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MEASLES SERODIAGNOSIS: PRODUCTION AND EVALUATION OF THE IGM-MEASLES ELISA^{IAL} REAGENT

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ABSTRACT

Recent measles outbreaks in different countries led to an increase of laboratory measles diagnosis. Thus, we developed the IgM-Measles ELISA^{IAL}, using measles virus antigens obtained from cell cultured in microcarriers in order to supply reagent kits to Brazilian public health laboratories. A batch of antigenic reagent was produced and evaluated in the enzyme immunoassay in comparison with clinical diagnosis and with as reference assay (IgM Capture ELISA^{CDC}) data. This study was performed in a positive panel with 70 serum samples from patients with measles, and a negative panel with 132 samples from patients with unrelated diseases and without recent measles or vaccination history. In relation to other diagnostic methods, the IgM ELISA^{IAL} presented sensitivity higher than 97.1%, specificity and precision of 97%, and agreement kappa (k) index higher than 0.94 ($P < 0.05$). Moreover, the IgM antibody profile from measles acute phase revealed by the assay was similar to the reference assay. A practical analysis system for checking the quality of new reagent batches was proposed based on the diagnostic features and agreement kappa index. Our findings suggest that measles antigenic reagents can be produced with reliable quality control system, and supplied to public health laboratories for routine serodiagnosis or population surveys.

Key words: measles, IgM-measles reagent, reagent quality control, serodiagnosis, seroepidemiology

INTRODUCTION

In recent years, unexpected outbreaks of measles have been reported in different countries including Brazil (1,2,3). Thus, requests for measles serodiagnosis have increased considerably at the public health laboratories.

There are many serologic techniques available but enzyme immunoassays have been preferred because of their sensitivity and convenience for detection of IgM antibodies, which are the main immunologic markers for current or recent measles virus (MV) infection (4,5).

In public health laboratories, the measles serodiagnosis requires a large amount of MV antigen, but the use of a microcarrier system that we had previously standardized at the Instituto Butantan (IB) obviated this problem (6). The so obtained MV permitted us to prepare different types of antigen, and in particular a deoxycholate-based antigen showed to be suitable for enzyme immunoassays such as IgG ELISA and IgG dot-ELISA, as well as IgM dot- ELISA (7).

The Instituto Adolfo Lutz Institute (IAL) produces diagnostic reagents for its own use and to supply other member laboratories of the São Paulo State Secretariat of Health. In a collaborative study between this institution and the IB, an enzyme immunoassay, IgM-Measles ELISA^{IAL} using a reagent prepared with the deoxycholate-based measles antigen, was designed.

In the present work, the standardized antigenic reagent for the IgM-Measles ELISA^{IAL} was produced in large volume, and its diagnostic features were evaluated. Moreover, a system for

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controlling the quality of new reagent batches was proposed with basis on diagnostic features and agreement κ index, which differs from other known systems (8,9,10).

MATERIALS AND METHODS

Panel of serum samples from patients with measles

Thirty-seven paired serum samples were collected from patients with clinical measles at acute- and convalescent- phases. The acute-phase-specimens were obtained between the first day of rash onset to 7 days afterwards, and convalescent-phase specimens were collected approximately two to three weeks after rash onset. All patients had clinical symptoms of measles and most of them had IgM antibodies (IgM-IFI and IgM dot-ELISA), including those with seroconversions by hemagglutination-inhibition and IgG dot-ELISA. Two positive control sera, one of these with high and other with low antibody level, were prepared by pooling the patient sera.

Panel of serum samples from patients without measles

This panel of serum samples comprised 132 serum samples from patients with unrelated infections and without the history of recent measles infection or vaccination, having well defined clinical and serological diagnosis. This panel had samples from 9 patients with rubella, 5 patients with cytomegalovirus infection, 5 patients with dengue virus infection, 6 with mononucleosis, 4 with *Streptococcus pyogenes* infection, 5 with syphilis, 5 with leptospirosis, 5 with South American blastomycosis, 6 with Chagas' disease, 5 with acute toxoplasmosis, 5 with mucocutaneous leishmaniasis, 6 with schistosomiasis mansoni, 6 with cysticercosis, and 29 with connective tissue diseases, with high levels of rheumatoid factor. Also, serum samples from 6 clinically healthy adult blood donors and 5 clinically healthy children, without IgM measles antibodies, were included in this panel. Two negative control sera were also prepared by pooling sera from non-measles patients.

All serum samples from the positive and negative panels had clinical and serological data.

Measles virus antigens

Antigens for IgM-Measles ELISA^{IAL} was prepared as previously described (6,7), using measles virus (Edmonston strain) from Vero cells cultured in the bioreactor 3.7. The protein contents of the antigen extracts were determined according to Bradford (11) and Lowry *et al.* (12).

Plastic microplates

Three commercially available microplate batches, P1 and P2 manufactured abroad and P3 made in Brazil, were evaluated in terms of plastic capability to fix virus antigen, reproducibility and type of regression line of the optical densities (OD) and serum dilutions. Student's *t* test (13) was used to compare OD means.

IgM-Measles ELISA^{IAL}

This enzyme immunoassay was performed as previously described (14) with some modifications. Briefly, the microplate wells were sensitized with measles antigen and uninfected cell extract. Plates were blocked with skim milk and incubated with serum, which had previously been treated with human gammaglobulin aggregates (RF absorbent-Behring, Germany) to remove rheumatoid factor. Plates were incubated with peroxidase-labeled goat anti-human IgM conjugate. The absorbance value (P) (Multiskan plus version 2.02 ELISA reader) of the color produced by O-phenylenediamine for each serum was substracted by the absorbance value of the uninfected cell extract (N). To determine the cutoff value for the IgM-Measles ELISA^{IAL}, 126 serum samples from infants without detectable antibodies by hemagglutination inhibition (HI) or indirect immunofluorescence (IFI) tests were assayed. The cutoff chosen was the mean value of the absorbances 0.015 found for these serum samples plus 3 standard deviations (0.03), i.e. the optical density of 0.045. In each run, two positive control sera and two negative control sera were included.

IgM capture ELISA^{CDC}

This assay was carried out as described previously (15). The cutoff value for this assay was determined with the same above referred to126 serum specimens from infants. The cutoff value was taken as the mean plus three standard deviations of the net absorbance values of a set of known measles-negative sera. Four afore mentioned control sera were also included in each run to assure the reproducibility of the assay.

Validity of the IgM-Measles ELISA^{IAL} reagent

The data obtained by the IgM-Measles ELISA^{IAL} was compared individually with the clinical and serological (IgM capture ELISA^{CDC}) diagnosis. The study was performed in panels of positive and negative serum samples, determining values of sensitivity, specificity and precision with their 95% confidence intervals (16). Also, the agreement kappa (κ) index was determined (17,18,19).

Reproducibility of the reagent

The reproducibility of the reagent was first inspected by intra- and inter-assays with a panel of about 20 positive and 20 negative serum samples in three different days. Then their mean OD values were compared by the statistical Student *t* test. However, the proposed inspection for the reproducibility of new reagents to be successively produced had basis on the diagnostic parameters of sensitivity and specificity, in addition to the agreement κ index.

Calculations for the diagnostic validity and reproducibility of the assay

The equations used for calculating the diagnostic parameters and agreement κ index were as follows:

		Clinical diagnosis or IgM capture ELISA^{CDC}			
		+		-	
IgM Measles ELISA^{IAL}	+	a	b	e	Sensitivity = a/g
	-	c	d	f	Specificity = d/h
		g	h	l	Precision (P_O) = (a + d)/l

kappa index (κ) = (Po – Pc)/(1-Pc), in which Pc = (eg + fh)/l^2
To test the significance of κ:
κ variance (var.) = 1/l (1 – Pc) [(Pc + Pc^2) – (e_1 g_1) (e_1 + g_1) + (f_1 h_1) (f_1 + h_1)], being e_1 = e/I; f_1 = f/l; g_1 = g/l; and h_1 = h/l.
κ standard deviation (sd) = $\sqrt{}$ var. and z_0 = κ/sd.
The critical *z* at 0.05 level corresponds to1.96 (17,18).

The strength of agreement κ index was ranked as *poor* for the values from 0 to 0.20, *slight* from 0.21 to 0.40, *moderate* from 0.41 to 0.60, *substantial* from 0.61 to 0.80 and *almost perfect* from 0.81 to 1.00 (19).

RESULTS AND DISCUSSION

The present work was carried out in sequential steps as follows: a) production of measles antigen virus and reagent kits; b) preparation of panels of measles positive and negative serum samples; c) selection of plastic plates; d) evaluation of the IgM-Measles ELISA^{IAL} *vs* clinical diagnosis, and *vs* IgM ELISA^{CDC}, in terms of sensitivity, specificity, precision and agreement k index; e) comparison of IgM-Measles ELISA^{IAL} with IgM ELISA^{CDC} in the natural course of measles infection; and f) proposal for the quality control inspection of new reagent kits successilvely produced.

A batch of MV antigen was produced in the standardized microcarrier system, starting with 3.6 liters of liquid medium that yielded almost 8 ml (5.01 mg/ml) virus antigen, which had a titer for use of 2,400 per mg antigen. We estimated that the MV antigen batch was enough for about 94,000 IgM-Measles ELISA^{IAL} testings. Eighty reagent kits were prepared and each kit with 0.1 ml MV antigen permitted to perform about 240 testings, including 4 control sera. The remaining antigen was stored for the production of new antigenic reagent batches.

The analysis of plastic microplates was seen to be essential for obtaining sensitive results. Three types of microplate were studied, and P1 and P2 plates showed similar capability to fix viral antigens (OD means of 0.679 and 0.605; P > 0.05). However, the P3 plates gave significantly lower capability to fix antigen (OD mean of 0.290; P < 0.05) than of the P1 and P2 plates. On the other hand, plates from the same manufacturer presented reproducible results. When same type of plates (P1, P2 or P3) were assayed in two different days gave comparable OD means between them (P1: 0.685 and 0.632, P > 0.05; P2: 0.604 and 0.618, P > 0.05; and P3: 0.2383 and 0.269, P > 0.05). The P1 and P2 plates showed similar type of regression line, but plates P3 gave less declined line in comparison to the plates P1 and P2. Thus, the plate P1 (Corning Lab. Sci. Co., N. York, USA) was chosen for the present work by providing sensitive results and being easily available.

Also, the antigenic reagent was assayed in the same day and in two different days with P1 plate, and the intra- and inter-assay reproducibilities were shown to be good, since the OD means obtained were close (P > 0.05).

The validity of the reagent was inspected in the panels of positive serum samples (70) and negative serum samples (132). The diagnostic features of the IgM-Measles ELISA^{IAL} in comparison with the clinical diagnosis, and serological diagnosis by IgM capture ELISA^{CDC} are presented in Table 1. The data show no statistical difference if confidence intervals at 95% level are observed. The over all sensitivity and specificity were within

Table 1. Diagnostic features of the IgM-Measles ELISA^{IAL} and IgM capture ELISA^{CDC} in the study of 202 serum samples from patients with measles and without measles.

Evaluation criterion	Sensitivity %	Specificity %	Precision %	Agreement κ index
IgM-Measles ELISA^{IAL} *vs* Clinical diagnosis	97.1 (90.2 – 99.2)	97.0 (92.5 – 98.8)	97.0 (93.7 – 98.6 0)	0.94*
IgM capture ELISA^{CDC} *vs* Clinical diagnosis	97.1 (90.2 – 00.2)	98.5 (95.7 – 99.5)	98.5 (95.7 – 99.5)	0.97*
IgM-Measles ELISA^{IAL} *vs* IgM capture ELISA^{CDC}	98.6 (92.2 – 99.7)	97.0 (92.5 – 98.8)	97.5 (94.3 – 98.9)	0.95*

* (P < 0.05).

the reported (4,5,20,21) ranges of 82.8% to 100% and 80% to 98.2%, respectively, either for home-made or commercially available IgM immunoenzymatic assay (IEA). We found 2 false negative and 4 false positive results by the IgM-Measles ELISA^{IAL} in comparison with the clinical diagnosis. But in relation to the IgM capture ELISA^{CDC}, the assay showed one false negative and 3 false positive results. Those 4 false positive serum samples belonged to patients with high RF titers, > 640, having concomitantly high titers of IgG anti-measles antibodies. This problem may be obviated by using enzyme conjugate with antibody fragments $F(ab')_2$, which will contribute to improve the specificity of the assay.

The agreement κ index found for the assay with other diagnostic methods were high, i.e. higher than 0.8 ($P < 0.05$) and ranked as *almost perfect*.

In the course of measles infection, the IgM-Measles ELISA^{IAL} revealed antibody profile (Fig. 1) looking like that of the IgM capture ELISA^{CDC}, except a mild difference seen at the initial phase of rash onset. This profile also seems to agree with those found by investigators (15,22), working with similar type of IEA. The sensitivity of the assay at acute-phase of measles, corresponding a period from day one up to 4 days after the rash onset, was found to be 87.5% (14/16). This finding was also consistent with previous data, which varied from 72% to 89% by IgM IEA, for a similar period of time. We observed that the sensitivity of an assay depends on the phase of measles infection, nevertheless, the positivity reaches 100% from 4 or 5 days to 30 days after rash onset, confirming reported data (4,5,23,24).

The diagnostic features of the IgM ELISA^{IA} permitted to stipulate that the acceptable levels for sensitivity and specificity were equal or higher than 97% (Table 1). Thus we built the

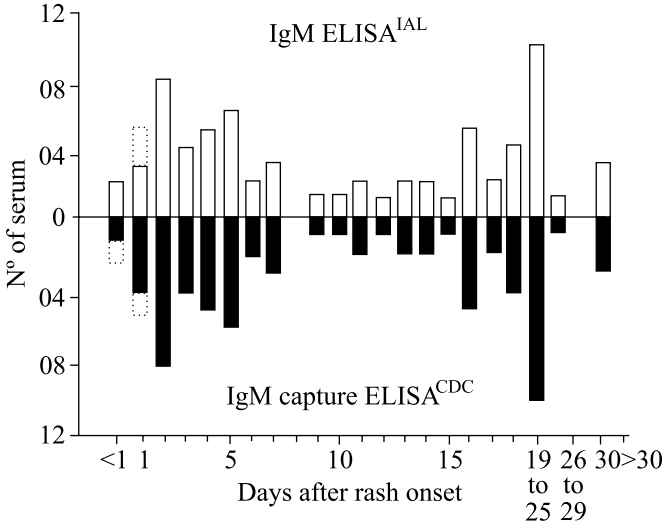

Figure 1. Specific antibodies detected in measles patients after rash onset by the IgM-Measles ELISA^{IAL} and IgM capture ELISA^{CD}.

Table 2. Control limit and warning limit of discrepant results calculated according to the number of positive or negative serum samples to measles, for the inspection of the IgM-Measles ELISA^{IAL} reagent by qualitative assayings.

Number of Serum Samples (n)	Discrepant Results	
	Control Limit (X1) (X1 = 0.03 n)	Warning Limit (X2) (X2 = 0.05 n)
20	0	1
30	0	1
40	1	2
50	1	2
60	1	3
70	2	3
80	2	4
90	2	4
100	3	5
110	3	5
120	3	6
130	4	6
140	4	7
150	4	7

Table 2, which is applicable for both positive and negative panels of serum samples, in the quality control analysis of new reagents. It is practical because the number of discrepant results was already calculated for the use of different number of positive and negative serum samples. When the discrepant results varies from 0 to 3% (control limit) in a positive or negative panel of samples, the reagent will be approved. However, if the discrepancies range from 3.1% up to 5% (warning limit), the reagent has to be re-assayed with additional number of serum samples, in order to leave the warning zone. Then the number of new discrepant results will provide basis for a decision whether the reagent will be accepted or rejected. Whenever the discrepant results are higher than 5%, the reagent will be rejected.

Three percent and 5% false positive or negative results were calculated according to the total number of samples (n) to be assayed, using the equations: Y1 = 0.03 n and Y2 = 0.05 n, considering only integer numbers. So, the Table 2 permit to select panels having different numbers of positive and negative serum samples, under the condition that up to 3% discrepant result, the agreement κ index is still equal or higher than 0.8 ($P < 0.05$). In this work, a panel of 70 positive serum samples and 132 negative serum samples were studied accordingly.

The data from Table 2 could also be represented by a graph with two regression lines (not shown), after transforming the number of discrepant results x 10 = $\log_{10}$. Thus, the regression line corresponding to the control limit had the equation $y_1 = 0.630 + 0.007x$ and that to the warning limit had the equation $y_2 = 0.973 + 0.007x$. These lines looked line of the sequential

analysis (8), however, they differ because the agreement level plus the sensitivity and specificity were considered, and it is applicable for a low number of serum samples of at least 20. This system of analysis is more practical than the sequential analysis, which is based on the sensitivity and specificity, and requires at least 75 serum samples.

The stipulated values for control and warning limits of the Table 2 are not definite and may be corrected with time, as more information about discrepant results become available. Also it will be possible to confirm whether 3% discrepant results, considered here reasonable, are or not a realistic estimate.

The IgM ELISA^{IAL} showed that 3% from the positive panel had values lower than the cutoff OD (0.045), 6% had values varying from 0.045 to 0.099, 28% from 0.100 to 0.199, 24% from 0.200 to 0.299, 16% from 0.300 to 0.399, 13% from 0.400 to 0.499, 10% higher than 0.500. Whereas the OD for 132 serum samples from the negative panel varied from 0.001 to 0.036, and were lower than the referred to cutoff DO, except for those 4 samples with high levels of RF. The positive serum samples were divided into 3 categories based on their OD: the first category comprised samples with low antibody levels (0.045 to 0.099), the second category had samples with medium antibody levels (0.100 to 0.299), and the third category presented high antibody levels (> 0.300). When a serum sample was used up, other one belonging to the same category replaced it. Positive serum samples with low and medium antibody levels are considered more sensitive to detect variation in the reagent reactivity rather than those with high antibody levels (9). Thus, a careful selection of positive samples is advisable. The patient humoral immune response also may vary and, depending on the phase of the measles infection or the patient immune status, false negative results may be found by serological assay. Taking into account that serum samples with low levels of IgM anti-measles antibodies are difficult to be obtained, the positive panel can be prepared mostly with samples with medium antibody levels, which are sensitive enough to detect small antigenic variation in the successively produced reagents (9).

On the other hand, false positive result may also occur with unrelated diseases because of antigen cross-reactivities. Thus, our negative panel of serum samples should be improved by adding samples from infected patients with herpesvirus 6 and parvovirus B19, which seem to cause some cross-reactivity with measles virus (5, 25).

Our results show that the reagent prepared with virus antigen obtained from microcarrier system presents features similar to those of the nucleoprotein recombinant antigen of the IgM capture ELISA^{CDC}. Other IgM enzyme immunoassays similar to the IgM-Measles ELISA^{IAL} were also shown to be as sensitive as the different types of IgM capture EIA (20).

Thus, we conclude that the IgM-Measles ELISA^{IAL} has diagnostic value, due to the sensitivity and specificity be higher than 97% and its agreement k indices with other diagnostic methods be also higher than 0.8, ranking as almost perfect. The proposed quality control method seems practical and applicable for assuring the reproducibility of new reagents in respect to their diagnostic features. Institutions can produce this reagent for their own use or supplying other public health laboratories or even research laboratories, since implementing a reliable quality control system.

RESUMO

Sorodiagnóstico de sarampo: produção e avaliação da técnica de ELISA IgM^{IAL}

Recentes epidemias de sarampo em diferentes países induziram aumento de sorodiagnóstico do sarampo, inclusive em laboratórios de saúde pública brasileiros. Desta forma, desenvolvemos uma técnica de ELISA IgM^{IAL}, utilizando antígenos de vírus do sarampo obtidos de células cultivadas em microcarregadores, com o objetivo de fornecer "kits" de reagentes para diferentes laboratórios. Foi produzida uma partida de reagente para esta técnica imunoenzimática, e os parâmetros diagnósticos foram avaliados em comparação com dados clínicos e de ensaio de referência (ELISA de captura IgM). O reagente foi estudado em um painel positivo tendo 70 soros de pacientes com sarampo e painel negativo com 132 soros de pacientes com doenças não relacionadas e sem história de sarampo recente ou vacinação. A técnica de ELISA IgM^{IAL} demonstrou sensibilidade de 97,1% e 98,6%, especificidades de 97%, e índices kapa de concordância de 0,94 e 0,95 ($p < 0,05$). O perfil de anticorpo IgM de sarampo da fase aguda foi também semelhante ao do ensaio de referência. Foi proposto ainda um sistema prático de análise para o controle de qualidade de reagentes com bases nos achados de validadação do reagente. Nossos achados sugerem que partidas de reagentes do vírus do sarampo podem ser produzidas sucessivamente e utilizadas em laboratórios de saúde pública para sorodiagnóstico de rotina ou inquérito soroepidemiológico de população.

Palavras-chave: sarampo, reagente de sarampo-IgM, controle de qualidade de reagente, sorodiagnóstico, soroepidemiologia

REFERENCES

1. Centers For Disease Control and Prevention. Advances in global measles control and elimination. Summary of the 1997, *International Meeting, MMWR*, 47 (RR-11): 1-23, 1998.
2. Brasil, Ministério da Saúde. Fundação Nacional de Saúde, Centro Nacional de Epidemiologia. Baixa cobertura vacinal favoreceu epidemia. *Bol. Epidemiol.* 2, 1997.
3. São Paulo, Secretaria do Estado da Saúde do Estado de São Paulo. Centro de Vigilância Epidemiológica "Professor Alexandre Vranjac". *Plano de Erradicação do Sarampo*. Grupo Técnico de Doenças de Transmissão Respiratória. São Paulo, 1999.

4. Arista, S.; Ferraro, D.; Cascio, A.; Vizzi, E.; Di Stefano, R. Detection of IgM antibodies specific for measles virus by capture and indirect enzyme immunoassays. *Res. Virol.*, 146:225-232, 1995.
5. Ratnam S.; Tipples G.; Head, C.; Fauvel, M.; Fearon, M.; Ward, B.J. Performance of indirect immunoglobulin M (IgM) serology tests and IgM capture assays for laboratory diagnosis of measles. *J. Clin. Microbiol.*, 38: 99-104, 2000.
6. Mendonça, R.Z.; Vaz de Lima, L.R.A.; Oliveira, M.I.; Pereira, C.A.; Hoshino-Shimizu, S. Studies on the efficiency of measles virus antigen production using Vero cell culture in a microcarrier system. *Brazilian J. Med. Biol. Res.*, 27: 1575-1587, 1994.
7. Vaz-de-Lima, L.R.A.; Hoshino-Shimizu, S.; Souza, V.A.V.F.; Pannuti, C.S.; Andrade, J.R.H.F.; Sumita, L.M.; Ferreira, A.W. Measles serodiagnosis : Standardization and evaluation of a Dot-ELISA. *Rev. Inst. Med. Trop. São Paulo*, 36: 139-147, 1994.
8. Hoshino-Shimizu, S.; Camargo, M.E.; Shimizu T.; Nagasse, T.K. A study on the reproducibility of a stable, lyophilized reagent for the Chagas' disease hemagglutination test: Proposals for quality control analysis. *Rev. Inst. Med. Trop. S. Paulo*, 24:61-68, 1983.
9. Hoshino-Shimizu, S.; Nagasse-Sugahara, T.K.; Castilho, E.A.; Camargo, M.E.; Shimizu T. A control chart method for evaluating hemagglutination reagent used in Chagas' disease diagnosis. *Bull. Pan Amer. Org.*, 20: 170-178, 1986.
10. Hoshino-Shimizu, S.; Nakamura, P.M.; Souza, E.M. Produção e controle de qualidade do reagente de hemaglutinação indireta para o diagnóstico da doença de Chagas. *Farm. Quím.*, 32: 37-44, 1999.
11. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analyt. Biochem*., 72: 248-254, 1976.
12. Lowry, O.H.; Rosembrough, H.J.; Farr, L.; Randall, R. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 263-275, 1951.
13. *Probability and Statistics for Engineering and Scientists*. Walpole, R.E.; Myers, R.H. eds., Macmillan Publishing Co., USA., 1993.
14. Voller, A.; Bidwell, D.E. Enzyme-immunoassay for antibodies in measles, cytomegalovirus infection and after rubella vaccination. *Br. J. Exp. Pathol*., 57: 243-147, 1976.
15. Erdman, D.D.; Anderson, L.J.; Adams, D.R.; Stewart, J.A.; Markowitz, L.E.; Bellini, W.J. Evaluation of monoclonal antibody based capture enzyme immunoassays for detection of specific antibodies to measles virus. *J. Clin. Microbiol.*, 29: 1466-1471, 1991.
16. Rothman K.J.; Boice J.D. *Epidemiologic Analyses with a Programmable Calculator*. Epidemiology Resources Inc., Boston, MA, USA.
17. Fleiss, J.L. *Statistical Methods for Rates and Proportions*. N. York, John Wiley & Sons. 1981, p.217-225.
18. Maclure, M.; Willet, W.C. *Misinterpretation and misuse of the kappa statistic*. Amer. J. Epidemiol., 126: 161-169, 1987.
19. Feinstein, A.R. *Clinical Epidemiology. The Architecture of Clinical Research*. Philadelphia, USA, Saunders Co., 1985, p. 185-186.
20. Cremer, N.E.; Cossen, C.K.; Shell, G.; Diggs, J.; Gallo, D.; Schmidt, N.J. Enzyme immunoassay versus plaque neutralization and other methods for determination of immune status to measles and varicella-zoster viruses and versus complement fixation for serodiagnosis of infections with those viruses. *J. Clin. Microbiol.*, 21: 869-874, 1985.
21. Lievens, A.W.; Brunell, P.A. Specific immunoglobulin M enzyme-linked immunosorbent assay for confirming the diagnosis of measles. *J. Clin. Microbiol.*, 24: 391-394, 1986.
22. Rossier E.; Miller, H.; McCulloch, B.; Sullivan, L.; Ward, K. Comparison of immunofluorescence and enzyme immunoassay for detection of measles-specific-immunoglobulin M antibody. *J. Clin. Microbiol.*, 29: 1069-1071, 1991.
23. Hummel, K.B.; Erdman, D.D.; Heath, J.; Bellini, W.J. – Baculovirus expression of the nucleoprotein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassays. *J. Clin. Microbiol.*, 30: 2874-2880, 1992.
24. Helfand, R.F.; Heath, J.L.; Anderson, L.J.; Maes, E.F.; Guris, D.; Bellini W.J. Diagnosis of measles with an IgM capture EUA: The optimal timing of specimen collection after rash onset. *J. Infect. Dis.*, 175: 195-199, 1997.
25. Thomas, H.I.J.; Barret, E.; Hesketh, L.M.; Wynne, A.; Morgan-Capner, P. Simultaneous IgM reactivity by EIA against more than one virus in measles, parvovirus B19 and rubella infection. *J. Clin. Virol.*, 14: 107-118, 1999.