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ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR MEASLES ANTIBODY. A COMPARISON WITH HAEMAGGLUTINATION INHIBITION, IMMUNOFLUORESCENCE AND PLAQUE NEUTRALIZATION TESTS

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SUMMARY

An enzyme-linked immunosorbent assay (ELISA) for measles antibodies was compared with Plaque Neutralization (PRN), Haemagglutination inhibition (HI) and Fluorescent antibody (IFA) tests in 181 sera from vaccinated children and umbilical cord. Of 179 positive samples by the sensitive PRN, only two, with titers of 8, were negative by ELISA (copositivity of 98.9%). IFA and HI presented, respectively, copositivities of 93.3% and 82.7%.

The ELISA presented a high sensitivity as well as a good reproducibility and represents an alternative for the time consuming PRN for detection of low measles antibodies.

KEY WORDS: Measles; ELISA; Plaque neutralization; Immunofluorescence; Hemagglutination inhibition.

INTRODUCTION

Measles serology has been routinely performed by haemagglutination-inhibition test (HI), considered as an adequate assay to determine immune status[6]. The main restriction to this test is the need of monkey red blood cells, not available for many laboratories. The indirect immunofluorescence assay (IFA) has been shown to be a convenient alternative to the HI, since it is slightly more sensitive, easier to perform and presents a good reproducibility[10, 11]. However, the IFA, as the HI, does not substitute the Plaque neutralization test (PRN) when low measles antibody titers are sought[11]. PRN is considered a highly sensitive assay for measles antibody[1] but it is time consuming and expensive. The Enzyme-linked immunosorbent assay (ELISA), first described for measles antibody by VOLLER et al[12] has been shown to be a sensitive and reliable assay and could represent an alternative to the PRN[2, 4, 5, 13].

In the present study we compared ELISA for detection of measles antibody, with PRN, HI and IFA.

MATERIAL AND METHODS

Sera: 181 serum samples obtained from vac-

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cinated children and umbilical cord, at Hospital do Servidor Público Estadual "Francisco Morato de Oliveira", were used for comparison between the tests. Thirty two pre-vaccination sera seronegative by IFA, obtained from children 10 months to 5 years old, without history of measles and who seroconverted after measles vaccination were tested at dilution 1:20 in order to determine the seronegative range of ELISA.

ELISA: For antigen preparation, the Toyoshima strain of measles virus was grown on Vero cells in Minimum Essential Medium (MEM) supplemented with 2% fetal calf serum, 100 U/ml Penicillin, 100 μg/ml Streptomycin and 2 μg/ml Amphotericin B. After 4 to 5 days of incubation at 37°C, when extensive CPE was observed, the cells were scraped into the fluid with glass beads and then washed three times with phosphate buffered saline (PBS) 0.01 M, pH 7.2, by 10 minutes centrifugation at 800 g. The sediment was resuspended in PBS in a volume equivalent to 1:50 of the original medium. Antigen was extracted by adding equal volume of 0.2% sodium desoxycholate (Difco) in PBS, sonicating for 3 minutes in ice bath and then clarified by 20 minutes centrifugation at 1800 g at 4°C. The supernatant was stored at -70°C. Control antigen was similarly prepared with uninfected Vero cells. The optimal dilution of viral and control antigens for sensitizing ELISA microplates was found by checkerboard titration after adjusting protein concentrations determined by the method of LOWRY[7]. The optimal concentration of viral and control antigens was approximately 10 μg/ml.

The microtiter plates (Hemobag - São Paulo, Brazil) were coated with 50 μl of viral or control antigens diluted in PBS, incubated overnight at room temperature, washed three times with PBS containing 0.1% Tween 80 (PBST), air dried and used in the same day or stored at -70°C in sealed plastic bags. In order to block free protein adsorption sites, 100 μl of 1% Bovine serum albumin (Sigma, St. Louis, Mo) in PBST (BSA) were added in each well and incubated at 37°C covered with moistened filter paper. From 1:20 starting dilution, serial fourfold dilutions in BSA were prepared in a volume of 50 μl, in plates coated with viral and control antigens. The plates were incubated 60 minutes at 37°C and washed four times with PBST. Then, 50μl of optimum dilution of peroxidase labelled anti-human IgG (Sigma) in BSA were added. After 40 minutes incubation at 37°C and four washes, 50 μl of substrate consisting of hydrogen peroxide and o-phenylenediamine (Sigma) (0.4 mg/ml) in 0.1M Citrate buffer, pH 5.0, were added. The enzymatic reaction was stopped with 50μl of 2.5N sulfuric acid after 20 min incubation in the dark at room temperature. Absorbance at 492 nm was measured with Titertek Multiskan Plus MK (Flow Lab.) and the results, expressed as DOD, were calculated as the difference between the mean OD of two antigen coated wells minus the mean OD of two wells coated with control. In all set of tests, four positive controls were titered and four negative controls were tested.

Indirect immunofluorescence assay: Edmonston B measles virus infected Vero cells and uninfected controls were washed twice and resuspended in PBS and acetone fixed onto slides at concentration of about 50 cells per 400x high power field. Thereafter, doubling dilutions of serum samples starting from 1:5 were added to the slides and incubated for 40 minutes at 37°C in a wet chamber. Then, the slides were washed three times with PBS and incubated with fluorescein labelled anti human IgG (bioMérieux) for 40 minutes at 37°C, washed, mounted with glycerol and read with Nikon FT fluorescence microscope.

Haemagglutination inhibition test: The standard HI test[3] was used with Edmonston B strain measles virus antigen obtained from sonicated infected Vero cells and 0.5% Rhesus monkey red blood cells.

Plaque reduction neutralization test: Was performed by Dr. Paul Albrecht, Division of Virology, FDA, Bethesda, MD, as previously described[1].

RESULTS

Determination of seronegative limit for ELISA: The mean DOD of 32 sera obtained from children before vaccination was 0.035 with a standard deviation of 0.025. Sera with DOD values that were three standard deviations above the mean, i.e., those above 0.12 were considered

positive. In order to assure absence of maternal antibodies, it was used only prevaccination sera from children who seroconverted after measles vaccination.

Correlation between DOD at one dilution and serum titers: Titers of 44 sera tested in the same day were compared with DOD at dilution 1:20 (Fig. 1). Correlation coefficient between titers and DOD at dilution 1:20 was 0.922. The high degree of correlation between DOD and titer allows the determination of presumptive serum titers testing only one dilution.

Reproducibility inter tests: Four positive control sera titered in six sets of tests presented no significant differences. Only difference at one dilution was sometimes observed. Four negative control sera showed DOD below the positive range.

Comparison of ELISA with PRN, IFA and HI: Of 181 sera tested, 179 (98.9%) were positive by PRN, with Geometric Mean Titer (GMT) of 916. Positivity by ELISA was 97.8% (177/181), with a GMT of 1548 and by IFA was 92.2%

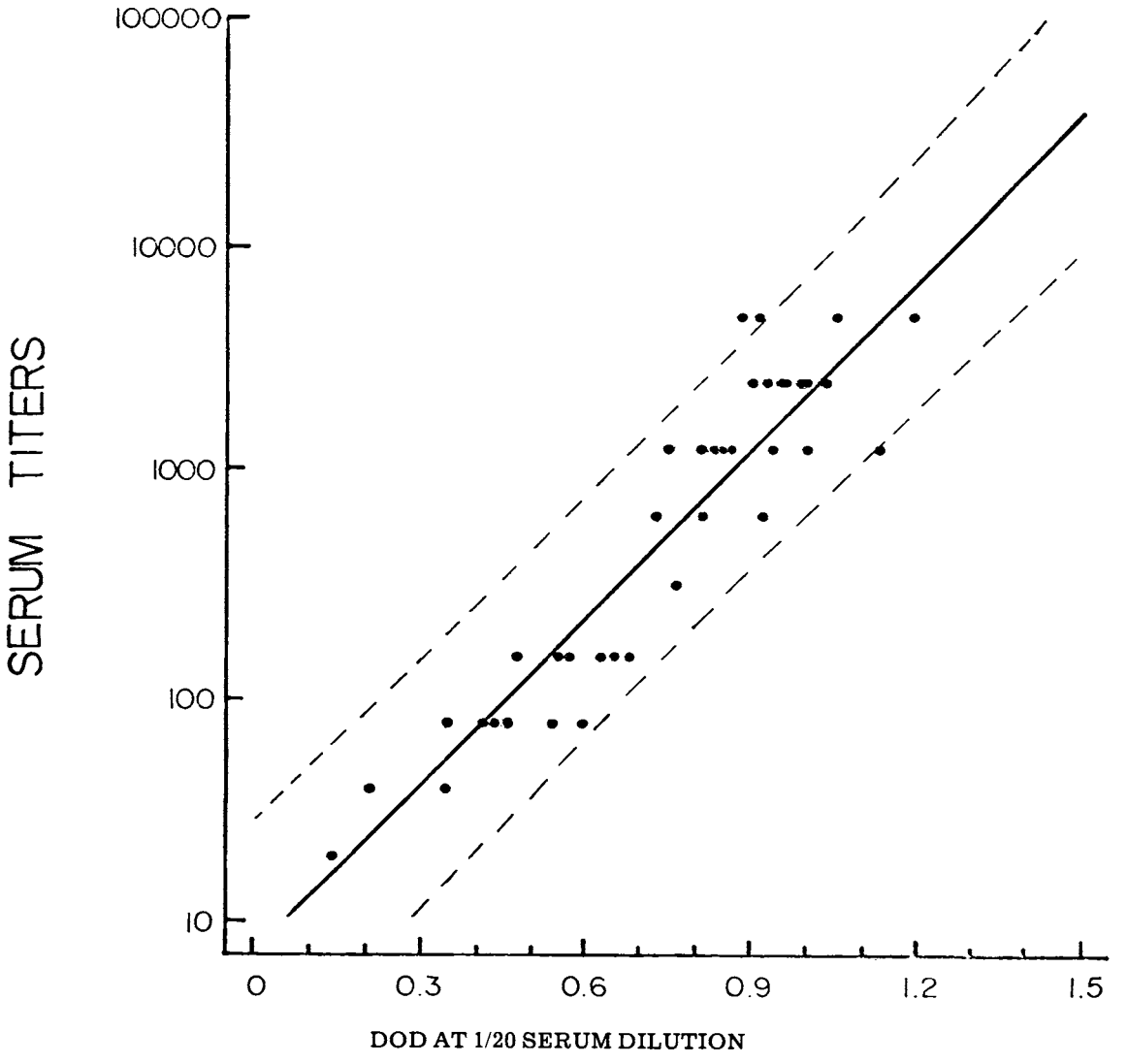

Fig. 1 — Correlation between ELISA titers and DOD at 1/20 serum dilution. The solid line represents the regression line. Broken lines represents two standard errors of the estimate.

(167/181), with a GMT of 31. The HI showed a positivity of 81.8% (148/181) with GMT of 9 (Table 1). When compared with PRN titers, ELISA showed 0.81 of correlation. With IFA and HI titers ELISA presented respectively 0.80 and 0.71 of correlation. Considering PRN as the reference test due to its high sensitivity and specificity[1], copositivity of ELISA, IFA and HI were respectively 98.9, 93.3 and 82.7%. The conegativity was not calculated due to the small number of negative samples by PRN (only two).

DISCUSSION

In the present study, ELISA showed a sensitivity similar to the complex PRN, and higher than HI and IFA. Of 179 positive samples by PRN, only two, with antibody titers of 8, were negative by ELISA. However, the correlation between low antibody titers by PRN and protection is not completely clear. Titers as low as 8 by PRN seems not to interfere on measles vaccination[1] and may not provide protection to infection.

When testing paired sera for diagnosis of measles, ELISA has been shown to be similar to HI, IFA, and CF[2, 4, 5]. For this purpose a highly sensitive test is not required, since antibodies rise to levels easily detected by any of the available methods. However, a high sensitive assay may be critically important for detection of low antibody titers, as is the case of passive maternal antibodies that can interfere on measles vaccination or when assessing the rate of seropositivity later after vaccination, when antibodies could have declined to undetectable levels by conventional methods[8]. Although the knowledge about the PRN or ELISA titers that provide protection is limited, it was demonstrated that persons with antibodies not detected by HI, but detected by PRN, are protected against measles[9].

TABLE 1
Comparison of ELISA, HI and IFA with PRN test for detection of measles antibodies.

PRN	ELISA No. Positive	IFA No. Positive	HI No. Positive
Pos. 179 (98.9%)	177/179 (97.8%)	167/179 (92.2%)	148/179 (81.7%)
Neg. 2 (1.1%)	0/2 (0.0%)	0/2 (0.0%)	0/2 (0.0%)
Tot. 181 (100.0%)	177/179 (97.8%)	167/179 (92.2%)	148/179 (81.7%)

Pos. titers: 8 for PRN, 20 for ELISA, 5 for HI and IFA.

In the present study, ELISA was performed with Toyoshima strain of measles virus and the other tests with Edmonston B strain. At present no data is available regarding the possible influence of different measles virus strain on the sensitivity and specificity of ELISA for detection of measles antibodies.

The ELISA can be used for testing great number of samples and may be a useful method for measles seroepidemiological surveys. Due to its high sensitivity and reproducibility, this assay can also be employed for evaluation of vaccine efficacy years after vaccination, when the PRN assay is not suitable considering the complexity of the method.

RESUMO

Reação imunoenzimática (ELISA) para detecção de anticorpos para o vírus do sarampo. Comparação com reações de inibição da hemaglutinação, imunofluorescência indireta e neutralização de placas.

A reação imunoenzimática (ELISA) para determinação de anticorpos para o vírus do sarampo foi comparada com a reação de neutralização de placas (RNP), inibição da hemaglutinação (RIH) e imunofluorescência indireta (RIF). Das 179 amostras positivas pela RNP, somente 2, com títulos iguais a 8, se apresentaram negativas por ELISA (copositividade de 98.9%). A RIF e RIH apresentaram, respectivamente, copositividade de 93.3 e 82.7%. ELISA apresentou sensibilidade equivalente à complexa RNP, boa reprodutibilidade e representa uma alternativa para a detecção de baixos títulos de anticorpos contra o sarampo.

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REFERENCES

1. ALBRECHT, P.; HERRMANN, K. & BURNS, G. R. — Role of virus strain in conventional and enhanced measles plaque neutralization test. **J. virol. Meth., 3**: 251-260, 1981.

2. BOTELER, W. L.; LUIPERSBECK, P. M.; FUCCILLO, D. A. & O'BEIRNE, A. J. — Enzyme-linked immunosorbent assay for detection of measles antibody. **J. clin. Microbiol., 17**: 814-818, 1983.

3. GERSHON, A. A. & KRUGMAN, S. — Measles virus. In: LENNETTE, E. H. & SCHMIDT, N. S., ed. Diagnostic procedures for viral and rickettsial infections. 5th ed. New York, American Public Health Association, 1979. p. 665-693.

4. KAHANE, S.; GOLDSTEIN, V. & SAROV, I. — Detection of IgG antibodies specific for measles virus by Enzyme-linked immunosorbent assay (ELISA). **Intervirology, 12**: 39-46, 1979.

5. KLEIMAN, M. B.; BLACKBURN, C. K. L.; ZIMMERMAN, S. E. & FRENCH, M. L. V. — Comparison of Enzyme-linked immunosorbent assay for acute measles with Haemagglutination inhibition, Complement fixation, and Fluorescent-antibody methods. **J. clin. Microbiol., 14**: 147-152, 1981.

6. KRUGMAN, S.; GILES, J. P.; FRIEDMAN, H. & STONE, S. — Studies on immunity to measles. **J. Pediat., 66**: 471-488, 1965.

7. LOWRY, O. H.; ROSEBROUGH, N. J.; FARR, A. L. & RANDALL, R. J. — Protein measurement with the Folin phenol reagent. **J. biol. Chem., 193**: 265-275, 1951.

8. MARKS, J. S.; HAYDEN, G. F. & ORESTEIN, W. A. — Methodologic issues in the evaluation of vaccine effectiveness. **Amer. J. Epidem., 116**: 510-522, 1982.

9. ORESTEIN, W.; ALBRECHT, P.; HERRMANN, K. L.; BERNIER, R.; BART, K. J. & ROVIRA, E. Z. — The plaque neutralization test as a measure of prior exposure to measles virus. **J. infect. Dis., 155**: 146-149, 1987.

10. PANNUTI, C. S.; SOUZA, V. A. U. F.; TAKAOKA, N.; LEME, S. T. S.; PEREIRA, C. R.; CARVALHO, R. P. S. & AMATO NETO, V. — Interferência entre vacinas anti-sarampo e antipoliomielite. **Bol. Ofic. sanit. panamer., 103**: 227-232, 1987.

11. SOUZA, V. A. U. F.; PANNUTI, C. S.; GODOY, C. V.F.: ALBRECHT, P.; LOPES, M. H. & MENDONÇA, J. S. — Comparison of indirect immunofluorescence test for measles antibodies with Haemagglutination inhibition and Plaque neutralization test. **Rev. Inst. Med. trop. S. Paulo, 31** (supl. 7): S80, 1989.

12. VOLLER, A. & BIDWELL, D. E. — Enzyme-immunoassays for antibodies in measles, cytomegalovirus infections and after rubella vaccination. **Brit. J. exp. Path., 57**: 243-247, 1976.

13. WEIGLE, K. A.; MURPHY, M. D. & BRUNELL, P. A. — Enzyme-linked immunosorbent assay for evaluation of immunity to measles virus. **J. clin. Microbiol., 19**: 376-379, 1984.

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