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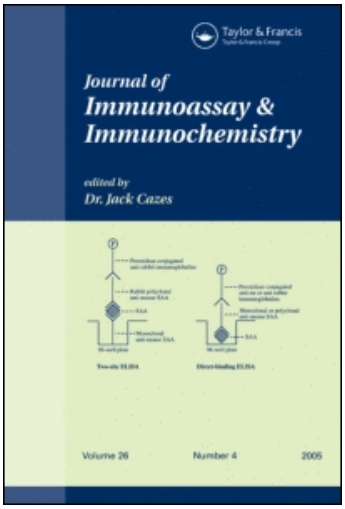

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Comparison of Two Commercial Immunoassays for the Detection of Anti-Rubella IgM and IgG in Pediatric Patients

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Comparison of Two Commercial Immunoassays for the Detection of Anti-Rubella IgM and IgG in Pediatric Patients

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Abstract: The only threatening modality of rubella is the Congenital Rubella Syndrome that affects fetuses of women who acquire infection during early pregnancy. Laboratory diagnosis is based on serological parameters. We compared anti-rubella IgM and IgG detection of two commercial immunoassay kits (Abbott and Roche). Although we observed an agreement of 97.8% for IgM and 95.7% for IgG when the categories positive, negative and indeterminate were considered, mean titers of IgG and the absorbance/cut off of IgM were statistically different for both kits, thus corroborating the idea that serological results depend very much on the methodology and must be carefully interpreted.

Keywords: Serology, Anti-Rubella IgG and IgM, Laboratory kit performance

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INTRODUCTION

Rubella infection, also known as German measles, is caused by a single-stranded RNA togavirus, which is the only member of the genus rubivirus. Infection is normally benign and is spread by a respiratory route. After an incubation period of 13 to 20 days, patients may be completely asymptomatic or present with low fever, a mild rash, and lymphadenopathy. Although primary rubella infection also varies from asymptomatic to mild disease in pregnant women,[1] severe fetal damage can occur leading to the Congenital Rubella Syndrome (CRS) because intrauterine rubella infection is a result of the vertical transmission of infection.[2] Laboratory diagnosis of CRS is confirmed by isolation of the virus in body fluids (nasal secretions, urine or CSF), or detection of anti-rubella virus IgM and IgG antibodies in serum samples.[3] Postnatal persistence of rubella virus-specific IgG corroborates the laboratory diagnosis of CRS and has to be evaluated due to prolonged detection of IgM antibodies after the acute phase of infection.[4,5]

Postnatal CRS antiviral therapy is not routinely recommended due to its ineffectiveness and toxicity; thus, infection has to be prevented by routine vaccination of young children and childbearing age women. Vaccination programs have been set up since 1980 and have reduced CRS in different countries.[6] In Brazil, systematic vaccination of children and young women began in 1994 and has apparently caused a positive impact on the incidence of CRS.[7]

Serologic screening of women can determine the risk of fetal infection and should be performed ideally before pregnancy, or as soon as possible during prenatal care.[8] The presence of maternal antibody before conception or at the time of rubella exposure does protect the fetus as the medical literature has not yet reported CRS cases caused by re-infection or vaccination.[9,10]

There are several serological commercial kits available, from immunoassays,[11] haemmaglutination tests,[12] time resolved immufluorimetric assays (TRFIA),[13] to microparticule immunoassays.[14] Other confirmatory methods, such as IgG avidity have been used to distinguish acute recent from non-acute recent infections in patients presenting with high IgG titers.[15,16] More recently, molecular methods such as RT-PCR have proved to be useful to diagnose CRS,[17,18] due to a higher sensitivity and specificity. However, molecular methods require specialized laboratories and personnel, so that RT-PCR is not yet routinely performed in clinical laboratories, and has been reserved to define diagnosis in more complex cases. Therefore, routine laboratory diagnosis is still made comparing maternal IgG and IgM titers[13,19,20] with fetal or neonatal antibody titers. Therefore, it is of utmost importance to evaluate the reliability of results produced by widely used serological kits.

The present study has aimed at comparing rubella-specific IgG and IgM titers of two commercial immunoassay kits.

EXPERIMENTAL

Patients and Methods

This study was approved by the Ethics Committee of the School of Medicine, University of São Paulo, Brazil. Ninety-two serum samples were collected from patients assisted in several units of the Child's Institute (São Paulo, Brazil), a pediatric tertiary-care hospital, after informed consent of parents. The study enrolled children and adolescents with complex diseases such as Systemic Erythematous Lupus, Juvenile Rheumatoid Arthritis, and HIV carriers. Four milliliters of peripheral blood were taken into serum separator tubes (BD Vacutainer SST 367696). Blood samples were centrifuged for 10 minutes at 1,800 g (Rotofix 32 – Hettich), in order to obtain serum samples which were then stored at 4ºC for 48 hours, until the time of analysis.

All serological tests were performed by means of a microparticle enzyme immunoassay (MEIA), using the Axsym-Abbott equipment and its corresponding kits (Axsym Rubella IgG and Axsym Rubella IgM) for detection of IgG and IgM antibodies anti-rubella-virus. Serum samples were also submitted to analysis by another MEIA IgG and IgM kit (Cobas-Core, Roche). All assays followed their manufacturer's instructions.

According to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1992),[21] IgG titers were classified in three categories:

—negative: if the levels of antibodies are <5 UI/mL;
—indeterminate: if the antibody level is ≥ 5 UI/mL and <10 UI/mL; and
—positive: if the levels of antibodies are ≥ 10 UI/mL.

As the two evaluated commercial IgM MEIA kits have similar, but not identical, principles, we have considered the absorbance/cut-off ratio instead of IgM titers in order to compare the results. As a qualitative test, IgM levels were interpreted according to the manufacturer's criteria as being:

—negative: if the absorbance/cut-off <0.8;
—indeterminate: if the absorbance/cut-off ≥ 0.8 and <1.0; and
—positive: if the absorbance/cut-off >1.0.

Statistical Analysis

The immunological response variable was considered as a qualitative test; the agreement of the two kits was evaluated using the kappa coefficient. For the other response variable considered (IgM absorbance/cut off titers), the correlation between the two commercial kits was verified using the Pearson's

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correlation coefficient (r), the paired-samples t test was used to compare mean values obtained by the two kits and a linear regression through the origin was used to estimate the difference between the two kits. We also considered a 95% confidence interval (IC 95%) for the paired difference mean and for the coefficient between the kits obtained from the linear regression analysis. We considered significant a p-value <0.05.

RESULTS AND DISCUSSION

In the present study, we evaluated the Abbott Axsym and the Cobas-Core analyzer and their corresponding IgG and IgM anti-rubella virus kits using 92 serum samples. For the IgM analysis, two serum samples were disregard: one due to insufficiency of volume and another due to a strongly positive result that was considered discrepant with respect to all other samples.

Table 1 shows IgG results, considering the categories of titers. We observed that 95.7% (88/92) of the samples presented with concordant results between Abbott and Roche kits. The kappa coefficient indicates a significant agreement between these kits (kappa = 0.77).

Regarding the 4 samples (4.3%) in which we observed disagreement of results, one was negative by Abbott and indeterminate by Roche, and 3 were positive by Roche and indeterminate by Abbott (i.e., Abbott = 7.1 UI/mL and Roche = 37.1 UI/mL, with a cut-off of 10 UI/mL for both kits). Although, in these particular cases, clinical interpretation did not change, IgG titers obtained by the Abbott kit were significantly higher than those of Roche's when titers above 200 UI/mL are considered. These discrepancies might lead to misinterpretation of serological results, exactly, in more complex cases, such as pregnant women, fetuses, neonates, and immunocompromised patients.

When we considered the IgG titers, we observed, as shown in Figure 1, that the dispersion plot for the Abbott and Roche IgG had several points far from an imaginary 45° line, indicating that some serum samples results presented with remarkably different titers values for these two kits. The

Table 1. Observed frequency for the IgG anti-rubella virus qualitative test

		Roche		
Abbott	Negative	Indeterminate	Positive	Total
Negative	5	1	0	6
Indeterminate	0	2	3	5
Positive	0	0	81	81
Total	5	3	84	92

Agreement: 95.7%, Kappa: 0.77.

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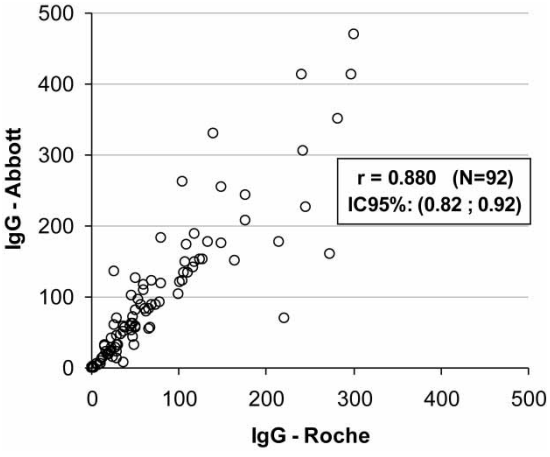

Figure 1. Dispersion plot for the Abbott and Roche IgG anti-rubella antibodies (UI/ml) and the Pearson's correlation coefficient (r).

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Pearson's correlation was r = 88.0%. The means of IgG titers were different for the Abbott and Roche MEIA kits ($p < 0.001$), and the estimated difference was 24.07 Ul/mL (IC 95%: min.13.94; max. 34.22 Ul/mL). Despite the clinical interpretation, these results have shown that the difference between these kits are due to the methodology and/or the manufacturer, and must be considered during results interpretation.

Table 2 shows the results for the IgM qualitative test and we observed that 97.8% (89/91) of samples had agreement results for both Abbott and Roche MEIA kits although the kappa coefficient indicated a moderate agreement (kappa = 0.49).

Considering the absorbance/cut-off ratio for IgM results, Figure 2 shows the dispersion plot for the Abbott and Roche, indicating that there is not a linear trend. The linear correlation was weak (r = 17.5%). The means of IgM absorbance/cut-off ratios were statistically different for Abbott and

Table 2. Observed frequency for the IgM anti-rubella virus qualitative test

		Roche			
		Negative	Indeterminate	Positive	Total
Abbott	Negative	88	1	0	89
	Indeterminate	1	0	0	1
	Positive	0	0	1	1
	Total	89	1	1	91

Agreement: 97.8%, Kappa: 0.49.

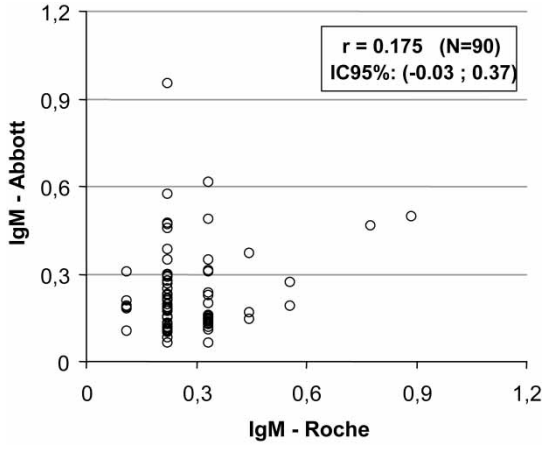

Figure 2. Dispersion plot for the Abbott and Roche IgM absorbance/cut-off and the Pearson's correlation coefficient (r). The sample excluded due to a strongly positive result (not shown in the figure) has IgM value of 2.44 for Roche and 3.54 for Abbott kit.

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Roche kits ($p = 0.004$) and the estimated difference was -0.05 (IC 95%: min. -0.09; max. -0.02) although, in this case, this difference is not clinically meaningful. This fact generally does not lead to misinterpretation of results because the final results are based on the qualitative test, but they must be carefully analyzed when IgM absorbances are near the cut-off. In these cases, IgM results should be analyzed together with IgG titers.

Although serological diagnosis of rubella can be both, sensitive and specific, reliability of serological results relies on the kit methodology.[22] Detection of IgM can be troublesome, especially in countries with low prevalence of disease due to false-positive results; absence of complementary tests to confirm laboratory diagnosis such as the IgG avidity; limited experience of the laboratory personnel to deal with more complex cases; besides inadequate interpretation of results by clinicians.[23] Another interesting fact is that rubella virus evolves to chronic infection very slowly in fetuses and neonates, and is eliminated in urine, stools, and secretions for prolonged periods after birth.[23] Therefore, accurate laboratory diagnosis of rubella is mandatory.

The presence of IgG antibodies indicates naturally or artificially acquired immunity (vaccinated individuals). After disappearance of exanthema, IgG titers increase quickly, reaching maximum levels in 10 to 20 days, being detectable for many years. Conversely, IgM antibodies appear during the acute phase of infection and normally disappear within 8 to 12 weeks, but might, in some cases, be detected for more than one year, depending on the detection method. It is also important to bear in mind that IgM production may eventually be induced by vaccination or, more rarely, after reactivation or re-infection with another rubella virus strain, even if the patient remains

Table 3. Descriptive measures for the IgG titles anti-rubella virus antibodies by MEIA kits (N = 92 serum samples)

	Descriptive measures				
IgG (UI/mL)	Mean	Standard deviation	Median	Minimum value	Maximum value
Abbott	101.76	99.11	71.05	0.00	470.50
Roche	77.69	73.89	50.25	0.71	300.00

Paired t test: $p < 0.001$.

asymptomatic.[24] Besides, false-positive results still occur, albeit the use of recombinant antigens.[25]

In pregnant women, the presence of IgM antibodies needs to be interpreted with caution, especially in cases in which there is antecedent of recent vaccination, or in asymptomatic patients living in countries with low prevalence of disease.[4]

All evaluated samples in our study had concordant results with respect to the clinical symptoms, but technical performance of the two immunoassays was slightly different. IgG titer had a higher mean with Abbott's than with Roche's kit (Table 3). These results might lead to misinterpretation, e.g., in pregnant women with high IgG titers during prenatal care due to a higher sensitivity of the serological method used and not to the presence of true infection. Concerning the IgG titers, Abbott tests were performed with a purified antigen, while Roche's were run with a recombinant antigen. Moreover, Roche's kit includes monoclonal antibodies while Abbott's uses microparticles that increase the reaction speed. Consequently, Abbott's kit can be more sensitive but less specific than Roche's.

Considering IgM detection, the majority of samples were negative. Nevertheless, the means of the absorbance/cut-off ratio showed significant statistical difference, being lower with Abbott than with Roche (Table 4). Differences

Table 4. Descriptive measures for the IgM absorbance/cut-off by MEIA kits (N = 90 serum samples)

	Descriptive measures				
IgM (A/C)	Mean	Standard deviation	Median	Minimum value	Maximum value
Abbott	0.22	0.14	0.18	0.06	0.95
Roche	0.28	0.12	0.22	0.11	0.89

Paired t test: $p = 0.004$.

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Table 5. Estimated coefficient Abbott/Roche with respect to IgG and IgM antibodies

Model Abbott = coefficient[a] Roche	Estimation coefficient	IC 95%	
IgG (n = 92)	1.25	1.16	1.34
IgM (n = 90)	0.71	0.61	0.82

[a]Linear regression through the origin.

between the two commercial immunoassays may have been caused by reagents and procedures of the kits. Different studies have shown slight differences among results of commercial or in-house serological kits, depending on the methodology.[13,14,22] Corroborating these studies, we observed by the linear regression that the difference between the kits depends on the methodology, as the coefficient Abbott/Roche was different according to the antibodies determination, being 1.25 for IgG and 0.71 for IgM (IC 95%: min. 1, 16, max. 1, 34 for IgG; and min. 0, 61, max. 0, 82 for IgM) (Table 5).

Likely, Axsym-Abbott IgM detection system includes a purified antigen, while Roche's kit uses monoclonal antibodies and the capture methodology. Hudson and Morgan-Capner,[26] have found superiority of the IgM capture assays. Yet, there are differences that can be attributed to biological samples' characteristics which may also interfere in the pre-analytical phase, such as sample collection and the type of collection tube.[27–29]

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CONCLUSION

The present study has corroborated data on the variability of serological results depending on the methodology. Although discrepancies of results might not have interfered with medical decisions, special attention must be paid when monitoring antibody titers in at-risk patients, such as pregnant women, fetuses, neonates, and immunocompromised patients. Especially, in these cases, it is important that a single and reliable standardized method is used during the entire follow-up in order to avoid variations caused by different methodologies.

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