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

Vanda Akico Ueda Fick de SOUZA (1), Claudio Sergio PANNUTI (1), Laura Massami SUMITA (1) & Paul ALBRECHT (2)

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; Haemagglutination inhibition.

INTRODUCTION

Measles serology has been routinely performed by haemagglutination-inhibition test (HI), considered as an adequate assay to determine immune status. 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. However, the IFA, as the HI, does not substitute the Plaque neutralization test (PRN) when low measles antibody titers are sought. PRN is considered a highly sensitive assay for measles antibody but it is time consuming and expensive. The Enzyme-linked immunosorbent assay (ELISA), first described for measles antibody by VOLLE et al. has been shown to be a sensitive and reliable assay and could represent an alternative to the PRN.

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-
cinated children and umbilical cord, at Hospital do Servidor Público Estadual "Francisco Mora
do de Oliveira", were used for comparison be
tween 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 vaccina-
tion were tested at dilution 1:20 in order to deter-
mine the seronegative range of ELISA.

ELISA: For antigen preparation, the Toyo-
shima strain of measles virus was grown on Vero
cells in Minimum Essential Medium (MEM) sup-
plemented 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 minu-
tes centrifugation at 800 g. The sediment was
resuspended in PBS in a volume equivalent to
1:50 of the original medium. Antigen was extrac-
ted by adding equal volume of 0.2% sodium deso-
ycholate (Difco) in PBS, sonication for 3 min-
utes 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 simil-
arily 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
LOWRY1. 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 albu-
min (Sigma, St. Louis, Mo) in PBST (BSA) were
added in each well and incubated at 37°C cove-
red with moistened filter paper. From 1:20 start-
ing dilution, serial fourfold dilutions in BSA we-
re 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 dilu-
tion of peroxidase labelled anti-human IgG (Sig-
ma) in BSA were added. After 40 minutes incuba-
tion at 37°C and four washes, 50 μl of substrate
consisting of hydrogen peroxide and o-phenyle-
mediamine (Sigma) (0.4 mg/ml) in 0.1M Citrate
buffer, pH 5.0, were added. The enzymatic reac-
tion was stopped with 50μl of 2.5N sulfuric acid
after 20 min incubation in the dark at room tem-
perature. Absorbance at 492 nm was measured
with Titertek Multiskan Plus MK (Flow Lab.)
and the results, expressed as DOD, were calcu-
lated 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: Ed-
monston B measles virus infected Vero cells and
uninfected controls were washed twice and re-
suspended in PBS and acetone fixed onto slides
at concentration of about 50 cells per 400x high
power field. Thereafter, doubling dilutions of se-
rum 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 fluo-
rescein 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 test2 was used with Edmonston B
strain measles virus antigen obtained from soni-
cated infected Vero cells and 0.5% Rhesus mon-
key red blood cells.

Plaque reduction neutralization test: Was
performed by Dr. Paul Albrecht, Division of Vi-
rology, FDA, Bethesda, MD, as previously descri-
bed1.

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 va-
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%
(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, the 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 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 CP. 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. 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.

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 asssay 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 reproduibilidade e representa uma alternativa para a detecção de baixos títulos de anticorpos contra o sarampo.

**ACKNOWLEDGEMENTS**

We thank Dr. H. F. Andrade Jr., Mrs. Maria Cristina D. S. Fink, Almir Robson Ferreira and Mrs. Jussara Clemente S. P. Moraes for their valuable cooperation.
REFERENCES


Aceptó para publicación en 18/9/1990.