Production of rabies fluorescent conjugate by immunization of rabbits with purified rabies antigen*

C. V. TRIMARCHI [1] & J. G. DEBBIE [2]

Fluorescein-labelled antirabies virus conjugate was produced by hyperimmunizing rabbits with a purified rabies virus (CVS-27). The conjugate had a working dilution of ⩾1 : 500 in the rabies fluorescent antibody test. Stability under lyophilization was satisfactory.

The fluorescent antibody test (FAT) for diagnosis of rabies is an established procedure of proven accuracy and specificity. The conventional procedure for conjugate preparation, as well as for the performance of the test, is given in detail by Dean & Abelseth (1). The production of high-titre conjugates using rabies virus nucleocapsid has been reported by Atanasiu et al. (2). A new method of preparing the antirabies conjugate by immunizing rabbits with purified CVS rabies virus is described here.

MATERIALS AND METHODS

Virus

The antigen used for immunization was a 20% (w/v) weanling mouse brain suspension of CVS-27 in physiological saline. The titre of the virus suspension was $10^{6.8}$ mouse intracerebral LD_{50} ($MICLD_{50}$) per 0.03 ml in Nya:NYLAR mice weighing 10–12 g.

Purification

Five hundred ml of a 20% antigen suspension in phosphate-buffered saline (PBS) at pH 7.4 was centrifuged at 1 000 *g* for 15 min. The supernatant was purified and concentrated by batch adsorption on aluminium phosphate gel at pH 7.1–7.3, followed by elution at pH 8.0, as described by Schneider (3). The final virus preparation was suspended in 5 ml of tris–EDTA–NaCl buffer. The titre in 10–12-g mice was $10^{8.7}$ $MICLD_{50}$/0.03 ml.

Inactivation

The virus suspension was inactivated with a final concentration of 0.2% β-propiolactone in bicarbonate buffered saline at 37°C for 2 h.

Immunization

The rabbits were 6-week-old Flemish giant-grey chinchilla crosses from a closed colony maintained at the Division of Laboratories and Research. The immunization procedure involved 4 weekly intraperitoneal injections of a thoroughly mixed suspension of 1 ml of inactivated virus and 1 ml of Freund's incomplete adjuvant, followed by 2 intraperitoneal injections of 1 ml of live virus at weekly intervals. Ten days after the last injection the rabbits were anaesthetized and exsanguinated by cardiac puncture. After clot retraction the serum was separated by centrifugation at 1 500 *g* for 15 min.

Conjugation

The globulin fraction of the serum was precipitated at 4°C by the addition of an equal volume of saturated ammonium sulfate. After 1 h the preparation was centrifuged at 4 000 *g* for 30 min. The protein was labelled with fluorescein isothiocyanate by the method described by Dean & Abelseth (1) with the exception that conventional dialysis was replaced by diafiltration with an Amicon [a] Model 202 stirred ultrafiltration cell with a PM 30 selective membrane.

Fluorescent antibody test

The FAT was performed with fluorescent microscope equipment as described by Dean & Abelseth (1).

* From the Division of Laboratories and Research, New York State Department of Health, Albany, NY, USA.

[1] Senior Bacteriologist.

[2] Associate Research Scientist.

[a] Amicon Corp., Lexington, MA, USA.

RESULTS

Prior to conjugation the antisera had neutralization titres of $\geqslant 1:5000$ by the tissue culture-fluorescent antibody method of Debbie et al. (4). Fig. 1 illustrates that the optimum dilution of the conjugate for the FAT as determined by microfluorimetry (5) was in the range 1 : 510–1 : 570. Conjugated antisera were lyophilized in appropriate amounts (generally 1 ml) and stored at −70°C. Before use the conjugate was reconstituted with PBS containing thiomersal 1 : 10 000, buffered to pH 7.6. Lyophilization caused no measurable loss of immunofluorescence, and the quality of the conjugate was stable for several weeks when stored at a working dilution of 1 : 500 at 4°C. Our laboratory has used this conjugate routinely for over 1 000 field cases submitted for rabies diagnosis.

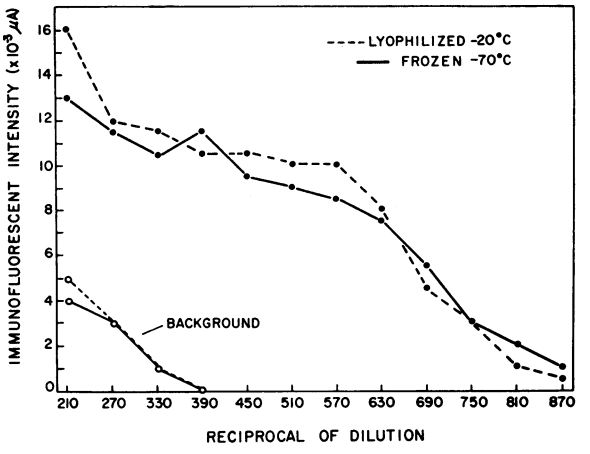

Fig. 1. Fluorescence of rabbit antirabies serum labelled with fluorescein isothiocyanate, lyophilized, and stored at −20°C or −70°C.

DISCUSSION

The use of rabbits to produce hyperimmune antisera is feasible for many laboratories that lack facilities for the large number of hamsters necessary to provide an equivalent amount of serum or for housing goats or equines.

Production of conjugate by the use of purified virus antigen has three major advantages over conventional methods: (1) The immunizing antigen has a lower level of tissue protein, which obviates the need for normal mouse brain adsorption; (2) the greater dilution of the conjugate reduces the chance of nonspecific fluorescence; and (3) the high working dilution affords a darker background and greater contrast.

The potency of the antibody is such that up to 3×10^4 ml of working conjugate can be obtained from a single rabbit, which permits consistent results at low cost. A large number of laboratories can be supplied with lyophilized conjugate of consistent quality in amounts large enough to enable many examinations to be made from a single lot.

RÉSUMÉ

PRODUCTION D'UN CONJUGUÉ RABIQUE FLUORESCENT PAR IMMUNISATION DE LAPINS AU MOYEN DE VIRUS RABIQUE PURIFIÉ

Le virus rabique CVS purifié par adsorption et élution sur gel de phosphate d'aluminium est utilisé afin d'immuniser des lapins pour la production de sérums antirabiques hyperimmuns. Les antisérums sont ensuite conjugués avec de l'isothiocyanate de fluorescéine. La dilution optimale du conjugué aux fins de l'épreuve des anticorps rabiques fluorescents est légèrement supérieure à 1 : 500. Les avantages de ce conjugué rabique par rapport aux conjugués conventionnels sont les suivants: activité accrue, de sorte que davantage d'épreuves peuvent être effectuées à partir d'un lot unique; qualité supérieure, la moindre quantité de protéines tissulaires dans l'antigène immunisant rendant moins nécessaire l'adsorption normale sur cerveau de souris; enfin utilité à une dilution plus élevée, qui réduit le risque de fluorescence non spécifique et assure un meilleur contraste dans l'épreuve des anticorps fluorescents.

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