THE STABILITY OF THE PREMIXED ANTIRABIES FLUORESCENT ANTIBODY CONJUGATE STORED IN AN ORDINARY FREEZER

FUMIO HONMA ITO
Professor Associado
Faculdade de Medicina Veterinária e Zootecnia da USP

SILVIO ARRUDA VASCONCELLOS
Professor Associado
Faculdade de Medicina Veterinária e Zootecnia da USP

MARCIA ESTER PARREIRA VASCONCELLOS
Pós-Graduanda
Faculdade de Medicina Veterinária e Zootecnia da USP

AVELINO ALBAS
Pesquisador Científico I
Instituto Butantan

JOSE DE ANGELIS CORTES
Professor Titular
Faculdade de Medicina Veterinária e Zootecnia da USP

ITO, F.H.; VASCONCELLOS, S.A.; VASCONCELLOS, M.E.P.; ALBAS, A.; CORTES, J.A. The stability of the premixed antirabies fluorescent antibody conjugate stored in an ordinary freezer. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo, 26(1): 35-37, 1989.

SUMMARY: The stability of fluorescein - conjugated hamster's antirabies globulin premixed to normal and rabid mouse brain suspensions was investigated, using the 50% glycerin as an additional preservative. The premixed conjugate, with and whithout addition of glycerin was dispensed in 0.5 ml aliquots and stored at -20° C. The tests were run each 3 month interval and after 2 years the results were quite satisfactory, althougn the addition of glycerin did not reveal any substantial difference to the fluorescence of the known rabies smears and to the storage life of the premixed conjugate.

UNITERMS: Rabies, virus; Fluorescent antibody technique

* RABVAC, PFIZER CO.

INTRODUCTION

Accurate and reliable diagnosis is essential for rabies, but for a laboratory that deals with a small number of diagnostic cases, the stability of reagents is questioned, if stored for a long period in an ordinary freezer.

MARKSON & UPCOTT, 4 have investigated the effect of premixed antirabies fluorescent antibody (FA) conjugate and the mouse brain suspensions for a storage period of 9 months and they found reliable results. The rabid mouse brain (RMB) suspension, if not fresh or properly stored, is said to be not able in inhibiting the antirabies conjugate and weeks later the RMB suspension looses its ability to absorb the conjugate (4).

The glycerin, in combination with low temperature, is a worldwide well known preservative of biologicals (1, 5), and in this experiment it was set up a design to test the storage life of the normal mouse brain (NMB) and RMB-premixed antirabies conjugate at temperature of -20° C with addition of 50% glycerin.

MATERIAL AND METHODS

The fluorescein-conjugated antirabies hamster's globulin was prepared in our laboratory facility by immunizing 30 hamsters, the procedures adopted for the hiperimmunization and for the preparation of FITC (Sigma) - antirabies IgG conjugate were essentially the same to that described by LARGHI, 3 with slight modification, i.e., the antigen used was substituted to a commercially available inactivated antirabies vaccine prepared in BHK cell culture (Rabivac)*.

The CVS (Challenge virus standard) and the normal mouse brain suspensions were prepared in a dilution of 1 in 5 using 0.01M phosphate buffered saline as diluent, the pH adjusted to 8.0 with phosphate buffer, centrifuged at 3,000 rpm for 15 minutes and the conjugate was diluted using these suspensions to give a final dilution of 1 in 60, and then centrifuged at 10,000 rpm for 30 minutes. The supernatants were dispensed in individual aliquots of 0.5 ml and stored at -20° C until required.

The glycerin - mixtured conjugate was prepared by adding to the supernatants of the NMB and RMB, equal volume of glycerin, the pH adjusted and the conjugate diluted and dispensed in 0.5 ml aliquots and stored as above.

The paired aliquots of NMB and RMB - diluted conjugate were thawed each 3

month interval and tested on smears of mice's brains that had been inoculated with a known rabies virus. The FA testing procedure was according to KAPLAN, 2, and the microscopic evaluation was made under a Zeiss binocular microscope, using an HBO 200 mercury vapor lamp fitted with a set of excitation and absorption filters.

RESULTS AND DISCUSSION

Although entirely subjective, the evaluation of the FA test was based upon the presence of specific fluorescence of intracytoplasmic inclusion bodies and the fluorescent sandlike particles routinely seen in rabies positive smears.

The premixed conjugate added with 50% glycerin after freezing at -20° C, in some bottles there was found a formation of 2 phases, the liquid phase at the bottom formed by glycerin, but after thawing, no interference to the reaction was observed. This could be related to the fluctuation of the temperature of the ordinary freezer, in hot summer season the temperature reached around -10° C.

The storage at -20° C gave an excellent fluorescence, and for the last pair of aliquots tested after 24 months, some fine fluorescent deposits of the conjugate were observed, especially for the NMB-premixed conjugate, similar to sandlike particicles, and these deposits would interfere to the reliability of the test when applied to doubtfull diagnostic tests. The same deposits formation was also observed for the NMB-premixed conjugate added with 50% glycerin, but when centrifuged at 10,000 rpm the results were quite satisfactory. In contrast to the NMB-premixed conjugate, to the RMB-premixed conjugate there was a negligible formation of deposits, but the absorption of the conjugate was complete even after 24 months of conservation. The formation of these deposits may be related to the quality of the conjugate, the higher the working dilution more stable would be the premixed conjugate.

The practicability of the premixed conjugate, as describe by MARKSON & UPCOTT, 4 was fully confirmed through the 24 months period of the FA testing, and this system of storage without addition of glycerin has now been introduced into our routine FA test for rabies diagnosis, in combination to the mouse inoculation test.

ACKNOWLEDGEMENT

The authors are grateful to Miss Rosana Cleide Paick Utiama for her careful assistance.

ITO, F.H.; VASCONCELLOS, S.A.; VASCONCELLOS, M.E.P.; ALBAS, A.; CORTES, J.A. Estabilidade do conjugado anti-rábico para reação de imunofluorescência direta pré-diluído e armazenado em freezer, comum. Rev. Fac. Med. Vet. Zootec. Univ. S. Paulo, 26(1): 35-37, 1989.

RESUMO A estabilidade do conjugado anti-rábico para reação de Imunofluorescência Direta, preparado a partir de imunoglobulina de hamsters e pré-diluido com suspensões de cérebros de camundongos normais e camundongos infectados com virus CVS (Challenge Virus Standard) foi investigada, utilizando-se a glicerina 50% como preservativo adicional. O conjugado pré-diluido, com e sem glicerina, foi dispensado em aliquotas de 0,5 ml e armazenado a -20° C. As reações de Imunofluorescência Direta foram realizadas em intervalo de cada 3 meses e após 2 anos, os resultados obtidos foram satisfatórios; no entanto, a adição de glicerina não revelou nenhuma diferença substancial na fluorescência e nem quanto ao tempo de armazenamento do conjugado pré-diluido.

UNITERMOS: Raiva, virus; Imunofluorescência

LITERATURE

1 - CORTES, J.A. & NILSSON, M.R. Nota sobre o emprego da glicerina na conservação do virus da raiva amostra C.V.S. Rev. Fac. Med.

Vet. Zootec. Univ. S. Paulo, 12:235-240, 1975.

2 - KAPLAN, M.M. Evaluación de las tecnicas de laboratorio aplicables al diagnostico y la preservación de la rabia y a las investigaciones antirabicas. In: KAPLAN, M.M. & KOPROWSKI, H. La rabia: tecnicas de laboratório. 3.ed. Ginebra, Organizacion Mundial de la Salud, 1976. p.19-26. (Serie de monografias, 23)

3 - LARGHI, O.P. Prueba de anticuerpos fluorescentes para rabia. Buenos Aires, Centro Panamericano de Zoonosis, 1975. p.9-21. (Nota tecnica, 8)

4 - MARKSON, L.M. & UPCOTT, D.H. The storage life of biological reagents used in the fluorescent antibody test for rabies. Trop. anim. Hlth. prod., 3: 86-88, 1971.

5 - MERYMAN, H.T. General principles of freezing and freezing injury in celullar material: freezing and drying of biological materials. Ann. N. Y. Acad. Sci., 85:503-569, 1960.

Recebido para publicação em 02/02/89
Aprovado para publicação em 09/05/89