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Comparative evaluation of a new chemiluminiscent assay and an ELISA for the detection of IgM against measles

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Background: The primary test for the laboratory confirmation of measles is immunoglobulin M (IgM) serology. It is therefore important to evaluate new commercial measles IgM immunoassays to ensure high-quality measles diagnostic testing. The purpose of this study was to evaluate the diagnostic performance of LIAISON IgM measles (DiaSorin, Saluggia, Italy), a new automated chemiluminescence immunoassay (CLIA), in comparison with enzyme immunoassay (EIA) Enzygnost (Siemens, Marburg, Germany). *Methods:* Sensitivity was examined using a panel of measles laboratory confirmed sera; specificity was addressed by testing sera from healthy populations and subjects with infections that may interfere with measles IgM serology. *Results:* The diagnostic performances of the two assays were very similar: both Enzygnost EIA and LIAISON CLIA performed with a sensitivity of 93.7% and 98.8%, whereas the specificity was 96.8% and 97.9%, respectively. *Conclusion:* We concluded that LIAISON IgM measles can be a good alternative to the other immunoassay for laboratory measles confirmation. J. Clin. Lab. Anal. 27:477–480, 2013.

Key words: chemiluminescence; serology; measles

INTRODUCTION

Measles is a vaccine-preventable disease that causes extensive morbidity and mortality in large parts of the world. Endemic measles has been eliminated in the Americas but continues to be endemic in the African and Southeast Asian regions, where vaccine coverage is low (1).

In Europe the situation of eradication programs is advanced, however, many countries in EU, also reported a considerable increase in the number of cases, with significant outbreaks, during 2010 and 2011 (2).

In Andalusia, Spain, following the recommendations of the World Health Organization (WHO), a Plan of Action for Measles/Rubella Elimination was approved in 2001 (3). The two strategic goals of the Plan are the maintenance of high vaccination coverage and early detection of cases to prevent transmission.

Laboratory confirmation of cases of measles is a vital aspect of surveillance because a number of other infections can be present with a rash resembling measles. The laboratory confirmation is done primarily through detection of specific immunoglobulin M (IgM) in serum samples collected 3–28 days after rash onset. The recommended assays for IgM determination are indirect or capture enzyme immunoassay (EIA; (4)). In addition to IgM antibody detection, measles can be diagnosed using other methods including a significant rise in measles IgG antibody level in paired sera, reverse transcription (RT)-PCR to detect measles virus ribonucleic acid (RNA), or isolation of measles virus in oral fluid, nasopharyngeal secretions, or urine (4).

Several different EIAs are described in the literature (5, 6) but there is no information about the performance of chemiluminescence immunoassays (CLIAs) to detect specific IgM antimeasles virus.

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The purpose of this study was to evaluate diagnostic performance of LIAISON® measles IgM (DiaSorin, Saluggia, Italy), a new automated CLIA, in comparison with EIA Enzygnost® antimeasles virus IgM (Siemens, Marburg, Germany). We used two test panels comprising a total of 285 sera from patients involved in measles outbreaks and obtained from persons with diseases that may cause false-positive reactions.

MATERIALS AND METHODS

Test Panel

Two panels of stored (−20°C) sera were used for the study: positive panel comprised single serum samples obtained from 190 patients who had laboratory-confirmed cases and who were involved in two outbreaks happened in Andalusia, Spain, in 2010 and 2011 (7,8). All sera were from patients with positive culture and/or RT-PCR from urine or nasopharyngeal exudates. The samples (blood, urine, and nasopharyngeal exudates) were collected simultaneously on a mean of 5 days (range: 0–9) after the onset of rash and sent to the laboratory following WHO recommendations (4). Culture for measles virus was done with the B95a cell line (9) and real-time RT-PCR was carried out as previously described, which targeted a fragment of the F gene (10).

Samples were divided into two subgroups according to time since onset of rash: days 0–3 (subgroup I, 45 samples) and days 4–9 (subgroup II, 145 samples). The percentage of correctly identified samples was calculated for each group and assay. Negative panel comprised a total of 95 serum samples distributed as follows: 25 from healthy adults who had no history of measles and received measles immunity investigation (all sera were positive for IgG antimeasles by ELISA Enzygnost (Siemens)), and 70 sera from subjects with IgM positive for other infectious agents such as Epstein–Barr virus (EBV) viral capsid antigen (VCA) ($n = 25$), *Mycoplasma pneumoniae* ($n = 15$), cytomegalovirus (CMV, $n = 9$), parvovirus B19 ($n = 18$), and rubella virus ($n = 3$). Parvovirus B19 and rubella positive IgM sera were obtained from patients involved in outbreaks in Granada and Seville as part of the Measles/Rubella Surveillance Program in Andalusia, Spain (3).

Commercially available EIA Enzygnost kits (Siemens) were used to detect the presence of specific IgM to CMV (Enzygnost anti-CMV IgM), and rubella virus (Enzygnost antirubella virus IgM). Rubella primary infection was confirmed by IgG avidity assay (Rubella IgG avidity EIA Well, Radim, Rome, Italy). Novagnost EIA kits (Siemens) were used for IgM against parvovirus B19 (Novagnost Parvovirus B19 IgM). Detection of IgM antibodies to *M. pneumoniae* was done by ELISA (SeroMP IgM, Savyon Diagnostic, Ashdod, Israel) and EBV-specific VCA IgM antibodies were detected by indirect fluorescence assay (IFA EBV VCA IgM Assay, Scimedx, Denville, NJ).

Serological Test

All serum samples were tested by Enzygnost EIA and LIAISON CLIA following the manufacturer's instructions. Before testing, all the sera were allowed to thaw at room temperature and prior to use, they were mixed thoroughly by vortexing.

LIAISON measles IgM is a qualitative, fully automated method for determination of specific IgM antibodies to measles virus in human serum or plasma. This new method is a two-step chemiluminescent enzyme IgM antibody μ-capture immunoassay. The solid phase, magnetic particle, is coated with a monoclonal murine anti-IgM antibody. During the first incubation, IgM antibodies to measles present in calibrators, samples, or controls bind to the solid phase. In the second cycle, a recombinant isoluminol-conjugated measles antigen binds to the specific IgM. The unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced and measured by a photomultiplier as relative light units (RLUs). A direct relationship exists between the amount of IgM antimeasles in the sample and the RLUs detected by the LIAISON immunoassay optical system.

Enzygnost antimeasles virus IgM is an indirect EIA used widely (6,11). The assay was processed by automated instrumentation (Tecan Freedom EVO/BEP III).

Serological results were interpreted in qualitative terms as positive, negative, or indeterminate. For sensitivity and specificity calculation, indeterminate results were considered the most adverse.

Sensitivity and specificity percentages were calculated with their 95% confidence intervals (95% CI). The z-test, to compare two proportions, was used to determine the statistical significance between the parameters of the two assays. Values with $P < 0.05$ were considered statistically significant.

RESULTS

Concordant results were obtained for 276 (96.8%) of the 285 sera tested by measles-specific IgM indirect EIA and CLIA. In the positive panel of 182 sera that tested positive in CLIA, 178 also tested positive and four were indeterminated by EIA. In the negative panel, 90 samples were tested negative by both methods and five sera were discordant (two indeterminate IgM results by CLIA and three by EIA).

TABLE 1. Sensitivity and Specificity Values (%) of CLIA LIAISON and EIA Enzygnost

	Sensitivity				Specificity	
	Number of positive/total	Overall (95% CI)	Subgroup I (%)[a]	Subgroup II (%)[b]	Number of negative/total	Overall (95% CI)
CLIA LIASION	182/190	98.8% (92.7–98.9)	84.4	99.3	93/95	97.9% (94.5–100)
ELISA Enzygnost	178/190	93.7% (89.9–97.4)	77.7	98.6	92/95	96.8% (92.8–100)

[a]Sensitivity was calculated only using samples collected 0–3 days post rash onset ($n = 45$).
[b]Sensitivity was calculated only using samples collected 4–9 days post rash onset ($n = 145$).

The measles IgM sensitivity value for each technique was determined using the positive panel and the results are shown in Table 1. The difference in sensitivities between LIAISON CLIA and Enzygnost EIA are not statistically significant ($P = 0.5$). False-negative measles cases included five indeterminate and three negative IgM results for LIAISON CLIA, and 11 indeterminate and one negative for Enzygnost EIA (Table 2).

The measles IgM specificity assessments were done using the negative panel and the results are shown in Table 1. CLIA specificity was greater than EIA specificity, but there were no significant differences ($P = 1$). Distribution of false-positive results are shown in Table 2.

DISCUSSION

Chemiluminescent immunoassays have proved to be at least as sensitive and specific as the conventional colorimetric methods in serological diagnosis of many infectious diseases (12, 13). Moreover, CLIA is very simple to perform and cost saving. The purpose of the present study was to assess the performance of the new chemiluminescent automated assay, LIAISON measles IgM. Results were compared to those obtained by the Enzygnost.

Evaluation of measles IgM assays requires well-defined panels of sera. In this study, the positive panel was composed by sera from rash illness cases in the context of two outbreaks in Andalusia, and culture and/or RT-PCR confirmed. In addition, for the specificity assessment, we included a number of single serum samples from subjects with diseases that may give false-positive IgM in measles IgM serology (6).

Our evaluation demonstrated a high level of concordance between LIAISON CLIA and Enzygnost EIA. The LIAISON CLIA tested in our study has good sensitivity and the results were better when compared with the results of Enzygnost EIA (98.8% vs. 93.7%). The results obtained in positive panel confirmed previously described observations related to a lower sensitivity in early stage of measles infection. In fact, of 45 serum samples collected between 0 and 3 days after the onset of rash, seven (16%) for CLIA and ten (22%) for EIA gave a negative or an indeterminate IgM result (Table 2). On the other hand, in samples collected more than 3 days after the appearance of the rash, more than 99% gave IgM-positive results for CLIA and 98.5% gave for EIA (Table 1). In agreement with our results, researchers in other studies found similar rates of false-negative IgM results by Enzygnost in specimens collected during the first 3 days after onset of the rash, and a sensitivity approaching 100% in samples collected 3 days post rash onset (5, 6, 14). The lowest rate of false-negative results in CLIA assay can be attributed

TABLE 2. Distribution of False Negatives and Positives Measles IgM Results

	Number of negative or indeterminate samples in positive panel			Number of positive or indeterminate samples in negative panel						
Assay	Subgroup I[a] ($n = 45$)	Subgroup II[b] ($n = 145$)	Total ($n = 190$)	Healthy persons ($n = 25$)	EBV[c] ($n = 25$)	CMV[d] ($n = 25$)	*Mycoplasma pneumoniae* ($n = 15$)	Parvovirus B19 ($n = 18$)	Rubella ($n = 3$)	Total ($n = 95$)
CLIA LIAISON	7[e]	1[g]	8	0	2[g]	0	0	0	0	2
ELISA Enzygnost	10[f]	2[g]	12	0	0	1[g]	0	2[g]	0	3

[a]Samples collected 0–3 days post rash onset.
[b]Samples collected 4–9 days post rash onset.
[c]Epstein–Barr virus.
[d]*Cytomegalovirus*.
[e]Four indeterminate and three negative results.
[f]Nine indeterminate and one negative results.
[g]Indeterminate results.

to an antibody capture configuration; in fact, it has been stated in publications that capture EIA is more sensitive than indirect EIA, mainly in serum specimens taken too early with respect to symptoms after rash onset (6).

Specificity should prevail in diagnostic methods for surveillance of low-incidence diseases, such as measles, in developed countries to ensure a good positive predictive value (11). The specificity of both LIAISON and EIA was excellent (97.9% and 96.8%, respectively) and similar to the values reported in other studies for Enzygnost EIA (5, 6). Specificity results obtained in our study are particularly relevant considering that we included sera from patients with conditions reported to cause measles IgM false-positive results (6).

False-positive or equivocal results have also been noted with sera from patients with EBV, CMV, human herpesvirus 6, and mycoplasma (6). In addition, the possibility of IgM cross-reactivity between measles, rubella, and other viruses, which causes exanthematic diseases, has previously been identified (6, 15). In our study, we had no false positives in patients with rubella infection, although the number of sera tested was low. The Enzygnost assay was most problematic for parvovirus B19 infection (1 of 18) and CMV (2 of 9) while CLIA assay gave false-positive results in EBV infections (2 of 25; Table 2). In summary, LIAISON IgM measles can be a good alternative to the traditional immunoassays to laboratory measles diagnostic, since it was found to be as specific and sensitive as Enzygnost EIA.

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