Vaccine control

Feasibility of a serological potency assay for rabies vaccines for human use

The method currently recommended to determine the potency of rabies vaccines, the NIH mouse protection test, is demanding to perform and causes significant suffering to experimental animals. WHO initiated a feasibility study in order to determine whether a simpler serological assay developed at the Paul-Ehrlich-Institut (PEI) may be a suitable alternative for use by vaccine control laboratories globally. The findings suggest that the serological assay is promising but needs further verification.

Introduction
The National Institute of Health (NIH) potency test, which is based on a mouse protection assay, is used to determine the potency of rabies vaccines.\(^1\) However, only a limited number of national control laboratories are performing this assay due to its high requirements in terms of safety and technical capability. Moreover, this assay requires high animal numbers and causes severe suffering to the test animals. It is also known to have high variability and can be problematic in terms of meeting all of the validity criteria.\(^2\)

In the interest of refining, reducing and replacing animal testing (3R principle) the Paul-Ehrlich-Institut (PEI) has developed an alternative method \(^3, 4\) that could potentially replace the rabies mouse challenge test. The proposed method is a multi-dose serological assay, based on vaccination of mice and subsequent determination of neutralizing antibodies \textit{in vitro}.

The WHO Technical Assistance and Laboratory Services (TAL) Group initiated and coordinated a small scale feasibility study to evaluate whether this alternative assay can be successfully used for the potency determination of rabies vaccines for human use, and whether the test protocol can be transferred and applied at other laboratories.

This short communication was contributed by Dr Ute Roskopf of the WHO Regulatory Systems Strengthening Team with input from Mrs Monika Zweygarth. It reflects the outcomes of a WHO-PEI Feasibility study to determine the potency of rabies vaccines by serology.

The testing was conducted at the Institute of Biological Products (IBP), Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand, and at the Paul-Ehrlich-Institut (PEI), Laboratory for Product Testing of Immunological Medicinal Products for Veterinary Use, Langen, Germany.

Study directors:
Dr Ute Roskopf, WHO Technical Assistance and Laboratory Services (TAL) Group (rosskopfu@who.int): Study initiation and coordination.
Dr Beate Krämer, Paul-Ehrlich-Institut (Federal Institute for Vaccines and Biomedicines): Development of study protocol and training of technical staff.
Method
The study was performed at PEI and at the Institute of Biological Products (IBP) in Thailand. Training was provided to ensure the appropriate and timely performance of the testing.

Each of the two participating laboratories was asked to test a panel of three prequalified rabies vaccine samples against a standard vaccine (the WHO 6th International Standard for rabies vaccine), using the serological assay. One of the three test vaccines was heat-treated in order to induce a sub-potent quality. This sub-potent vaccine was tested using both serology and the NIH mouse protection test.

Each test vaccine was investigated in two independent test runs at four dilutions, against four equivalent dilutions of the standard vaccine. A fifth dilution of the standard vaccine equivalent to the minimum required potency (2.5 IU/ml) was added to investigate the suitability of a single-dose approach (cut-off test).

Groups of mice were immunized twice with either the test vaccine or the standard vaccine. Each dilution of each vaccine was administered into 10 mice.

Seven days after the second immunization blood samples were taken and the sera were tested for rabies antibodies using the proposed virus neutralization assay, a modified rapid fluorescent focus inhibition test (RFFIT).

The statistical analysis was performed at PEI and by Dr Stanley Norris Deming, Statistical Designs, Houston. Individual titers as well as pool sera were evaluated with a parallel line model to obtain a potency value for each sample relative to the WHO standard vaccine (with an assigned potency of 8 IU/ml).

Findings – in brief
While differences with regard to estimated potencies were observed between the participating laboratories, a vaccine dose-dependent immune response of rabies antibody titres could be shown in almost all of the tests. The results of the NIH tests (manufacturer’s results at time point of release) could only partly be confirmed by the serological assay. This comes as little surprise since the comparison is based on the measurement of two different parameters: survival rates are enumerated with the NIH test, whereas antibody responses are determined in the serological assay.

Development of a single dilution assay (cut-off test) did not seem feasible as the serum activities obtained with the standard, adjusted to the minimum potency, were higher than those obtained with the test vaccines in all tests.

When potencies were calculated for individual sera, 3 out of 12 tests gave estimated curves that fulfilled the validity criteria for both linearity and parallelism. When potencies were calculated for serum pools, parallelism was observed in all cases, provided that deviations from linearity were accepted.

The sub-potent vaccine was correctly classified by means of the NIH test at both laboratories. In the serological assay, when analyzing serum pools, the sub-potent quality of the vaccines was correctly assigned in 3 out of 4 cases.

Conclusions
Routine testing of individual sera using the serological assay does not seem appropriate due to the high number of plates to be handled and the difficulty to meet the current validity criteria. Testing of serum pools seems preferable. This
was a first feasibility study. The suitability of the serological assay for the potency quantification of rabies vaccines for human use would need to be further proven.

A further, exhaustive publication on the study is in preparation.

References


