REFER P/N	Anti-Measles Virus Reference P/N	1 x 0.4 mL
DILUENT	Sample Buffer POD	2 x 50 mL
	Polyethylene bag	1 pc.
	Barcode table of values	1 pc.
	Instructions for Use	1 pc.

The test plate, the conjugate, the conjugate buffer as well as Anti-Measles Virus Reference P/N must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values.

Materials required but not provided

Supplementary Reagents for Enzygnost®/TMB (REF OUVP)

The reagents Chromogen TMB and Buffer/Substrate TMB must be used only in the combination of lots stated for the Supplementary Reagents kit. The applicable lot numbers are the 6-digit lot numbers listed on the package.

Composition

Enzygnost® Anti-Measles Virus/IgG test plate: Microtitration plate coated with inactivated Measles virus antigen. The wells in the left row of each strip are coated with antigen derived from permanent simian

kidney cells infected with measles virus, and the wells in the right row are coated with antigen from non-infected cells (= control antigen).

Anti-Human IgG/POD Conjugate: F(ab)' fragment of a rabbit antibody to human IgG, conjugated with peroxidase, in TRIS/HCl buffer with polygeline. The conjugate is colored green.

Dyes: Patent Blue
tartrazine

Preservative: phenol (≤ 1 g/L)

Conjugate Buffer Microbiol: EDTA in phosphate buffer with TETRONIC and BSA

Preservatives: gentamicin (~ 100 mg/L)
5-chloro-2-methyl-isothiazole-3-one (~ 6 mg/L)
2-methyl-4-isothiazole-3-one (~ 2 mg/L)

Anti-Measles Virus Reference P/N: Human serum containing IgG antibodies to measles virus antigens, contained in TRIS/HCl buffer with HUMANALBIN

Preservatives: amphotericin B (~ 5 mg/L)
gentamicin (~ 100 mg/L)

Sample Buffer POD: TRIS/HCl buffer with Tween 20, polygeline and bovine serum

Preservatives: amphotericin B (~ 5 mg/L)
gentamicin (~ 100 mg/L)

Warnings and Precautions

For *in-vitro* diagnostic use only.

The test was developed for testing individual samples, not for pooled samples.

CAUTION! POTENTIAL BIOHAZARD
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

MICROBIOL G
Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction. Safety data sheet available on request.

It is advisable to wear protective gloves throughout the entire test procedure. Please follow the recommendations of the manufacturer concerning the compatibility between gloves and exposed materials.

For disposal, it is recommended that solid infectious materials should be autoclaved for at least 1 hour at 121 °C. All aspirated liquids should be collected in two receptacles connected in series. Both should contain a disinfectant suitable for inactivating human pathogens. The concentrations and times specified by the manufacturer must be observed.

Buffer/Substrate TMB, Chromogen Working Solution and Stopping Solution POD must not be allowed to come into contact with heavy metal ions or oxidizing substances (do not use pipettes with metal parts which are in direct contact with the liquid). The substrate reaction steps must not be performed in the vicinity of disinfectants containing hypochlorite. If the Chromogen Working Solution has spontaneously developed a blue color before being transferred into the test plate, this indicates that the solution is contaminated; in such cases, prepare a fresh solution in a clean container. Skin contact with the above mentioned solutions is to be avoided.

Preparation of the Reagents

Bring all reagents and test samples to 18 to 25 °C before starting with the test. Do not remove the foil pouch from the test plates during this step. Before starting the test processing, remove not required strips from the holder and store these in the enclosed polyethylene bag for later use (see Table 1). If reagents or working reagent solutions need to be mixed, avoid foam formation.

Dilute Anti-Human IgG/POD Conjugate 1+50 with Conjugate Buffer Microbiol, e.g., for one test plate, add 250 µL conjugate into a vial with 12.5 mL Conjugate Buffer Microbiol. Shake gently to mix.

For diluting samples, 50 mL Sample Buffer POD can be dyed blue-violet by adding 2.5 mL of Colour Solution blue for Enzygnost® (from the kit Supplementary Reagents for Enzygnost®/TMB). Shake gently to mix. Do not use this blue-violet dyed Sample Buffer POD for pre-dispensing into the microtitration plate. In the Sample Buffer POD, the occurrence of a slight, insoluble deposit is unproblematic.

For each test plate, dilute 20 mL of Washing Solution POD from the kit Supplementary Reagents for Enzygnost®/TMB with distilled or deionized water to 400 mL.

For each test plate, dilute 1 mL of Chromogen TMB with 10 mL of Buffer/Substrate TMB from the kit Supplementary Reagents for Enzygnost®/TMB using the supplied empty plastic bottle (Chromogen Working Solution). Store protected from light. After use, carefully rinse the bottle with distilled or deionized water. For technical reasons (overfill), it is not permissible to pour together the full contents of the Chromogen TMB vial and the full contents of the Buffer/Substrate TMB vial.

Storage and Stability

Stored unopened at 2 to 8 °C, all components of the test kit may be used up to the expiry dates given on the labels.

For complete stability and storage data for reagents that have been opened or diluted, see Table 1.

Equipment Required

BEP® III:	For automatic processing of the test after dispensing the samples as well as for evaluation
BEP® 2000/BEP 2000 *Advance*®:	For fully automatic processing and evaluation of the test
Pipettes:	Piston-type pipettes with fixed or variable volumes, or single-and multi-channel pipettes with adjustable volumes

The following items are required additionally if the test is not processed automatically:

Incubator:	Incubator for 37 ±1 °C with increased humidity, e.g. obtained by inserting a dish of water, or similar incubation method
Washing device:	Microtitration plate washer
Photometer:	Photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate)

For quantitative evaluation of the test: a pocket calculator with exponential and logarithmic functions.

All the equipment used in the test must have been validated.

Specimens

Suitable specimens are individual samples (human sera or citrated/EDTA/heparinized plasma) obtained by standard laboratory techniques. The samples should be stored for no more than 3 days at 2 to 8 °C. If the samples are to be stored for a longer period of time, they must be frozen.

Procedure

Non-automated Test Procedure

1. **Dilute samples:** Dilute all samples as well as the Anti-Measles Virus Reference P/N in a ratio of 1+20 using (blue-violet dyed) Sample Buffer POD, e.g. by pipetting 20 µL sample into a dilution tube and adding 400 µL of Sample Buffer POD. Shake gently to ensure a thorough mixture. The diluted samples can be stored, sealed, overnight at 2 to 8 °C in tubes with low protein-binding capacity.
2. **Assay scheme:** The necessary number of test plate well pairs is given by the number of test samples plus the number of determinations (n = 2) for Anti-Measles Virus Reference P/N.
3. **Pre-dispense buffer:** Pre-dispense 200 µL of non-dyed Sample Buffer POD into each required well of the test plate.
4. **Dispense samples:** Dispense 20 µL of diluted Anti-Measles Virus Reference P/N (1+20) into each well of the first pair (A1: Measles Virus antigen; A2: Measles Virus control antigen), and 20 µL of diluted sample (1+20) into each well of the subsequent pairs. At the end of the series, respectively test plate, fill the last pair of wells with 20 µL each of diluted Anti-Measles Virus Reference P/N.

Important: It is not permitted to first pipette Anti-Measles Virus Reference P/N into the wells at the start and end of the sample series, and then put the samples in-between.

Mix thoroughly after dispensing by drawing up and expelling at least twice with the pipette. Each sample must be pipetted with its own pipette tip. The pipetting steps must be completed within 15 minutes per test plate. An 8-channel pipette simplifies and speeds up the transfer of diluted samples into the test plate. After completing the pipetting steps, seal the test plate with foil and place immediately into the incubator.

5. **Incubate samples:** Incubate for 60 ± 2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
6. **Wash:** Remove foil and aspirate all wells. Fill each well with approx. 0.3 mL diluted Washing Solution POD, aspirate the plate, and repeat the wash cycle three times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).
7. **Dispense conjugate:** Pipette 100 µL of diluted Anti-Human-IgG/POD Conjugate into each well. Then seal the test plate with fresh foil and place immediately into the incubator.
8. **Incubate conjugate:** Incubate for 60 ± 2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
9. **Wash:** As described in step 6.
10. **Dispense substrate:** Pipette 100 µL of Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.
11. **Incubate substrate:** Immediately after the substrate dispensing step, incubate at 18 to 25 °C for 30 ± 2 minutes, protected from light.
12. **Stop reaction:** Remove the foil. Add 100 µL Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing step.
13. **Measure:** Read the test plate at 450 nm within one hour. The recommended reference wavelength is 650 nm, or where appropriate between 615 and 690 nm.

Procedure for the BEP® III System

When using the BEP® III, the test plates must be prepared up to the sample dispensing step (steps 1 to 4 in the section "Non-automated Test Procedure").

Immediately afterwards place the uncovered test plates, i.e. not covered with foil, into the BEP® III. All subsequent processing steps are performed fully automatically by the instrument (see BEP® III Instruction Manual).

The settings for the incubation times in the BEP® III software may differ from the times in the section "Non-automated Test Procedure" for technical reasons (system speed) but have been validated for Enzygnost® on the BEP® III.

Procedure for the BEP® 2000 System

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see BEP® 2000 Instruction Manual).

Sample processing with the BEP® 2000 System may differ from the information given under "Non-automated Test Procedure", but has been validated for Enzygnost® on the BEP® 2000.

Internal Quality Control

Validation Criteria

Note: For all evaluations, the absorbance values obtained from the measurement with Measles virus antigen minus the absorbance value of the same sample obtained with Measles virus control antigen must be used. This value is given as ΔA.

To evaluate the test the following criteria must be fulfilled:

Anti-Measles Virus Reference P/N:

Each ΔA value must be within the lot-dependent lower and upper margin listed in the respective barcode table of values:

lower margin ≤ $\Delta A_{\text{Reference P/N}}$ ≤ upper margin

In addition the individual ΔA values (Anti-Measles Virus Reference P/N at the start and end of a series of measurements or test plate) must not differ by more than ±20 % from the mean calculated from these values.

If these conditions are not met, the test is not valid for evaluation. In this case, the software of BEP® III and BEP® 2000 will give notice of an invalid test result. The measurements must be repeated after investigating the cause.

Results

The evaluations are performed automatically in the BEP® III and the BEP® 2000. Please consult the relevant Instruction Manual. The following sections must be taken into account when performing measurements without software support.

Measurement Correction

For achieving an optimal reproducibility of the results, the measurements require correction, both for the quantitative evaluation using the α-method and for the qualitative evaluation of the test.

To determine the correction factor, the nominal value of Anti-Measles Virus Reference P/N (provided in the barcode table of values) is divided by the mean test result of Reference P/N:

$$\text{Correction factor} = \frac{\Delta A \text{ nominal value}}{\text{mean } \Delta A \text{ value}_{\text{Reference P/N}}}$$

The differences in absorbance (ΔA) of those test samples determined in the series must now be multiplied by this correction factor. If processing several test plates, the correction factor must be calculated and used for each individual test plate.

Qualitative Evaluation

Based on the criteria of the test, the samples are classified as follows:

Anti-Measles virus/IgG **negative**		ΔA < 0.100 (cut-off)
Anti-Measles virus/IgG **positive**		ΔA > 0.200
Anti-Measles virus/IgG **equivocal**	0.100 ≤	ΔA ≤ 0.200

Test samples with an equivocal result must be retested in duplicate. If the result is confirmed, the samples are classified as equivocal, otherwise as positive or negative.

Quantitative Evaluation with the Aid of the α-Method

Samples with IgG antibody activities higher than the cut-off value can be quantitatively analyzed using the α-method.

Do not use for calculation:

- Readings (ΔA) corrected < cut-off
- Readings (ΔA) uncorrected ≥ 2.5

The calculation is performed according to the following formula:

Log_{10} mIU/mL = $\alpha \times \Delta A^{\beta}$

The values for the lot-dependent constants α and β can be taken from the enclosed barcode table of values.

Samples with an absorbance value (ΔA uncorrected) ≥ 2.5 must be tested in a higher dilution, e.g. 1+2309 for a valid evaluation. Then, the result (not the reading) must be multiplied by the dilution factor (e.g., 10). The values are traceable to Anti-Measles Serum (International Reference Preparation, 1964) of the WHO[8].

Assessment of the Results

Determinations used to assess significant changes in activity should always be performed in the same run and in the same test dilution. In these cases a difference of more than a factor of 2 is indicative of such a change.

When comparing results from different runs, identical lots of reagent must be used and the test samples must be assessed in the same dilutions (1+230 or 1+2309). Under these conditions, differences of more than a factor of 3 indicate a significant change in activity.

A "negative" result means that virus-specific IgG antibodies cannot be detected.

If exposure to the virus is suspected despite a negative finding, a second sample should be collected no less than 2 to 3 weeks after the suspected time of virus exposure and should be tested together with the first sample.

Seroconversion from "negative" in the first sample to "positive" in the second sample is evidence of a recent infection, or of a successful vaccination, or of the administration of hyperimmune globulin, such as is recommended for recent measles infections in HIV-positive children[9].

A viral infection is indicated by an "equivocal" test result when confirmed by repeating the test. In this case, a second test sample must also be taken at least 7 days later and tested together with the first sample.

A "positive" result means that virus-specific IgG antibodies were detected. If the Enzygnost® Anti-Measles Virus/IgM test is run at the same time and does not detect virus-specific antibodies, it can be assumed that the patient was infected with measles in the past or has received immunoglobulin.

A significant increase in activity between a pair of samples collected at least 7 days apart is indicative of virus reactivation.

Neonates of vaccinated mothers usually have lower IgG activities against Measles virus than children of women immunized by a past infection; the antibody activities found in preterm infants (in Europe) are generally lower[10].

Detection of a single virus-specific antibody titer, even a high titer, does not provide proof of a recent infection as there are no reference values. Nevertheless, for a wide range of investigative purposes, the quantitative evaluation remains an indispensable diagnostic tool (e.g., therapeutic monitoring).

Limitations of the Procedure

1. Anticoagulants (citrate, EDTA, heparin) and rheumatoid factors do not interfere with the test result.
2. Lipemic, hemolytic and icteric samples do not interfere with the test.
3. Samples with substances that may interfere with test results: ANA, AMA, samples with elevated total IgG and IgM, samples from dialysis patients, HBsAg antibodies, Toxoplasmosis/IgM antibodies, Rubella/IgM antibodies, EBV/IgM antibodies and CMV/IgM antibodies were examined in the test. No influence on the test results was observed with the samples tested.
4. No interferences have been observed with heat-treated samples (30 minutes, 56 °C).
5. Incompletely coagulated sera and microbially contaminated test samples should not be used. Any particles (e.g. fibrin clots, erythrocytes) contained in the sample should be removed prior to assay.
6. If thawed samples are used, ensure that the material is thoroughly homogenized.
7. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
8. The Reference was produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
9. Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.
10. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Specific Performance Characteristics

With Enzygnost® Anti-Measles Virus/IgG, samples containing approximately 150 mIU/mL are found to be within the range of 0.100 to 0.200 ΔA.

Sensitivity

734 test samples were tested in parallel with Enzygnost® Anti-Measles Virus/IgG and with comparison methods. In this set of samples, Enzygnost® Anti-Measles Virus/IgG was shown to have a sensitivity of 99.6 %.

Specificity

Enzygnost® Anti-Measles Virus/IgG detects only IgG.

46 test samples were assessed in parallel with Enzygnost® Anti-Measles Virus/IgG and with comparison methods; in this study the test was shown to have a specificity of 100 %.

Precision

Three samples with different levels of anti-measles virus/IgG antibody activities were evaluated to determine the intra- and interassay coefficients of variation (CV). The following results were obtained:

Sample	Intraassay		Interassay	
	Mean absorbance (ΔmA)	CV (%)	Mean absorbance (ΔmA)	CV (%)
A	82	11.4	50	21.6
B	589	4.4	-	-
C	1403	5.8	1469	6.4

Note: The results refer to the groups of samples investigated.

BEP®, BEP 2000 *Advance*® and Enzygnost® are trademarks of Siemens Healthcare Diagnostics.
TETRONIC is a trademark of BASF.
HUMANALBIN is a trademark of CSL.

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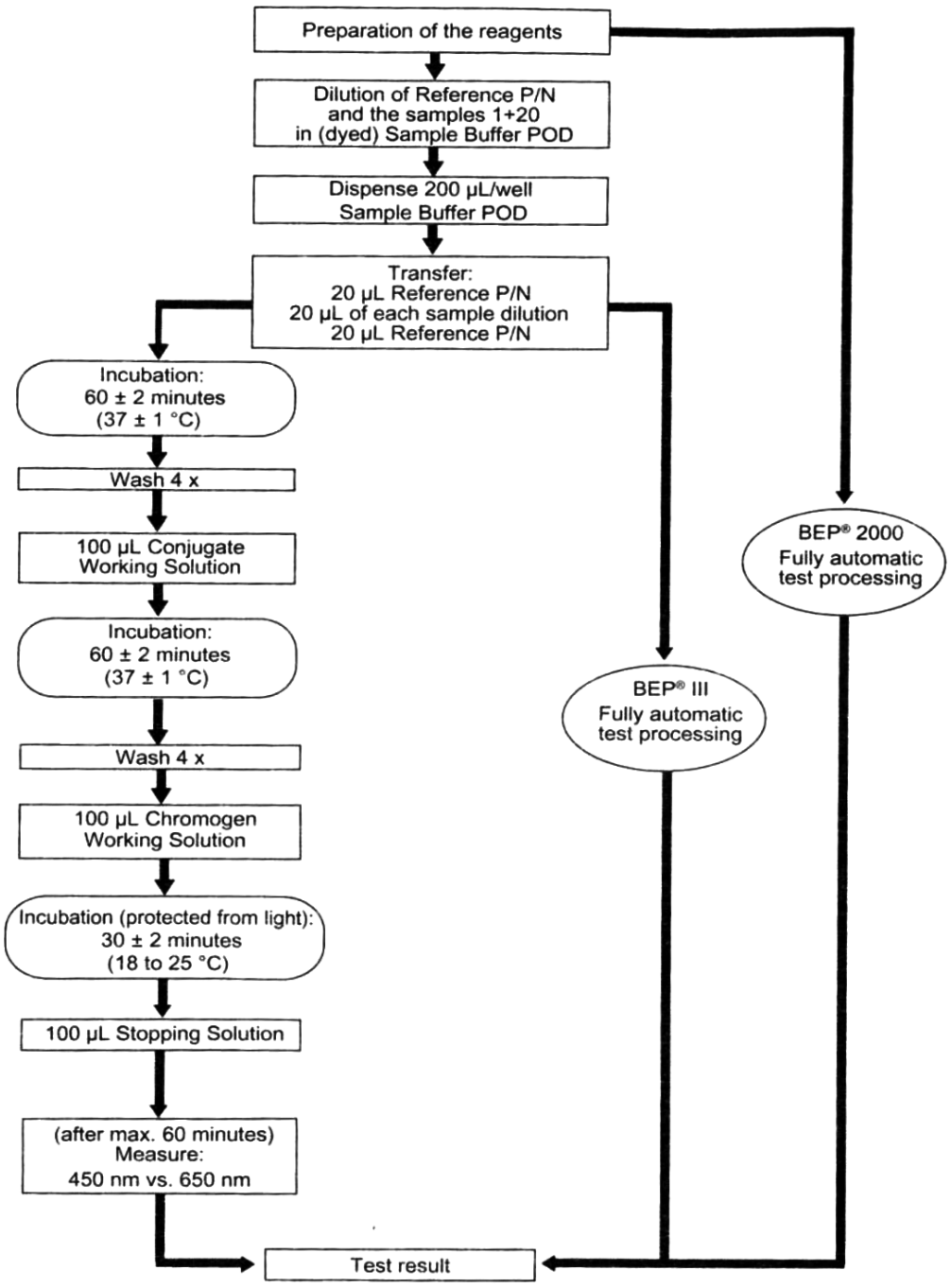
Table 1 Storage and Stability

Material/Reagent	State	Storage	Stability*
Enzygnost® Anti-Measles Virus/IgG test plate, remaining strips	once opened	2–8 °C in the bag with desiccant	8 weeks
Anti-Human IgG/POD Conjugate	once opened diluted 1+50	2–8 °C 2–8 °C 15–25 °C	12 months 4 weeks 1 day
Conjugate Buffer Microbiol	once opened	2–8 °C	8 weeks
Anti-Measles Virus Reference P/N	once opened diluted 1+20	2–8 °C 2–8 °C	12 months overnight **
Sample Buffer POD	once opened	2–8 °C	8 weeks
Chromogen TMB	once opened	2–8 °C	expiry date
Buffer/Substrate TMB	once opened	2–8 °C	expiry date
Chromogen Working Solution	diluted 1+10	2–8 °C 15–25 °C closed container protected from light	5 days 8 hours
Washing Solution POD	once opened diluted 1+19	2–8 °C 2–8 °C 18–25 °C	expiry date 1 week 1 day
Colour Solution blue for Enzygnost®	once opened	2–8 °C	expiry date
Stopping Solution POD	once opened	2–8 °C	expiry date

* use each component by the expiry date at the latest
** in closed dilution tubes with low protein-binding capacity

Table 2 Test Procedure



Enzygnost® Anti-Measles-Virus/IgM

MEASLES/IgM

Revision bar indicates update to previous version.

Intended Use

Enzyme immunoassay for the qualitative detection and quantitative determination of specific IgM antibodies to Measles virus in human serum and plasma.

The enzyme immunoassay can be processed using the ELISA processors, BEP® III System, BEP® 2000 System or the BEP 2000 *Advance*® System. A non-automated processing of the test is also possible.

Summary and Explanation

Testing for measles-specific IgM is used to monitor diseases associated with Measles virus infection.

Measles is the most common childhood infection in unvaccinated populations. In developing countries, about 10 % of hospitalized measles patients die as a result of the disease[1,2].

In industrialized countries, children with cancer are at special risk for measles as the infection often leads to fatal pulmonary inflammation or encephalitis[3].

A case of fatal measles-related pneumonia without a rash has been reported in a child with AIDS[4].

In adults, measles is often accompanied by temporary hepatic dysfunction[5,6].

Principle of the Method

To prevent false positive results due to rheumatoid factors interference a pretreatment with RF Absorbent is implemented. The RF Absorbent binds to the IgG present in the test sample. Any rheumatoid factors in the sample bind to the resulting immune complexes and are thus eliminated. The RF Absorbent precipitates up to 15 mg IgG/mL (calculated on the basis of the undiluted sample) and is also removing any virus-specific IgG. This side effect increases the sensitivity for the detection of IgM.

The specific IgM antibodies to Measles virus contained in the test sample bind to the antigen in the reaction wells of the test plate. The Anti-Human IgM/POD Conjugate binds to these specific antibodies. The enzyme portion of the conjugate causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow.

IgM against cellular antigens is detected in the same way in the wells coated with control antigen. The difference between the color intensity in the well coated with antigen and in the well coated with control antigen is a measure of the immunochemical reactivity of the Measles virus-specific IgM antibodies in the sample.

Reagents

Symbols	Materials provided	
MEASLES/IgM	*Enzygnost® Anti-Measles Virus/IgM*	*2 x 48*
MTP	Enzygnost® Anti-Measles Virus/IgM test plate	2 pcs.
CONJUGATE ANTI-M	Anti-Human IgM/POD Conjugate	1 x 1 mL
MICROBIOL M	Conjugate Buffer Microbiol	4 x 12.5 mL
REFER P/P	Anti-Measles Virus Reference P/P	1 x 0.65 mL
REFER P/N	Anti-Measles Virus Reference P/N	1 x 0.4 mL
DILUENT	Sample Buffer POD	2 x 50 mL
RF ABSORBENT	RF Absorbent	4 x → 5 mL
	Polyethylene bag	1 pc.
	Barcode table of values	1 pc.
	Instructions for Use	1 pc.

The test plate, the conjugate, the conjugate buffer as well as Anti-Measles Virus Reference P/P and Anti-Measles Virus Reference P/N must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values.

Materials required but not provided

Supplementary Reagents for Enzygnost®/TMB (REF OUVP)

The reagents Chromogen TMB and Buffer/Substrate TMB must be used only in the combination of lots stated for the Supplementary Reagents kit. The applicable lot numbers are the 6-digit lot numbers listed on the package.

Composition

Enzygnost® Anti-Measles Virus/IgM test plate: Microtitration plate coated with inactivated Measles virus antigen. The wells in the left row of each strip are coated with antigen derived from permanent simian kidney cells infected with Measles virus, and the wells in the right row are coated with antigen from non-infected cells (= control antigen).

Anti-Human IgM/POD Conjugate: Goat antibodies to human IgM, conjugated with peroxidase, in TRIS/HCl buffer with polygeline. The conjugate is colored red.

Dye: Brilliant Ponceau

Preservative: phenol (≤ 1 g/L)

Conjugate Buffer Microbiol: EDTA in phosphate buffer with TETRONIC and BSA

Preservatives: gentamicin (~100 mg/L)
5-chloro-2-methyl-isothiazole-3-one (~6 mg/L)
2-methyl-4-isothiazole-3-one (~2 mg/L)

Anti-Measles Virus Reference P/P: Human serum containing IgG antibodies to Measles virus antigens together with rheumatoid factors (RF) in TRIS/HCl buffer with HUMANALBIN

Preservatives: amphotericin B (~5 mg/L)
gentamicin (~100 mg/L)

Anti-Measles Virus Reference P/N: Human serum containing IgG antibodies to Measles virus antigens contained in TRIS/HCl buffer with HUMANALBIN

Preservatives: amphotericin B (~5 mg/L)
gentamicin (~100 mg/L)

Sample Buffer POD: TRIS/HCl buffer with Tween 20, polygeline and bovine serum

Preservatives: amphotericin B (~5 mg/L)
gentamicin (~100 mg/L)

RF Absorbent (lyophilized): Sheep antibodies against human IgG-Fc fragment in phosphate buffered saline

Preservative: sodium azide (≤ 0.4 g/L)

Warnings and Precautions

For *in-vitro* diagnostic use only.

The test was developed for testing individual samples, not for pooled samples.

Contains sodium azide (< 1 g/L) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

CAUTION! POTENTIAL BIOHAZARD
Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

MICROBIOL M
Contains reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1). May produce an allergic reaction. Safety data sheet available on request.

It is advisable to wear protective gloves throughout the entire test procedure. Please follow the recommendations of the manufacturer concerning the compatibility between gloves and exposed materials.

For disposal, it is recommended that solid infectious materials should be autoclaved for at least 1 hour at 121 °C. All aspirated liquids should be collected in two receptacles connected in series. Both should contain a disinfectant suitable for inactivating human pathogens. The concentrations and times specified by the manufacturer must be observed.

Buffer/Substrate TMB, Chromogen Working Solution and Stopping Solution POD must not be allowed to come into contact with heavy metal ions or oxidizing substances (do not use pipettes with metal parts which are in direct contact with the liquid). The substrate reaction steps must not be performed in the vicinity of disinfectants containing hypochlorite. If the Chromogen Working Solution has spontaneously developed a blue color before being transferred into the test plate, this indicates that the solution is contaminated; in such cases, prepare a fresh solution in a clean container. Skin contact with the above mentioned solutions is to be avoided.

Preparation of the Reagents

Bring all reagents and test samples to 18 to 25 °C before starting with the test. Do not remove the foil pouch from the test plates during this step. Before starting the test processing, remove not required strips from the holder and store these in the enclosed polyethylene bag for later use (see Table 1). If reagents or working reagent solutions need to be mixed, avoid foam formation.

Dilute Anti-Human IgM/POD Conjugate 1+50 with Conjugate Buffer Microbiol, e.g., for one test plate, add 250 µL conjugate into a vial with 12.5 mL Conjugate Buffer Microbiol. Shake gently to mix.

For diluting samples, 50 mL Sample Buffer POD can be dyed blue-violet by adding 2.5 mL of Colour Solution blue for Enzygnost® (from the kit Supplementary Reagents for Enzygnost®/TMB). Shake gently to mix. In the Sample Buffer POD, the occurrence of a slight, insoluble deposit is unproblematic.

For each test plate, dilute 20 mL of Washing Solution POD from the kit Supplementary Reagents for Enzygnost®/TMB with distilled or deionized water to 400 mL.

For each test plate, dilute 1 mL of Chromogen TMB with 10 mL of Buffer/Substrate TMB from the kit Supplementary Reagents for Enzygnost®/TMB using the supplied empty plastic bottle (Chromogen Working Solution). Store protected from light. After use, carefully rinse the bottle with distilled or deionized water. For technical reasons (overfill), it is not permissible to pour together the full contents of the Chromogen TMB vial and the full contents of the Buffer/Substrate TMB vial.

The RF Absorbent must be dissolved in 5 mL sterile, distilled water per vial, and then it is ready to use.

Storage and Stability

Stored unopened at 2 to 8 °C, all components of the test kit may be used up to the expiry dates given on the labels.

For complete stability and storage data of the opened, reconstituted, respectively diluted reagents, see Table 1.

Equipment Required

BEP® III:	For automatic processing of the test after dispensing the samples as well as for evaluation
BEP® 2000/BEP 2000 *Advance*®:	For fully automatic processing and evaluation of the test
Pipettes:	Piston-type pipettes with fixed or variable volumes, or single- and multi-channel pipettes with adjustable volumes

The following items are required additionally if the test is not processed automatically:

Incubator:	Incubator for 37 ±1 °C with increased humidity, e.g. obtained by inserting a dish of water, or similar incubation method
Washing device:	Microtitration plate washer
Photometer:	Photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate)

All the equipment used in the test must have been validated.

Specimens

Suitable specimens are individual samples (human sera or citrated/EDTA/heparinized plasma) obtained by standard laboratory techniques. The samples should be stored for no more than 3 days at 2 to 8 °C. If the samples are to be stored for a longer period of time, they must be frozen.

Procedure

Non-automated Test Procedure

1. **Dilute samples:** Dilute all samples as well as the Anti-Measles Virus Reference P/P and P/N in a ratio of 1+20 using (blue-violet dyed) Sample Buffer POD, e.g. by pipetting 20 µL sample into a dilution tube and adding 400 µL of Sample Buffer POD. Shake gently to ensure a thorough mixture. The diluted samples can be stored, sealed, overnight at 2 to 8 °C in tubes with low protein-binding capacity.
2. **RF absorption:** Add 0.2 mL of the 1+20 diluted test samples, but not of the equally diluted References, to 0.2 mL of the reconstituted RF Absorbent (sample dilution, 1+41). Incubate for 15 minutes at 15 to 25 °C or overnight at 2 to 8 °C.
3. **Assay scheme:** The necessary number of test plate well pairs is given by the number of test samples plus the number of determinations (n = 3) for Anti-Measles Virus Reference P/N and P/P.
4. **Dispense samples:** Dispense 150 µL of diluted Anti-Measles Virus Reference P/N (1+20) into each well of the first pair (A1: Measles virus antigen; A2: Measles virus control antigen), 150 µL of diluted Anti-Measles Virus Reference P/P (1+20) into each well of the next pair, and 150 µL of diluted sample (1+41) into each well of the subsequent pairs. At the end of the series, respectively test plate, fill the last pair of wells with 150 µL each of diluted Anti-Measles Virus Reference P/P.

   Important: It is not permitted to first pipette Anti-Measles Virus Reference P/P into the wells at the start and end of the sample series, and then put the samples in-between.

   Each sample must be pipetted with its own pipette tip. The pipetting steps must be completed within 15 minutes per test plate. An 8-channel pipette simplifies and speeds up the transfer of diluted samples into the test plate. After completing the pipetting steps, seal the test plate with foil and place immediately into the incubator.
5. **Incubate samples:** Incubate for 60 ± 2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
6. **Wash:** Remove foil and aspirate all wells. Fill each well with approx. 0.3 mL diluted Washing Solution POD, aspirate the plate, and repeat the wash cycle three times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).
7. **Dispense conjugate:** Pipette 100 µL of diluted Anti-Human-IgM/POD Conjugate into each well. Then seal the test plate with fresh foil and place immediately into the incubator.
8. **Incubate conjugate:** Incubate for 60 ± 2 minutes at 37 ±1 °C, then proceed immediately to the wash step.
9. **Wash:** As described in step 6.
10. **Dispense substrate:** Pipette 100 µL of Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.
11. **Incubate substrate:** Immediately after the substrate dispensing step, incubate at 18 to 25 °C for 30 ± 2 minutes, protected from light.
12. **Stop reaction:** Remove the foil. Add 100 µL Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing step.
13. **Measure:** Read the test plate at 450 nm within one hour. The recommended reference wavelength is 650 nm, or where appropriate between 615 and 690 nm.

Procedure for the BEP® III System

When using the BEP® III, the test plates must be prepared up to the sample dispensing step (steps 1 to 4 in the section "Non-automated Test Procedure"). Immediately afterwards place the uncovered test plates, i.e. not covered with foil, into the BEP® III. All subsequent processing steps are performed fully automatically by the instrument (see BEP® III Instruction Manual).

The settings for the incubation times in the BEP® III software may differ from the times in the section "Non-automated Test Procedure" for technical reasons (system speed) but have been validated for Enzygnost® on the BEP® III.

Procedure for the BEP® 2000 System

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see BEP® 2000 Instruction Manual).

Sample processing with the BEP® 2000 System may differ from the information given under "Non-automated Test Procedure", but has been validated for Enzygnost® on the BEP® 2000.

Internal Quality Control

Validation Criteria

Note: For all evaluations, the absorbance values obtained from the measurement with measles virus antigen minus the absorbance value of the same sample obtained with measles virus control antigen must be used. This value is given as ΔA.

To evaluate the test the following criteria must be fulfilled:

Anti-Measles Virus Reference P/P:

Each ΔA value must be within the lot-dependent lower and upper margin listed in the respective barcode table of values:

lower margin ≤ $\Delta A_{\text{Reference P/P}}$ ≤ upper margin

In addition the individual ΔA values (Anti-Measles Virus Reference P/P at the start and end of a series of measurements or test plate) must not differ by more than ±20 % from the mean calculated from these values.

Anti-Measles Virus Reference P/N:

$\Delta A_{\text{Reference P/N}} \leq 0.099$

If these conditions are not met, the test is not valid for evaluation. In this case, the software of BEP® III and BEP® 2000 will give notice of an invalid test result. The measurements must be repeated after investigating the cause.

Results

The evaluations are performed automatically in the BEP® III and the BEP® 2000. Please consult the relevant Instruction Manual. The following sections must be taken into account when performing measurements without software support.

Measurement Correction

For achieving an optimal reproducibility of the results, the measurements require correction.

To determine the correction factor, the nominal value of Anti-Measles Virus Reference P/P (provided in the barcode table of values) is divided by the mean test result of Reference P/P:

$$\text{Correction factor} = \frac{\Delta A \text{ nominal value}}{\text{mean } \Delta A \text{ value}_{\text{Reference P/P}}}$$

The differences in absorbance (ΔA) of those test samples determined in the series must now be multiplied by this correction factor. If processing several test plates, the correction factor must be calculated and used for each individual test plate.

Based on the criteria of the test, the samples are classified as follows:

Anti-Measles Virus/IgM **negative**	ΔA < 0.100 (cut-off)
Anti-Measles Virus/IgM **positive**	ΔA > 0.200
Anti-Measles Virus/IgM **equivocal**	0.100 ≤ ΔA ≤ 0.200

Test samples with an equivocal result must be retested in duplicate. If the result is confirmed, the samples are classified as equivocal, otherwise as positive or negative.

Sample Titration

If the sample is to be titrated, pipette 0.15 mL of Sample Buffer POD into each pair of antigen and control antigen wells, but leave the first pair of wells intended for the starting dilution empty. Fill each of the first two wells with 0.15 mL of the sample pretreated as described in step 1 and 2 under "Non-automated Test Procedure" (starting dilution 1+41). Titrate in 4-fold serial dilutions by transferring 0.05 mL from one well to the next, mixing thoroughly each time. Discard the surplus 0.05 mL from the last well of the titration series. The reaction volume of 0.10 mL remaining in the wells with the starting dilution (1+41) is still sufficient.

To determine the titer it is advisable to prepare the sample dilution curve by plotting the corrected absorbance differences against the titration scale on semi-logarithmic paper (ΔA linear).

The titer can then be read from the point at which the curve intersects the cut-off value of 0.10 ΔA. The above specified value classifying a sample as positive (ΔA > 0.20) does not apply to titrations.

Ratio Determination

The result of the IgM test can be easily quantified by calculating the quotient from the associated cut-off value ($\Delta A_{\text{sample}}/\Delta A_{\text{cut-off}}$).

Assessment of the Results

A **"negative"** result means that no virus-specific IgM was detected. The patient either is not acutely infected with Measles virus or, if infected or vaccinated, is unable to produce IgM specific for the virus. Thus, for example, it is often not possible to detect virus-specific IgM in persons who contract measles despite prior vaccination against the disease[7,8]. If exposure to the virus is suspected despite a negative finding, a second sample should be collected no less than 7 days later and should be tested together with the first sample.

A **"positive"** result means that virus-specific IgM was detected. This is proof of a recent infection. In over half the measles patients, anti-Measles virus IgM is detectable already on the first day of the rash[8]; these antibodies can then be detected for about 3 months[9]. Specific anti-Measles virus IgM is found in cases of chronic active hepatic inflammation not caused by hepatitis B virus[10]. Specific anti-Measles virus IgM cannot be regarded as a marker for subacute sclerosing panencephalitis (SSPE)[11].

If a result is classified as **"equivocal"** after a retest, this is indicative of virus infection. Here, a second sample must likewise be collected no less than 7 days later and tested together with the first sample.

Limitations of the Procedure

1. Anticoagulants (citrate, EDTA, heparin) and rheumatoid factors do not interfere with the test result.
2. Lipemic, hemolytic and icteric samples do not interfere with the test.
3. Samples with the following potentially interfering substances were tested: ANA, AMA, samples with elevated total IgG and IgM, samples from dialysis patients and samples containing antibodies against Toxoplasmosis/IgM, EBV/IgM, CMV/IgM, Rubella/IgM. No influence on the test results was observed with the samples tested.
4. No interferences have been observed with heat-treated samples (30 minutes, 56 °C).
5. Incompletely coagulated sera and microbially contaminated test samples should not be used. Any particles (e.g. fibrin clots, erythrocytes) contained in the sample should be removed prior to assay.
6. If thawed samples are used, ensure that the material is thoroughly homogenized.
7. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
8. The References were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
9. Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.
10. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Specific Performance Characteristics

Sensitivity

47 test samples were tested in parallel in Enzygnost® Anti-Measles Virus/IgM and in a comparison method. In this set of samples, Enzygnost® Anti-Measles Virus/IgM was shown to have a sensitivity of 100 %.

Specificity

Enzygnost® Anti-Measles Virus/IgM detects only IgM. The RF Absorbent eliminates falsepositive results caused by certain rheumatoid factors and also false-negative results caused by high concentrations of virus-specific IgG[12-14].

536 test samples were assessed in parallel in Enzygnost® Anti-Measles Virus/IgM and in a comparison method; in this study the test was shown to have a specificity of 100 %.

Precision

A test sample containing antibody activity for the virus was evaluated to determine the intra- and inter-assay coefficients of variation (CV). The following results were obtained:

Intra-assay			Inter-assay		
Sample	**Mean Absorbance (ΔmA)**	**CV (%)**	**Sample**	**Mean Absorbance (ΔmA)**	**CV (%)**
A	830	4.6	A	916	3.5

The results refer to the groups of samples investigated.

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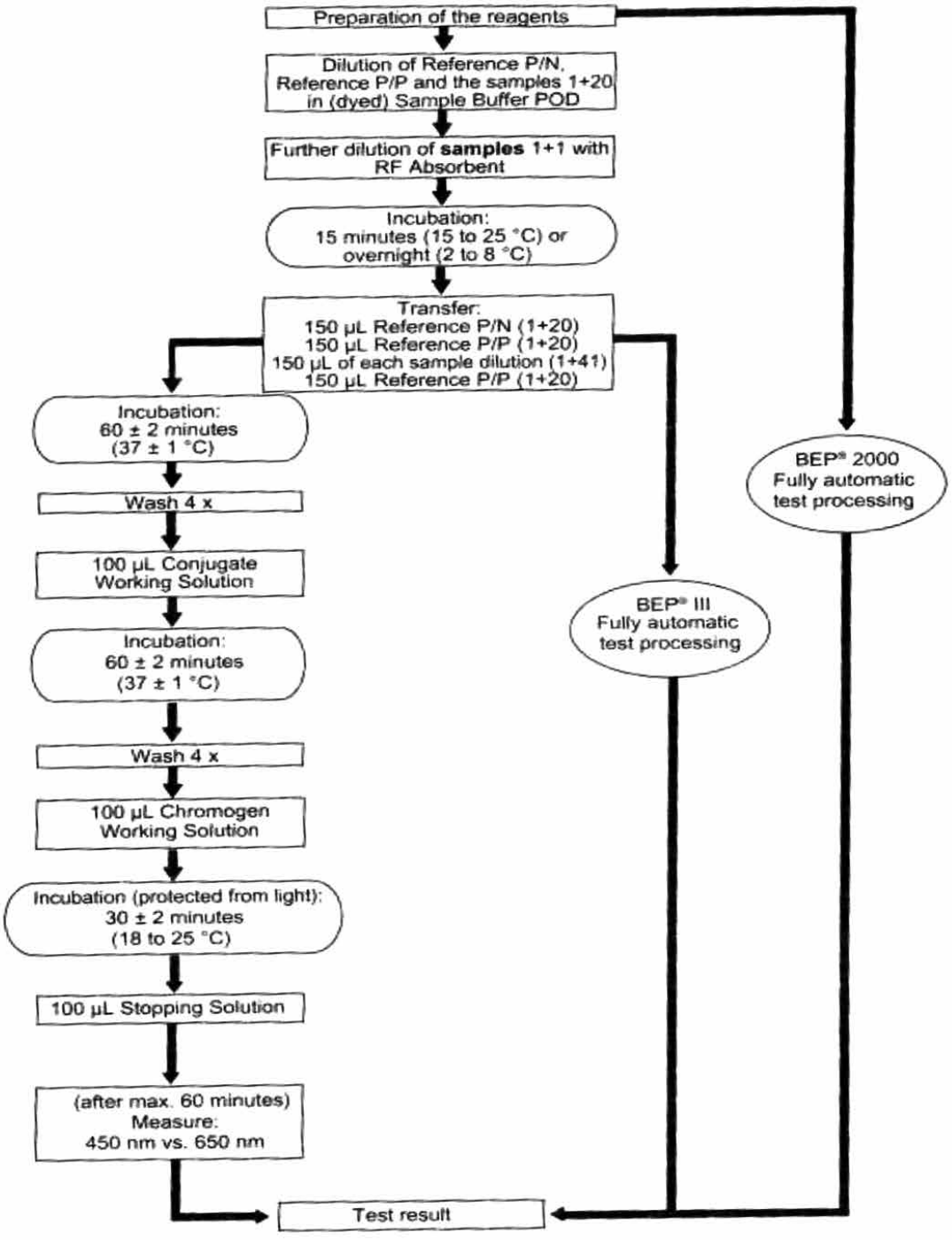
Table 1 Storage and Stability

Material/Reagent	State	Storage	Stability•
Enzygnost® Anti-Measles Virus/IgM test plate, remaining strips	once opened	2–8 °C in the bag with desiccant	8 weeks
Anti-Human IgM/POD Conjugate	once opened diluted 1+50	2–8 °C 2–8 °C 15–25 °C	12 months 4 weeks 1 day
Conjugate Buffer Microbiol	once opened	2–8 °C	8 weeks
Anti-Measles Virus Reference P/P Anti-Measles Virus Reference P/N	once opened diluted 1+20	2–8 °C 2–8 °C	12 months overnight••
Sample Buffer POD	once opened	2–8 °C	8 weeks
RF Absorbent	reconstituted	2–8 °C 15–25 °C	4 weeks 1 week
Chromogen TMB	once opened	2–8 °C	expiry date
Buffer/Substrate TMB	once opened	2–8 °C	expiry date
Chromogen Working Solution	diluted 1+10	2–8 °C 15–25 °C closed container protected from light	5 days 8 hours
Washing Solution POD	once opened diluted 1+19	2–8 °C 2–8 °C 18–25 °C	expiry date 1 week 1 day
Colour Solution blue for Enzygnost®	once opened	2–8 °C	expiry date
Stopping Solution POD	once opened	2–8 °C	expiry date

• use each component by the expiry date at the latest
•• in closed dilution tubes with low protein-binding capacity

Table 2 Test Procedure



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