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COMPARISON OF INDIRECT IMMUNOFLUORESCENCE TEST FOR MEASLES ANTIBODIES WITH HAEMAGGLUTINATION INHIBITION AND PLAQUE NEUTRALIZATION TESTS

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SUMMARY

Indirect Immunofluorescence (IFA), Plaque Reduction Neutralization (PRN) and Haemagglutination Inhibition (HI) tests for measles antibodies were carried out in 197 sera obtained from umbilical cord and vaccinated children. The IFA was also applied to blood samples collected with filter paper.

IFA results demonstrated that the test is relatively simple to perform, with good reproducibility for different antigen lots. Good correlation was obtained between IFA, PRN and HI antibody titers. Better correlation was demonstrated with IFA and PRN than with HI and PRN tests.

Sensitivity of IFA in detecting antibody was less effective than PRN, however more effective than HI using rhesus monkey red blood cells. PRN antibody titers over 100 were detected by IFA but not by HI (9.7% with negative results). IFA may be of considerable practical use and able to substitute HI in seroepidemiological surveys and to evaluate vaccine efficacy. It also can be simplified by employing filter paper collected samples.

KEY WORDS: Measles; Indirect immunofluorescence; Plaque reduction, Haemagglutination inhibiton.

INTRODUCTION

Seroepidemiological surveys and correct evaluation of vaccine efficacy administered in different age groups are of primary importance for measles control, specially in developing countries.

The haemagglutination inhibition test (HI), routinely used for measles antibody detection has a limiting factor in the availability of monkey red blood cells, variability of results depending on the type of monkey red blood cells[6, 14] beside the need for pre-treatment of sera for agglutinin and non-specific haemagglutination inhibitors removal.

Plaque reduction neutralization test (PRN), considered one of the most sensitive assays for measles antibody detection[2] is not feasible for

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all laboratories considering the elaborated technical test procedures. Immunofluorescence assay, extensively applied in diagnostic virology, has been scarcely employed for measles antibody detection[4, 10] and few comparative trials with other methods are available[5, 9].

In the present study aspects of sensitivity and reproducibility of the indirect immunofluorescence assay (IFA) are reported, comparing it with HI and PRN tests.

MATERIAL AND METHODS

Sera: 197 serum samples were obtained during the period of 1979 to 1981 at the Hospital do Servidor Público do Estado de São Paulo. Of this total, 50 were from umbilical cord blood and 147 from children vaccinated against measles between 1975 and 1979. An additional lot of 62 samples from adults and 9 month unvaccinated children was collected simultaneously by venous puncture and finger prick[12] to evaluate the feasibility of the IFA with filter paper blood samples.

Indirect Immunofluorescence assay (IFA): The test was performed with Edmonston strain measles virus infected Vero cells slides and isothiocyanate labelled anti-human IgG from a comercial source (bioMérieux). Cells presenting 75% CPE were mechanically detached from the tissue culture flask and washed 3 times with PBS, pH 7.2. Cells were then resuspended in PBS to obtain smears with about 50 cells per microscopic field (400x). Slides were fixed for 10 minutes at -20°C in cold acetone and stored at -20°C[7]. All slides were used within 5 weeks, as a loss of antigen stability was observed beyond this period. As control, non infected cell slides were likewise prepared. Sera were tested in twofold dilutions starting from 1:5. Each serum was tested at starting dilution of 1:5 with control cells. Positive and negative control sera were included in each set of tests.

To ascertain the reproducibility of the IFA test with several antigen lots, 20 sera with different antibody titers were tested with 4 different antigen lots.

Haemagglutination Inhibition test (HI): The standard HI test[8] was used with Edmonston B strain measles virus antigen, obtained from sonicated infected Vero cells, and a 0.5% Rhesus monkey red blood cell suspension. Non-specific inhibitors were removed with heparin and $MnCl_2$[3]. In this assay, sera were tested at a starting dilution of 1:5, in twofold dilutions.

Plaque Reduction Neutralization test (PRN): Performed by Dr. Paul Albrecht, Division of Virology, FDA Bethesda, MD[2]. Sera were tested in fourfold dilution, starting from 1:8.

RESULTS

Of the 197 sera tested, 195 (99.0%) were considered positive by PRN, with titers ranging from 8 to 33,418 and a GMT of 900. When the same samples were tested by IFA, 180 (91.4%) were positive, with GMT of 31 (titers ranging from 5 to 320), whereas 159 (80.7%) were positive by HI with GMT of 9 (titers ranging from 5 to 160). Copositivity obtained by IFA as compared to PRN, considered as the reference test, was 92.31% with a correlation index of 0.84. For HI, copositivity was 81.54% and the correlation index 0.71, in relation to the same reference test.

Comparison of IFA and HI results in relation to different PRN antibody titers range is shown in Table 1.

Good reproducibility of IFA with 4 different antigen lots was obtained. Of the 20 sera tested all except 2 provided same antibody titers or variations corresponding to a single serum dilution. Variance analysis of antibody titers showed no statistical differences between the 4 antigen lots ($f = 0.0338$; $\alpha = 0.05$).

Comparative IFA antibody titers from sera obtained by the filter paper technique and standard venous puncture are plotted in Figure 1. Variance analysis of antibody titers did not demonstrate significant difference ($F = 1.5248$; $\alpha = 0.05$).

DISCUSSION

The present study confirms the greater sensitivity of PRN as compared with HI[2]. The results also demonstrate to be the same in relation to IFA. The differences are critically important

TABLE 1

Comparative results of **IFA** and **HI** with **PRN** antibody titers for measles virus in 197 sera

No. of sera	PRN Titers	GMT*	IFA		HI	
			Positive**	GMT*	Positive**	GMT
2	< 8	(< 8)	0	(< 5)	0	(< 5)
11	8- 50	(24)	0	(< 5)	0	(< 5)
9	51- 100	(69)	5	(< 5)	1	(< 5)
8	101- 200	(145)	8	(9)	3	(< 5)
26	201- 400	(303)	26	(14)	20	(6)
40	401- 800	(572)	40	(26)	37	(9)
26	801- 1600	(1191)	26	(52)	26	(10)
27	1601- 3200	(2108)	27	(65)	25	(10)
20	3201- 6400	(4591)	20	(88)	19	(17)
28	6400-33418	(11508)	28	(109)	28	(27)
Total 197		(900)	180	(31)	159	(9)

* Geometric mean titers

** Sera with titers ≥ 5

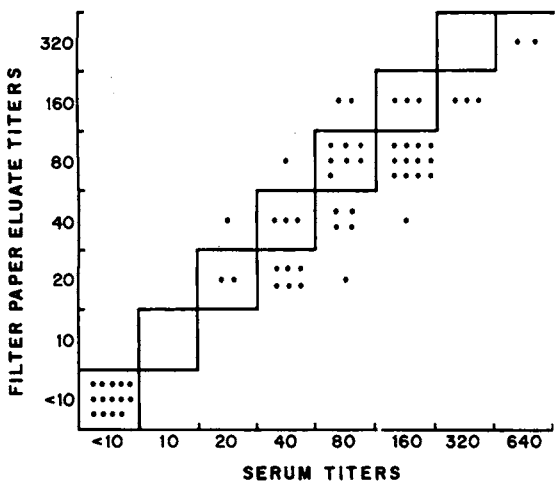

Figure 1: Comparison of IFA measles antibody titers in 62 samples collected simultaneously by venous puncture and finger prick filter paper.

to the detection of low antibody titers since PRN titers equal to or below 50, are not detected by either IFA or HI. There is evidence that low antibody titers revealed by PRN may interfere in measles vaccine efficacy of infants with passively transmitted maternal antibodies[1]. In the conditions this study was performed, PRN antibody titers equal to or above 100 were detected by IFA but not by HI (9.7% with negative results). Since there is not, presently, general agreement whether antibody titers lower than 100 by PRN are protective, IFA could figure as a valid alternative assay for the evaluation of measles serological immunity.

Furthermore, the results of IFA from sera obtained by finger prick paper technique were similar to those from standard venous puncture sera,implying that the test is useful for large scale serological studies, as already reported with HI test[11, 13].

Results of the present study demonstrated that IFA is not adequate for substituting the PRN when low measles antibody titers are sought as for the end point determination of passive antibody curve extinction. However it could represent a convenient alternative test for HI in laboratories able to maintain measles virus in tissue culture, considering the greater sensitivity and good reproducibility, discarding monkey red blood cells and sera pre-treatment.

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RESUMO

Comparação da reação de imunofluorescência indireta para o vírus do sarampo com as reações de inibição da hemaglutinação e neutralização por redução de placas.

As reações de imunofluorescência indireta (RIF), neutralização por redução de placas (RNP) e inibição da hemaglutinação (RIH) para detecção de anticorpos para o vírus do sarampo foram aplicadas a 197 soros provenientes de cordão umbilical e de crianças vacinadas contra o sarampo. Avaliou-se ainda a aplicação da RIF em amostras colhidas em papel de filtro.

A RIF apresentou-se como uma prova de execução relativamente simples, de boa reprodutibilidade com diferentes partidas de antígeno. Observou-se boa correlação entre os títulos de anticorpos obtidos por RIF, RNP e RIH. Com a RNP, a RIF apresentou maior correlação que a RIH.

A sensibilidade da RIF na detecção de anticorpos contra o sarampo foi menor que RNP, porém superior à da RIH com hemácias de macaco rhesus.

Anticorpos com títulos superiores a 100 pela RNP foram sistematicamente detectados pela RIF, mas não por RIH (9,7% de resultados negativos). A RIF pode ser de grande utilidade, podendo substituir com vantagem a RIH em inquéritos soroepidemiológicos e na avaliação de eficácia de vacinas. Além disso, mostrou-se adequada para aplicação em larga escala, já que permite processar amostras de sangue colhidas em papel de filtro.

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