

ENZYME LINKED IMMUNOSORBENT ASSAY FOR RUBELLA ANTIBODIES: A SIMPLE METHOD OF ANTIGEN PRODUCTION. A PRELIMINARY REPORT

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SUMMARY

A simple method of rubella antigen production by treatment with sodium desoxycholate for use in enzyme immunoassay (IMT-ELISA) is presented. When this assay was compared with a commercial test (Enzygnost-Rubella, Behring), in the study of 108 sera and 118 filter paper blood samples, 96.9% (219/226) overall agreement and correlation coefficient of 0.90 between absorbances were observed. Seven samples showed discordant results, negative by the commercial kit and positive by our test. Four of those 7 samples were available, being 3 positive by HI.

KEYWORDS: Rubella; ELISA; Antigen.

INTRODUCTION

Rubella virus infection usually presents as a mild self-limited disease but, when acquired during early pregnancy, fetal infection with development of many different congenital abnormalities is very frequent.

The evaluation of rubella immune status by the detection of rubella IgG antibodies in women in child-bearing age is an important measure for the prevention of congenital rubella. The prevalence of rubella virus in the community can be monitored by serological surveys as an index of the effectiveness of immunization strategies¹. There are currently several commercial tests available for these purposes^{3,8}. However the enzyme linked immunosorbent assay (ELISA) has been the most widely applied. Although ELISA is highly sensitive and specific⁴ and easy to perform, the large-scale application of the commercial tests in developing countries has been hampered by their high cost⁶. On the other hand, the in-house ELISA has a limited use,

because some studies have emphasized the need of laborious virus purification techniques by gradient density and ultra centrifugation for production of a reliable rubella antigen^{2,5}.

The purpose of this study is to evaluate a simple method of antigen production to be applied in ELISA for detection of rubella IgG antibodies, and to compare it with a commercial kit (Enzygnost-Rubella).

MATERIALS AND METHODS

SAMPLES: Sera from 108 patients submitted to rubella serology for diagnosis or prenatal care at Instituto Adolpho Lutz and Hospital do Servidor Público do Estado de São Paulo and 118 filter paper blood samples from children 2 to 14 years old collected for a measles serological survey⁷ were used for the standardization of the test.

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IMT-ELISA STANDARDIZATION

ANTIGEN: The HPV77 strain of rubella virus maintained at -70°C was first reactivated by three passages in Vero cells. The inoculum for antigen production was obtained after inoculation of Vero cells cultivated in 75 cm^2 flasks with 3 ml of the second passage of the virus and Minimum Essential Medium (MEM) with 2% fetal calf serum as maintenance medium. After 7 days incubation at 36°C , 5 ml of the whole culture suspension was inoculated in Vero cells grown in 225 cm^2 plastic flasks and incubated for 60 minutes at 36°C . After addition of 70 ml of MEM without serum, the infected cells were incubated for additional 10 days at 36°C . Then, the cells were scrapped from the bottles with glass beads and washed three times with phosphate buffered saline (PBS) pH 7.2. The resulting pellet was resuspended in a volume equivalent to 1:100 of original medium. After sonication, the same volume of PBS containing 0.2% sodium desoxycholate (Difco) was added and the mixture incubated for 1 hour at 4°C . The suspension was centrifuged for 1 minute at 6500g in a microcentrifuge and the supernatant stored at -70°C . Uninfected Vero cells were similarly prepared as a control antigen.

Optimal dilution of viral antigen and control for ELISA plate sensitization were determined by block titration, using negative and positive sera.

ELISA: Microtiter plate wells (NUNC - Polysorp) were sensitized by overnight incubation at 4°C with 50 μl of a 1:50 dilution of viral antigen or its control in PBS. After three washes with PBS containing 0.1% Tween 80 (PBST), the plates were incubated for 1 hour at 37°C with blocking solution (5% skim milk in PBST). Serum diluted at 1:100 was then added (50 μl) to each well and incubated for 1 hour at 37°C . After three washes, 50 μl of peroxidase anti-human IgG conjugate (Sigma) was added and incubated for 40 minutes at 37°C . Chromogenic substrate (o-phenylenediamine and hydrogen peroxide in citrate buffer, pH 5.0) was then added and the enzymatic reaction stopped with 2.5N H_2SO_4 after incubating at room temperature for 20 minutes in the dark. The test was considered as positive when the absorbance of the antigen well, read at 492 nm in Titertek Multiskan Plus (Labsystems), minus the control well absorbance (Differential optical density - DOD) was higher than 0.2.

The commercial ELISA was performed according to the manufacturer's instructions. Doubtful results were considered negative for comparison purposes.

RESULTS

The overall agreement between the IMT-ELISA using antigen obtained by the simplified extraction method and the commercial ELISA in the study of 108 sera and 118 filter paper blood samples was 96.9%

(219/226) with a correlation coefficient of 0.90 between respective DOD values. All samples positive by Enzygnost-rubella were also positive by IMT-ELISA (100% relative sensitivity) and 109 of 116 samples were negative (93.9% relative specificity). The positive predictive value was 94.0% and the negative predictive value 100%. Four sera with discordant results (positive by IMT test and negative by the commercial kit) were also tested by hemagglutination-inhibition test, and three of them were positive. The three discordant filter paper samples were not available for HI testing.

TABLE I

Comparison between IMT-ELISA and commercial ELISA for Rubella IgG antibodies in sera and filter paper samples

	Enzygnost	IMT-ELISA		
		Positive	Negative	Total
Sera	Positive	49	0	49
	Negative	4	55	59
	Total	53	55	108
Filter Paper	Positive	61	0	61
	Negative	3	54	57
	Total	64	54	118

DISCUSSION

In this study, the antigen prepared through extraction of crude infected cells by treatment with sodium desoxycholate and sonication showed results comparable to the commercial assay (Enzygnost-rubella). The latter assay was reported⁴ previously as presenting 99.26% sensitivity and 100% specificity when compared with hemagglutination-inhibition test.

Among 116 negative samples by the commercial ELISA, 7(6.0%) were positive by our test. These results seem to point out a higher reactivity of this new antigen, since HI test performed in 4 of those 7 discordant sera was positive in 3. The preliminary results observed in this study indicate that the IMT-ELISA may be a feasible alternative to the expensive commercial kits, since it is simple to perform, does not require special apparatus for virus purification and can be done by any virus laboratory. An extended study using a greater number of samples, obtained from patients with rubella and other diseases is advisable.

RESUMO

Reação imunoenzimática (ELISA) para detecção de anticorpos contra o vírus da Rubéola: um método simples de produção de antígeno. Nota prévia.

Um método simples de produção de antígeno de vírus da rubéola, por extração com desoxicolato de

sódio para aplicação no ensaio imunoenzimático, IMT-ELISA, é apresentado. Este ensaio comparado com ELISA comercial (Enzygnost-Rubella, Behring), no estudo de 108 soros e 118 amostras de papel de filtro apresentou 96,9% (219/226) de concordância e um coeficiente de correlação de 0,90 entre as absorvâncias. Sete amostras apresentaram resultados discordantes, negativos pelo ensaio comercial e positivos pelo IMT-ELISA. Destas, 4 foram testadas por RIH, observando-se positividade em 3.

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