

BRIEF COMMUNICATION

AN INDIRECT IMMUNOFLUORESCENCE ASSAY TO DETECT ANTIBODIES AGAINST ST. LOUIS ENCEPHALITIS VIRUS

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SUMMARY

An in house indirect immunofluorescence assay (IFA) in relation to neutralization (NT) reference test, was assessed as a fast and cheap method to carry out serological surveys for St. Louis Encephalitis virus (SLE). Sera obtained from 213 blood donors were analyzed by both tests. The prevalence of seropositivity obtained with IFA was lower than (30.98%) that observed on NT (41.78%). The relative specificity rate of IFA was 96.77% whereas its relative sensitivity rate was 69.66%. Kappa index showed a good correlation between both tests. The results indicate that neutralization assay is still the serological test with the highest sensitivity and specificity relative rates for detecting antibodies against SLE virus. Nevertheless, the IFA could be useful as an alternative test in order to learn the circulation of the Flavivirus genus in a certain area.

KEYWORDS: Indirect immunofluorescence assay; Neutralization test; St. Louis Encephalitis virus.

St. Louis encephalitis (SLE) virus is a member of the genus Flavivirus within the Flaviviridae family. This genus includes the dengue, the yellow fever (YF) and the Japanese encephalitis (JE) viruses, which are important human pathogens. The SLE virus has been isolated from *Culex* mosquito and birds, and it is responsible for a great number of human infections in the USA. Three clinical syndromes have been described: encephalitis, aseptic meningitis, and febrile headache. The severity of the illness increases with advancing age, and persons over 60 year-old present the highest frequency of encephalitis⁴.

In the Americas the SLE virus is found from southeast of Canada to Argentina.

In Argentina the virus was isolated from patients with systematic illness, mosquitoes, and wild rodents. At the same time a high prevalence of specific antibodies in humans was shown especially in population living in warm weather areas^{7,8}. The classic techniques of hemagglutination inhibition (HI) and neutralization test (NT) have particularly been useful, since this infection could be determined in diverse animal species and in humans from most different areas. The NT is considered to be the most specific test for arbovirus identification. In many cases it is used for positive identification after sufficient screening and grouping by the HI and other tests^{4,7}.

In recent years, immunoassays have been applied for the serodiagnosis of arbovirus infections, including a number of flaviviruses:

JE, YF, dengue and SLE, and on account of these techniques could be performed for virological diagnosis without special equipments or a highly qualified staff.

It is known that the immunofluorescence antibodies for the yellow fever virus appear together with the HI and NT antibodies in the first week the onset of illness⁴, whereas the appearance and duration of the immunofluorescence antibodies in the SLE infection as well as the use of the indirect immunofluorescence assay (IFA) are not well known. The need of using fast, easy and cheap techniques, especially for seroepidemiological studies led us to develop an in house IFA, and compare its rate of sensitivity and specificity relative with the NT, a reference test used in our laboratory for detecting antibodies against SLE virus.

For the present study, serum samples obtained from 213 blood donors were analyzed by both tests.

The IFA was slightly modified and performed as described by ROWE *et al.*⁶. Air dried acetone-fixed slides with SLE 78V6507 infected VERO cells were used as source of antigens in IFA. Noninfected VERO cells were included on the slides as a control of inespecificity. Sera were diluted at 1:5 in phosphate buffered saline (PBS). The reaction was accomplished adding anti-human IgG rabbit serum linked to fluorescein isothiocyanate (GIBCO, Grand Island Biological Company, Grand Island, NY, USA).

As a positive control an in-house human reference serum was included in every slide.

Sera were considered positive for SLE antibodies when the specific apple-green fluorescence was located in the cytoplasm or on the plasma membrane in approximately 25% of the total amount of cells, and no fluorescence staining on noninfected cell were observed.

The plaque reduction neutralization assay was performed in VERO cell line with agarose according to the procedures previously described². Sera were diluted at 1:5 in MEM (Minimum Essential Medium), and those that neutralized 80% of the plaque forming unites (pfu) included in the test were considered positive.

Of 213 samples, 89 sera (41.78%) were positive on NT whereas 66 sera (30.98%) were positive on IFA. The estimation of the relative sensitivity and specificity rates, positive predictive value, negative predictive value and Kappa index¹ for IFA are shown in Table 1. The Table shows that 4 samples are positive on IFA and negative on NT, and 27 samples are negative on IFA but positive on NT. These data may indicate that NT is able to detect long-lasting antibodies as it showed to be a more sensitive test. Four positive sera detected on IFA but negative on NT could be due to non-specific antibodies.

Table 1

Comparison of the results obtained with IFA and NT for detecting anti-SLE virus antibodies

Screening Test	Reference Test		Total
	Neutralization Test		
IFA	Positive	Negative	
Positive	62	4	66
Negative	27	120	147
Total	89	124	213

Rate of relative sensitivity: 69.66%; Rate of relative specificity: 96.77%; Kappa Index: 0.68; Positive predictive value: 93.94%; Negative predictive value: 81.63%.

Our results demonstrate that IFA is cheaper and technically easier to perform than the NT test but the latter showed to be more sensitive than the IFA. The predictive negative and positive values were 81.63% and 93.94% respectively. Similarly previous studies on IFA performed with yellow fever virus showed a low sensitivity, and a similar specificity to NT^{3,5}.

Nevertheless, the Kappa index (68%) showed a good correlation between both assays, and therefore the IFA could be useful as an alternative test in order to study the circulation of this virus genus in a specific area. However NT will always be the reference test for SLE seroepidemiological studies.

In conclusion, the limits of the low complexity techniques should be taken into account at the time of selecting a technique for epidemiological purposes. Neutralization assay is still the most sensitive and specific serological test for detecting antibodies against the SLE virus.

RESUMO

Teste de imunofluorescência indireta para a detecção de anticorpos contra o vírus da encefalite St.Louis

Com a finalidade de dispor de um método rápido e de baixo custo para realizar estudos séricos dirigidos ao vírus da encefalite St.Louis (ESL), avaliamos a técnica de imunofluorescência indireta (IFI) artesanal em relação ao teste referência de neutralização (NT).

Examinaram-se 213 soros de doadores de sangue. A prevalência dos anticorpos obtidas na técnica de IFI (30,98%) foi mais baixa do que a pesquisada com NT (41,78%).

A taxa de especificidade relativa da IFI foi 96,77%, enquanto sua taxa de sensibilidade relativa foi 69,66%. O índice Kappa demonstrou boa concordância entre os dois testes. Os resultados achados revelam que a NT continua sendo a técnica sorológica de maior taxa de especificidade e sensibilidade relativa para a detecção de anticorpos IgG para o vírus ESL. Contudo, a IFI poderia ser o teste de escolha em estudos da circulação desta arbovirose na população humana.

REFERENCES

1. CERQUEIRO, M.C. - Nociones de epidemiología aplicada a las infecciones virales. In: CARBALLAL, G. & OUBIÑA, J.R. *Virología médica*. 2. ed. Buenos Aires, 1996. p. 521-529.
2. GLOWACKI, G.; SPINSANTI, L.; BASUALDO, M.A.; DIAZ, G. & CONTIGIANI, M. - Prevalencia de anticuerpos contra Flavivirus en jóvenes voluntarios ingresantes al servicio militar de la provincia de Formosa, Argentina. *Rev. argent. Microbiol.*, **30**: 170-175, 1998.
3. MONATH, T.P.; CROPP, C.B.; MUTH, D.J. & CALISHER, C.H. - Indirect fluorescent antibody test for the diagnosis of yellow fever. *Trans. roy. Soc. trop. Med. Hyg.*, **75**: 282-286, 1981.
4. MONATH, T.P. & HEINZ, F. - Flavivirus. In: FIELDS, B.N.; KNIPE, D.M.; HOWLEY, P.M. *et al. Fields Virology*. 3. ed. Philadelphia, Lippincott- Raven, 1996. p. 961-984.
5. NIEDRIG, M.; LADEMANN, M.; EMMERICH, P. & LAFREZ, M. - Assessment of IgG antibodies against yellow fever virus after vaccination with 17D by different assays: neutralization test, haemagglutination inhibition test, immunofluorescence assay and ELISA. *Trop. Med. Int. Hlth.*, **4**: 867-871, 1999.
6. ROWE, W.P.; PUGH, W.E.; WEBB, P.A. & PETERS, C.J. - Serological relationships to the Tacaribe complex of virus to lymphocytic choriomeningitis virus. *J. Virol.*, **5**: 289-292, 1970.
7. SABATTINI, M.S.; AVILÉS, G. & MONATH, T.P. - Historical, epidemiological and ecological aspects of arbovirus in Argentina: Flaviviridae, Bunyaviridae and Rhabdoviridae. In: TRAVASSOS DA ROSA, A.P.A.; VASCONCELOS, P.F.C. & TRAVASSOS DA ROSA, J.F.S., ed. *Overview of arbovirology in Brazil and neighboring countries*. Belém, Instituto Evandro Chagas, 1998. p. 113-134.
8. SPINSANTI, L.; RÉ, V.; BASUALDO, M. A. *et al.* - Seroprevalencia de infección por el virus encefalitis San Luis en la provincia de Formosa. *Medicina (B.Aires)*, **60**: 474-476, 2000.

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