

COMPARISON OF A COMMERCIAL ENZYME IMMUNOASSAY WITH PLAQUE REDUCTION NEUTRALIZATION FOR MATERNAL AND INFANT MEASLES ANTIBODY MEASUREMENT

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SUMMARY

The most practicable assay for measurement of measles IgG (mIgG) in large numbers of sera is an enzyme immunoassay (EIA). To assess how EIA results would agree with those by the gold standard method of plaque reduction neutralization (PRN) we compared the results from the two methods in 43 pairs of maternal and umbilical cord sera, and sera from the corresponding infants when aged 11 - 14 months. In maternal-cord sera, the differences between mean antibody levels by EIA or PRN were not statistically significant, though in individual sera, differences could be large. However, agreement was less good for infants sera, in which levels of mIgG were very low. The conclusions of a study of transplacental transport of mIgG would not be affected by the use of either technique. When studying waning immunity in infants, PRN should be the method of choice, while results from studies using EIA should be interpreted with caution.

KEYWORDS: Measles; IgG; Enzyme immunoassay; Plaque neutralization.

INTRODUCTION

Measles-specific IgG (mIgG) may be measured by a variety of methods that differ in their sensitivity and specificity¹³. The plaque reduction neutralization (PRN) assay is considered to be the "gold standard" because of its high sensitivity and specificity²⁷. PRN assays can detect very low levels of antibody, and evidence showed that antibody titers less than 200 mIU/ml are not fully protective against clinical disease⁹. This assay is, however, too demanding of time and resources to use to screen large numbers of sera.

Commercial enzyme immunoassay (EIA) tests are widely available, and their relative ease of execution make them attractive for field studies^{10, 30}. EIA is generally more sensitive than HI and complement fixation (CF) tests²⁷. Many studies have shown that seropositivity in EIA assays correlates with protection from clinical measles^{20, 26, 31}, but that seronegatives are not necessarily susceptible, as only a small proportion of seronegative individuals acquired measles disease in these studies. The specificity of EIA assays also varies between laboratories and some assays have correlated poorly with the results of neutralization tests, particularly for the measurement of low levels of maternally derived measles antibody in 4-6 month old infants^{28, 29}.

Though the PRN assay is the "gold standard", EIA assays

are quicker and cheaper to perform, and require only very small volumes of sera which is an advantage in studies of young infants¹². Unfortunately, commercially available kits of EIA differ in terms of their sensitivity and specificity^{21, 27}, which poses an additional problem. The use of reference sera, calibrated against the International Standard for anti-measles serum (IS)¹⁷, in parallel with the sera under study, has been recommended by the Expanded Programme on Immunization (EPI) as a way of reducing variation in results between different tests and different laboratories¹². Nevertheless, even with the use of the IS, problems of comparability between results from different methods still arise⁴. Very few studies using EIA have used the IS or reported the results in milli-international units per millilitre (mIU/ml)^{19, 21, 22}.

We planned a study on transplacental transport efficiency of measles-specific IgG in over one thousand pairs of samples of maternal and umbilical cord sera. To assess the consequences of using an EIA assay, we compared the results obtained by EIA with those from PRN on a subsample of sera. To assess how the differences between the assays might affect the conclusions from different types of epidemiological study, we also used EIA and PRN to measure mIgG in the sera of infants when they reached 11-14 months of age (the age for routine measles vaccination in many industrialised countries).

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METHODS

After obtaining informed consent from women, venous blood samples were collected from the mother and the umbilical cord. Follow-up samples were obtained from 43 infants when they reached 11-14 months of age. For these 43 infants, sera from maternal and cord blood, and the corresponding sera from infants were tested by both EIA and PRN.

Levels of mIgG were measured using a commercial enzyme immunoassay (14458 Measles Virus IgG EIA, Diagnostica, Merck) at the Virology Department of the National Institute of Health (INSA), in Lisbon. A Portuguese standard serum (AH) was calibrated against the International Standard for anti-measles serum^{17,19}, and then used in each EIA, thus making it possible to report the results in mIU/ml¹⁹. In the calibration study, the AH standard serum was used in the dilutions 1/50 to 1/12800, in each EIA plate. The optical density (OD) response values were plotted to draw a curve relating the logarithm of the dilution (or the concentration in mIU/ml) with OD. A Basic computer programme (written by Prof Maia, University of Oporto) tested the fitting of a logistic curve to the observed OD values by dilution (or the concentration) fitted as natural logs. The fitting was always excellent with R² values close to 0.999 for all forty plates that were used (minimum of 0.99857). The programme also derived the parameters of the logistic curve with the best fit, which were used in an Epi-Info programme¹⁶ which converted values of OD into mIU/ml for all study sera tested.

The same sera were also tested by plaque reduction neutralization (PRN), following published procedures^{1,17,24} at the National Institute for Biological Standards and Control (NIBSC), U.K.. Results were expressed in mIU/ml.

Scatter plots were produced of the values obtained by the two methods. The differences between the two methods were then plotted against their mean^{7,18}. Because the differences in mIU/ml between the two methods increased as the concentration increased, the following further analysis was conducted on log-transformed concentrations.

The mean difference between the two methods, termed the "bias" of the EIA technique relative to PRN, was computed. The respective 95% "limits of agreement" include the difference between single measurements on the same serum by the two methods with 95% probability^{3,6,7}. The antilog of the bias represents the "proportional bias" between the two methods and the antilogs of the 95% limits of agreement gave us the range of "proportional agreement" (for individual measurements) between EIA and PRN^{3,5,6}. The standard error and 95% confidence interval of the bias were also computed and a paired t-test was performed to examine the hypothesis of zero bias (or proportional bias equal to 1).

To assess the effect of using EIA rather than PRN assays in the study of transplacental concentration of measles antibody, a regression model of cord level on maternal level, with measles IgG measurement method as a dummy variable, was fitted to the data in order to test if, in the EIA and PRN groups, the slopes were parallel, the intercepts were equal and finally if the models were coincident²³.

One-way analysis of variance was used to compare transplacental concentration ratios computed with the data from EIA and PRN methods.

Maternal antibody levels of 40-99 mIU/ml may delay the antibody response to measles vaccination, while levels of 100 mIU or more greatly reduce the response to standard titre vaccines²⁵. Thus, for infants sera, we assessed the sensitivity and specificity of EIA versus PRN using either 40 or 100 mIU/ml as the cut-off for seropositivity.

RESULTS

Measles antibody levels

The geometric mean concentrations of measles antibody levels were very similar by both assays (Table 1). Levels were higher in umbilical cord than maternal sera, as expected⁴.

TABLE 1

Measles IgG (mIgG) geometric mean concentration (GMC) in maternal and corresponding umbilical cord sera (n=43) measured by enzyme immunoassay (EIA) and plaque reduction neutralization (PRN)

Type of sera	Technique used to measure mIgG	
	EIA	PRN
Maternal sera		
Log (sd) *	7.04 (0.98)	7.05 (1.28)
mIU/ml †	1145	1153
Umbilical cord sera		
Log (sd) *	7.34 (1.15)	7.40 (1.31)
mIU/ml †	1539	1644

* GMC and standard deviation (sd) expressed as natural Logs

† GMC expressed in milli international units per millilitre, resulting from back transformation of Logs

Scatter plots of EIA by PRN values

Fig. 1 shows the skewness of the antibody levels and improvement after log transformation. Log transformed levels of measles IgG by EIA or PRN spread around the equality line (Fig. 1) in maternal and umbilical cord sera, suggesting a reasonable agreement between the two methods.

Scatter plots of the differences between the two methods (EIA-PRN) against the average of the two measurements

When results for individual sera were plotted without transformation, Fig. 2a shows that the disagreement between the two methods in either direction could be as much as 10000 mIU/ml (or even more for the outliers). Log transformed values were plotted

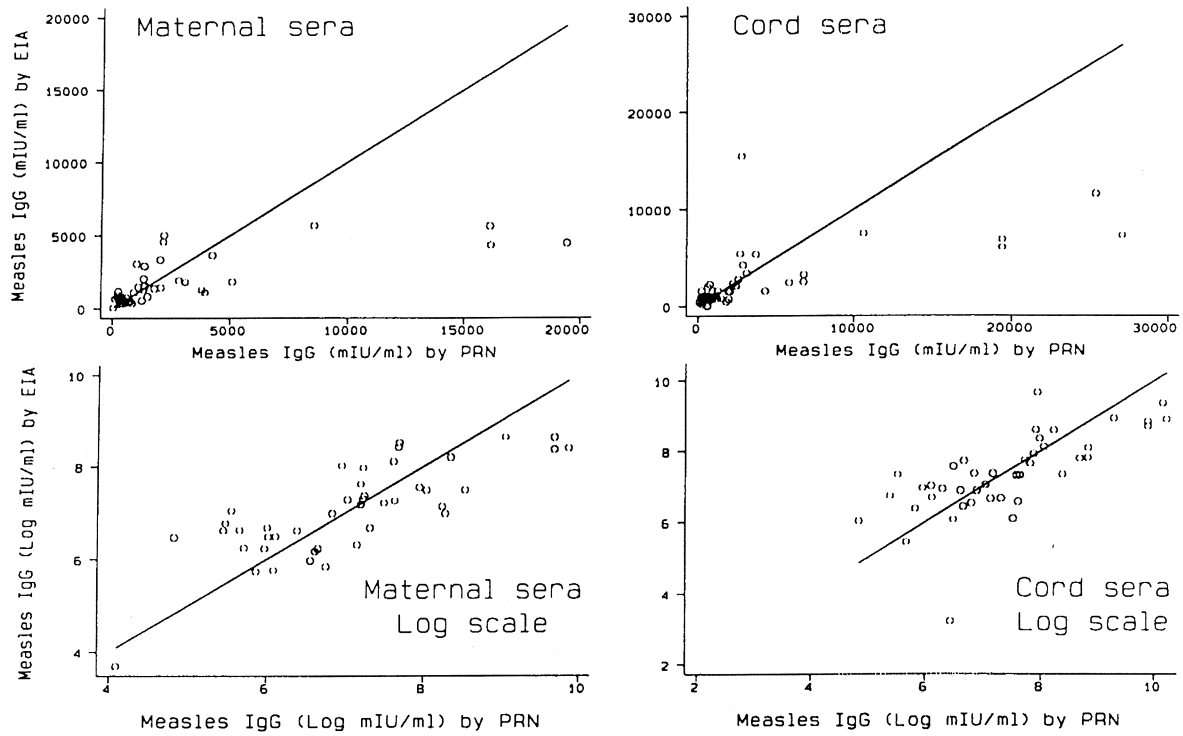


Fig. 1 - Comparison of EIA and PRN methods of measurement of measles IgG levels in maternal and umbilical cord sera (n=43), showing the equality lines.

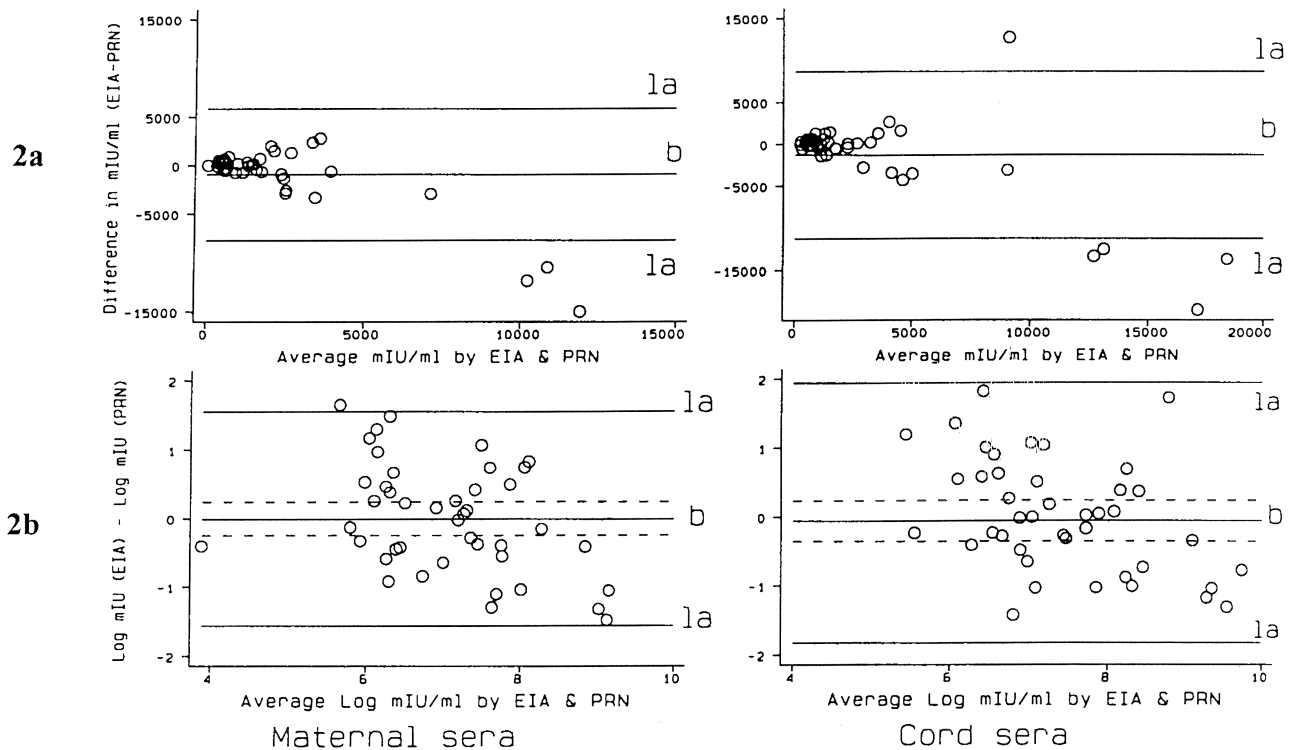


Fig. 2 - Differences between EIA and PRN measurements of measles IgG levels in maternal and umbilical cord sera. Arithmetic (Fig. 2a) and log (Fig. 2b) scales (n=43). The 95% CI limits of agreement (1a) for individual differences, between EIA and PRN measurements of measles IgG, are shown. The bias (b) and the 95% CI limits (dashed lines) of the bias are also represented.

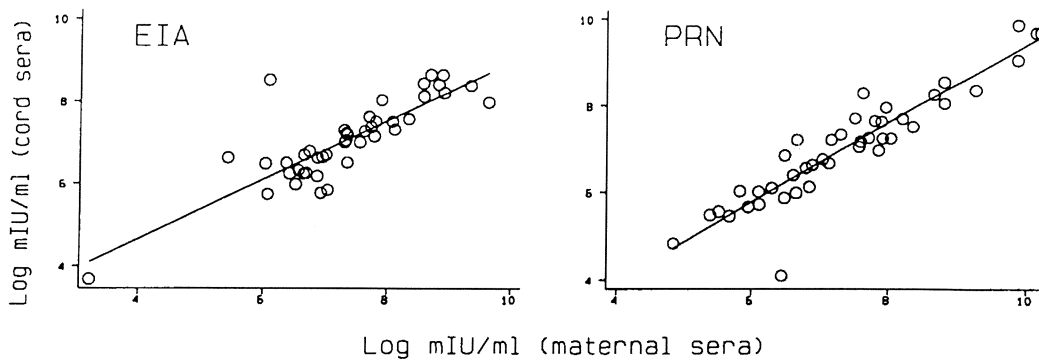


Fig. 3 - Regression lines of umbilical cord on maternal levels of measles IgG using EIA and PRN methods (n=43).
Slope for EIA: 0.9766, correlation coefficient (r): 0.83
Slope for PRN: 0.9477, correlation coefficient (r): 0.93

in Fig. 2b, which shows the 95% limits of agreement, and the limits of the 95% CIs (dotted lines) of the bias, for both maternal and umbilical cord sera.

In the case of **maternal sera**, EIA produced individual estimates of measles IgG levels (mIU/ml) between 0.21 to 4.70 times the results from PRN (taking the antilog of the limits of agreement shown in Fig. 2). For umbilical **cord sera**, the corresponding range of proportional agreement between the two methods, was 0.14-6.16. For **maternal sera**, mean EIA measurements had a proportional bias of 0.99 (95% CI: 0.78 to 1.27) (Fig. 2b) times the results of mean PRN measurements; for umbilical **cord sera**, the proportional bias was 0.94 (95% CI: 0.70 to 1.26) (Fig. 2b); in both instances the proportional bias was not significantly different from unity. In other words, though the differences of individual measurements may have a wide variation in both directions, the mean of the differences (bias) between the two methods is very close to 0 and the 95% CIs very

narrow (Fig. 2), showing that differences between measurements performed by the two methods are very likely due to chance.

Analysis of transplacental transport of measles IgG

As Fig. 3 shows, the method of antibody measurement did not influence the model relating umbilical cord with maternal IgG levels. The slopes of the two models were parallel and intercepts were not different. There was no significant difference in transplacental concentration ratios computed from EIA values (1.57; 95% CI: 1.30, 1.84) or from PRN values (1.67; 95% CI: 1.21, 2.12).

Sera from infants

In all sera of infants with undetectable measles IgG by PRN, EIA detected antibody, with concentrations ranging from 5 to 44 mIU/ml. However, if we consider as "positive" those sera with ≥ 40

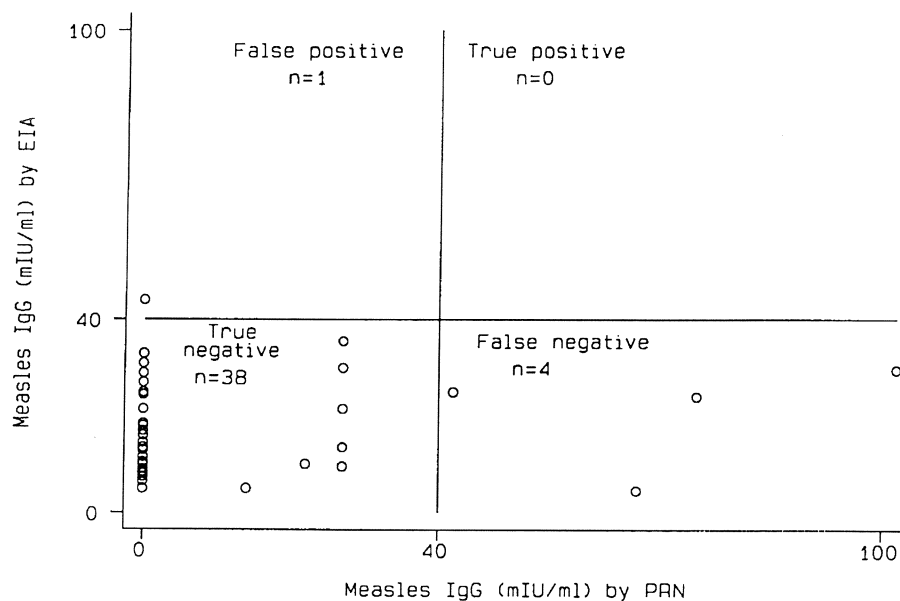


Fig. 4 - Comparison of measles IgG values by EIA or PRN, using the cut-off value of 40 mIU/ml. Sera from 43 infants.

mIU/ml and "negative" those with < 40 mIU/ml (Fig. 4)^{14, 25} the specificity of EIA compared to PRN was 97% (38 of 39 sera correctly identified as negative). All 4 samples with PRN values \geq 40 mIU/ml, were negative by EIA. If the cut-off value used is 100 mIU/ml, then the two methods only disagreed in one of the measurements, in which one serum had over 100 mIU/ml by PRN only.

DISCUSSION AND CONCLUSIONS

For **maternal and umbilical cord sera**, the two methods had a good proportional agreement. Though the geometric mean levels by EIA were slightly lower than those by PRN, the differences were not statistically significant. Nevertheless, for some individual sera, especially those with very high levels of mIgG, the differences in absolute value of mIU/ml might be large. If EIA were to be used, for example, to assess the need for (re)vaccination of an individual, this difference could have clinical importance. However, for the more common use of assessing immunity to measles at the population level, EIA would give valid results. In addition, for our original purpose, the use of EIA did not bias the analysis of transplacental transport of measles IgG (Fig. 3). In populations where a high proportion of individuals had vaccine-induced immunity, with lower antibody levels than those following natural infection, the correlation between EIA and PRN might, however, differ from that in our study.

For studies of passive immunity in infants, our results support previous findings that EIA does not correlate well with PRN^{15, 28}, although the numbers in our study were very small. Using a cut-off of 40 mIU, 4 of 43 sera gave false negative results by EIA. Hence, basing a decision on the optimum age for vaccination on results from EIA could lead to vaccination too early. Similarly, data from Kinshasa, Zaire, showed that in one study of 6 month old infants, only 2% were seropositive by a relatively insensitive commercial EIA assay¹², whereas in a study in a similar population in Kinshasa, around 60% of 6 month old infants had antibody levels over 50mIU by PRN (F.Cutts unpublished data 1994). Thus, PRN remains the method of choice in studies of maternal measles antibody. For large-scale studies of measles immunity in adults, the more practical EIA assay will usually suffice.

RESUMO

Comparação de uma técnica imunoenzimática comercial com a redução de neutralização em placa, usadas no doseamento de IgG anti-sarampo em soros maternos e dos respectivos filhos

A técnica de doseamento imunoenzimático (ELISA) de IgG anti-sarampo (IgG-AS) é a mais prática e conveniente para estudos com muitos soros. A técnica de referência é a redução de neutralização em placa (RNP). Para avaliar a concordância dos resultados obtidos por ELISA e pela técnica padrão RNP, comparámos os resultados de doseamentos efectuados pelas duas técnicas em 43 pares de soros de puérperas e respectivos recém-nascidos (cordão umbilical), e ainda nos soros dessas crianças em amostras colhidas entre os 11 e os 14 meses de idade. Nos soros maternos e do cordão umbilical, as

diferenças médias das concentrações de IgG-AS medidas por ELISA ou RNP não eram estatisticamente significativas. No entanto, a nível individual as diferenças eram por vezes grandes. A concordância não era tão boa no caso dos soros infantis, nos quais os níveis de anticorpos residuais eram muito baixos. As conclusões de um estudo sobre transporte placentar de IgG-AS não seriam afectadas pelo uso de uma ou outra técnica. No entanto, nos estudos de imunidade residual no 1º ano de vida, a RNP deveria ser a técnica escolhida, enquanto os resultados de estudos usando ELISA deveriam ser interpretados com cautela.

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