Results of an inquiry on potency testing of rabies vaccine by the NIH test: suggestions for further improvement

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Summary: The NIH test, which is used to evaluate the potency of rabies vaccines, has produced highly variable results. These have created doubts as to its validity.

An inquiry was therefore conducted on the NIH test in 19 laboratories. Data was obtained regarding current applications of the NIH test along with suggestions for improvement.

KEYWORDS: International cooperation - Potency testing - Rabies - Statistical methods - Vaccines - Viral diseases.

1. The principle of the NIH test is the immunisation of mice with different vaccine concentrations, followed by challenge infection with one virus concentration of 5 to 50 LD$_{50}$/dose of the challenge virus strain (CVS) derived from the Pasteur strain. The protection rates (ED$_{50}$) of a standard and the test vaccines are calculated, and the potency of the test vaccine is given in International Units (IU).

Due to the fact that different seed virus strains are used for the production of rabies vaccines, difficulties arise from the different strain relationship between the seed virus of the test vaccine as well as the challenge virus strain and the seed virus strain of the reference vaccine.

Another factor of concern is the inherent variability of the NIH test. Because of high variability in test results, the validity of the NIH test was questioned by several groups.

In several discussions, however, it was obvious that investigators rated the reliability of the results quite differently.

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2. Therefore, an inquiry was conducted on the NIH test in 19 institutions listed below1.

A questionnaire was sent to these 19 laboratories in order to obtain data about the judgment of the NIH test, description of the test protocol, remarks and suggestions for improvement.

3. According to this inquiry, the NIH test protocol is accepted by the majority of the participants, although there are objections to the simulation of the vaccination practice. Five institutes prefer to immunise mice only once according to the modified NIH test of the European Pharmacopoeia.

Half of the participants are satisfied with the reproducibility of the test. However, the participants accept a confidence limit (95%) of ED\(_{50}\) values of 1/3 to 1/4 and respectively of three to four times the ED\(_{50}\) mean-value.

Only one NIH test is normally carried out for the determination of the IU-value of one vaccine. In a few instances, depending on the result, two or more tests with the same test vaccine are carried out.

As for the animals, only white mice of both sexes are utilised, and they are microbiologically controlled.

Three to five dilutions with the dilution factor of 5 are applied by the intraperitoneal route. A few participants try to centre the dilutions around the anticipated ED\(_{50}\) value.

In all but three institutions, reference vaccines with the virus strain PM are used. In addition, other substandard vaccines adapted to the official reference vaccines are used. The PV strain is used in three institutions. LEP and ERA are sometimes used as additional substandard vaccines, especially for scientific studies on the NIH test.

The use of the challenge virus is the same in almost all of the different institutions. Except in one case (Belgium, using the LEP strain), the CVS strain is used. However, the LD\(_{50}\) concentration of the virus differs substantially from 5 to 500 LD\(_{50}\).

Concerning the evaluation of ED\(_{50}\), one third of the participants apply the method of Reed and Muench, whereas three participants use the method of Spearman and Kärber. Approximately 50% use a logit or probit analysis with the aid of a computer.

The participants are of the opinion that this in vivo method should not be abolished but should be modified to reduce the variability of the test and to adjust the method to the newer inactivated tissue culture rabies vaccines.

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1. Participating institutions: Paul-Ehrlich Institut, Frankfurt, FRG; Food and Drug Administration, Bethesda, USA; Mediterranean Zoonoses Control Centre, Athens, Greece; Behring-Werke, Marburg, FRG; Connaught Laboratories, Ontario, Canada; Norden Laboratories, Lincoln, USA; Institut Pasteur Production, Marnes-la-Coquette, France; Bundesamt für Veterinärwesen, Eidgen, Switzerland; Vakzine-Institut, Basel, Switzerland; Institut Mérieux, Charbonnières-les-Bains, France; Ministère de l'Agriculture, CNEVA, Laboratoire d’Études sur la Rage, Malzéville, France; IFFA Mérieux, Lyons, France; Fromm Laboratories, Grafton, USA; Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen, FRG; Institut Pasteur, Rabies Unit, Research Laboratories, Paris, France; Institut Pasteur, WHO Collaborating Centre for Reference and Research in Rabies, Paris, France; Impfstoffwerk Wellcome, Grossburgwedel, FRG; Institut für Medizinische Virologie und Immunologie, Klinikum Essen, FRG; Institut National de Recherches Vétérinaires, Brussels, Belgium.
They are also of the opinion that rigid selection of the breeding farm as well as the use of animals of the same age and sex may reduce the variability.

Concerning the vaccination scheme, the participants are in favour of a single vaccination and change to intramuscular application. Proposals to increase the vaccine dilutions as well as the animal numbers per dilution, which would increase the number of animal experiments, may also increase the reliability of the result.

For mathematical reasons, the Reed and Muench method should no longer be used for evaluation. The Spearman/Kärber method gives good results if the dilutions are centred around the mean ED$_{50}$ value. As computers are now available at low prices, the calculation of the IU value should not be reduced to the one point ratio, and logit, probit and parallel line bioassay (e.g. Finney) could be used. The Bayesian statistic might also be introduced, especially for standardisation of reference vaccines and substandards.

Laboratories which are engaged in NIH tests should show that the ED$_{50}$ value of the reference vaccine falls continuously in a range prescribed by an expert group. Only those reference vaccines which show a steep dose response curve in a collaborative study in *in vivo* and *in vitro* tests, should be licensed.