

Use of Hamsters for Potency Assay of Eastern and Western Equine Encephalitis Vaccines

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An antigen extinction test in hamsters is described. Comparative potency assays with guinea pigs and hamsters showed the latter to be a suitable, advantageous replacement animal in these assays.

The increasing occurrence of Eastern and Western equine encephalitis (EEE and WEE) in man and animals has stimulated interest in the development of improved vaccines for these diseases (4; L. F. Maire et al., *submitted for publication*). Unfortunately, there is no standard, quantitative potency test used for quality control of production lots of these vaccines.

Early workers (2) gave guinea pigs two weekly intradermal doses followed by intracerebral challenge 12 to 14 days later. Current Department of Agriculture standards (1) still require only a similar *qualitative* test. More recently, Robinson et al. (4) suggested a quantitative, antigen extinction-type test, again in guinea pigs. The intracerebral challenge required for guinea pigs with most EEE and WEE virus strains is cumbersome and time-consuming; it also bypasses most circulating antibody and normal body defenses. For these reasons another test animal was sought. This note describes a procedure for determination of the median effective dose (ED_{50}) of EEE and WEE vaccines that has been used in our laboratory for the past 3 years.

Lakeview strain Golden Syrian hamsters (85 to 95 g), obtained from Lakeview Hamster Colony, Newfield, N.J., were employed. Two 0.5-ml doses of fivefold dilutions of vaccine were given by the intraperitoneal route on days 0 and 7. The hamsters were challenged with 10^3 to 10^4 intraperitoneal median lethal doses (LD_{50}) at 21 days after the last vaccine dose. For the assay reported here, the challenge strains of EEE and WEE virus were PE-6 and B-11, respectively. Similar results have been obtained with other strains of these viruses.

Animals were observed for 14 days after challenge; titration end points were calculated by the method of Reed and Muench (3). The ED_{50} is expressed as that volume of undiluted vaccine given in each of two doses which protects 50% of the hamsters from death after challenge.

Table 1 shows typical results of antigen extinction-type assays of two vaccines; the requisite graded response to serial dilutions of vaccine is apparent.

TABLE 1. Antigen extinction-type potency assays of WEE and EEE vaccines in hamsters

Vaccine dilution	Response to challenge ^a after two doses of vaccine (survivors/total)	
	WEE-CE ^b	EEE #4 ^c
Undiluted	10/10	10/10
1/5	10/10	10/10
1/25	7/10	10/10
1/125	1/10	6/10
1/625	0/10	0/10

- ^a Intraperitoneal challenge with 10^3 - 10^4 LD_{50} .
- ^b The ED_{50} was 0.012 ml.
- ^c The ED_{50} was 0.0031 ml.

TABLE 2. WEE and EEE vaccine potency assays in hamsters and guinea pigs

Vaccine ^a	ED_{50} (ml)	
	Hamster ^b	Guinea pig ^c
WEE #6	0.0008	≤ 0.0008
WEE-TC	≤ 0.0008	0.012
WEE-7	0.045	< 0.5
EEE #4	0.0031	0.0049
EEE-TC	0.11	0.35
EEE-1-1966	0.009	0.012

- ^a All animals given two 0.5-ml doses: intraperitoneal route in hamsters; subcutaneous route in guinea pigs.
- ^b Intraperitoneal challenge with 10^3 to 10^4 LD_{50} contained in 0.5 ml
- ^c Intracerebral challenge with 10^3 to 10^4 LD_{50} contained in 0.15 ml.

Comparative guinea pig and hamster assays were performed on lots of vaccines of various potencies. Results with vaccines of high or moderate potency (e.g., WEE 6, EEE 4) were comparable in the two test animals; vaccines of low potency (e.g., WEE-7, EEE-TC) gave lower ED₅₀ values in the guinea pig than in the hamster (Table 2).

These data indicate that the hamster is suitable for use in EEE and WEE vaccine potency tests. Moreover, the use of hamsters offers several advantages: (i) the intraperitoneal challenge route may be used, eliminating the inherent difficulties in, and objections to, an intracerebral challenge; (ii) animal costs are reduced by more than 50%; and (iii) because of their size in contrast to guinea

pigs, a greater number of hamsters may be housed in a given space.

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