Trop. Anim. Hlth Prod. (1971) 3, 86–88

# THE STORAGE LIFE OF BIOLOGICAL REAGENTS USED IN THE FLUORESCENT ANTIBODY TEST FOR RABIES

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## SUMMARY

Three systems of storing fluorescein-conjugated antirabies globulin and mouse brain suspensions were investigated to find their effect on the reliability of the fluorescent antibody test for rabies. Premixed conjugate and mouse brain suspension, dispensed in test aliquots and maintained at −20°C until required, gave the most consistent results. After 9 months' storage the reagents gave a satisfactory test with known positive smears.

## INTRODUCTION

During 1969 the fluorescent antibody (FA) test was set up at the Central Veterinary Laboratory as part of the diagnostic procedure used to deal with suspected rabies cases. Essentially the method used was that of Goldwasser, Kemron and Nobel (1961). Early in the work a few discrepant results raised the question of the storage life of the mouse brain suspensions and the diluted conjugate. Goldwasser had written (personal communication) that he stored his rabid mouse brain suspension in small aliquots at −20°C, thawing and freezing as required without its apparent deterioration. In our experience this reagent soon lost its ability to inhibit a positive reaction. As the Central Veterinary Laboratory is required to deal with only a comparatively small number of rabies diagnostic cases (last year's total of about 200 is unusually high for us, but quite small compared to the thousands of examinations made annually by laboratories in countries where the disease is enzootic), it was thought desirable to set up an experiment to test the effect of different storage conditions on diluted conjugate and the mouse brain suspensions.

## MATERIALS AND METHODS

Normal and rabid mouse brain suspensions (NMB and RMB) were prepared in the usual way to a dilution of 1 in 5 using egg yolk buffer. The CVS (challenge virus standard) strain of rabies virus was used after one additional intracranial passage in mice. The same strain of virus was used to produce a stock of smears of brain from mice known to be rabid. The smears were fixed in acetone for 2 hours at −20°C and drained dry and stored at the same temperature. The fluorescein-conjugated antirabies equine globulin was the commercially available product made by Baltimore Biological Laboratories. This was reconstituted as directed by the manufacturers and the reconstituted stock conjugate dispensed in 0·5 ml aliquots which were stored at −20°C until required. The working dilution of conjugate was prepared by diluting one stock aliquot with 4·5 ml phosphate buffered saline to give a dilution of 1 in 10. For the tests the working dilution was mixed with an equal quantity of NMB or RMB to give a final conjugate dilution of 1 in 20. Three systems of storage were investigated:

1. A quantity of the working dilution of the conjugate was stored at −20°C. Every fortnight it was thawed, a sample taken to test on the known positive smears and the remainder refrozen. Bottles of the MB suspensions were similarly thawed, sampled and refrozen.
2. A quantity of the working conjugate was stored at 4°C and similarly tested every fortnight, but with single test aliquots of the MB which had been maintained frozen at −20°C, without fortnightly thawing.
3. Working dilution of conjugate was mixed in bulk with an equal quantity of one or other of the two MB suspensions and the two mixtures held at room temperature for 1 hour. During this time they were dispensed in bijou bottles, 4 or 5 drops to a bottle and the bottles labelled before being stored at −20°C. The contents of a pair of bottles (one of each mixture) were tested on known positive smears every fortnight.

The stained smears were mounted in Entallan (Merck)—a solution in toluene of a synthetic resin—and examined under a Reichert Zetopan microscope using an HBO 200 mercury vapour lamp, with immersion dark ground condenser, the E2 (UG1/1·5 mm) exciter filter and the Sp2 (2 × GG 13/1 mm + Wratten foil 2B) absorption filter.

## RESULTS

Assessment of the results was entirely subjective. Smears stained with the conjugate/RMB mixture would be negative if the rabies antigen had absorbed the conjugate. Whether the conjugate was stored at 4°C or repeatedly thawed and refrozen apparently made no difference to its efficacy; but by the fourteenth week the RMB suspension was losing its ability to absorb the conjugate and inhibit the reaction. Applied to doubtful diagnostic tests, this would have resulted in the inability to identify non-specific fluorescence as such. Premixing the conjugate and RMB suspension before freezing down in test aliquots gave excellent inhibition of positive reactions. The last pair of aliquots tested, after 9 months' storage at −20°C, gave excellent results; the smear stained with conjugate NMB mixture was clearly positive; the RMB aliquot gave the hoped for negative result. This third system of storage has now been incorporated into our routine FA test for rabies.

The method is also very convenient when one has to perform a single test. Take two bijou bottles from the freezer—one containing 0·2 ml conjugate/NMB, the other conjugate/RMB, thaw the contents, pipette on to the smears, then incubate, wash and mount in the usual way. Two pairs of bottles will suffice for three tests. Time is saved at the moment of operation, when the situation may be urgent. Stock can be assessed at a glance.

## ACKNOWLEDGEMENT

The authors are grateful to Mrs. Mary Robins for technical assistance.

## REFERENCES

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La durée de conservation des réactifs biologiques utilisés dans le diagnostic de la rage par immunofluorescence.

*Résumé*—Trois méthodes de conservation des globulines antirabiques conjugées à la fluorescéine et des suspensions de cerveaux de souris ont été étudiées afin de déterminer leur effet sur la sûreté du diagnostic de la rage par les anticorps fluorescents. Les résultats les plus fidèles ont été obtenus avec des mélanges preparés à l'avance de conjugé et de suspension de cerveaux de souris, distribués en quantités aliquotes et conservés à −20°C jusqu'à leur emploi. Après 9 mois de conservation, ces réactifs donnent des résultats satisfaisants sur des préparations témoins positives.

La conservación de reactivos biológicos usados en la prueba de los anticuerpos fluorescentes para el diagnostico de la rabia.

*Sumario*—Se investigaron tres sistemas de almacenamiento para globulina antirrábica conjugada con fluoresceina y para suspensiones de cerebro de ratón con el propósito de hallar el efecto que estos tienen sobre la prueba de los anticuerpos fluorescentes en rabia. El conjugado pre-mezclado y la suspensión de cerebro de ratón dispensados en cantidades alícuotas y mantenidas a −20°C hasta ser utilizadas, dieron los mejores resultados. Despues de 9 meses de almacenamiento los reactivos dieron una prueba satisfactoria con froticez positivos conocidos.