Enzygnost® Anti-Measles Virus/IgG

MEASLES/IgG

See shaded sections - updated information versus edition May 2008

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

1. For *in-vitro* diagnostic use only.
2. The test was developed for testing individual samples, not for pooled samples.
3. 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.
4. 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.
5. 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.
6. 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 $\leq \Delta A_{\text{Reference P/N}} \leq$ 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**	$\Delta A < 0.100$ (cut-off)
Anti-Measles virus/IgG **positive**	$\Delta A > 0.200$
Anti-Measles virus/IgG **equivocal**	$0.100 \leq \Delta A \leq 0.200$

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:

	Intraassay		Interassay	
Sample	**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.

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