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CRITICAL REVIEW OF THE NIH-METHOD FOR TESTING POTENCY OF INACTIVATED RABIES VACCINES

High lethality from rabies for people and animals determines a large value of prevention of this infection, where a leading role is taken by vaccines, from this, what vaccine is used and what its efficiency is, depend the success of the measure [3, 10].

In connection to this an important value is acquired by the development and use of facilities for the estimation of quality of the antirabic inactivated vaccines.

Efficiency of estimation of the inactivated antirabic vaccines during a long period of time is in the article of permanent researches, discussions and publications [1–3, 7, 10, 15]. It is difficult to find a simple method that would satisfactorily determine all necessary parameters of the inactivated vaccines. Therefore a question about the development of methods of control of the inactivated vaccines remains open.

Review. The most reliable method of testing of vaccines is the determination of protective activity of medication on animals that they are intended for (having special purpose animals). However the traditional analysis of vaccine preparations requires substantial quantity animal and material expenses. In addition to this, during many years the methods of testing vaccines on laboratory animals are used as an alternative by a having a special purpose animal.

At the same time some authors specify on the advantages of the use of laboratory animals, as the formed level of immunity for having a special purpose animal, even low levels, prevents the development of disease after an infection [16]. From laboratory animals white mice have been compared to having special purpose animals as they are more often used, easier to maintain and grow. As a reference-strain the recommended WHO is using strains of «CVS» [10, 12, 13].

Currently, there are several different methods of testing the immunogenicity of rabies vaccines, but the most effective is the Habel- method and the NIH-test (National Institutes of Health, USA) [9, 12, 13, 16]. The first type relates to a test carried out on a «breakthrough» immunity all animals which receive the same immunizing dose are then inoculated with various doses of the virus. NIH method refers to a type test based on titration of antigen, which varies the amount of vaccine administered, and the dose remains a constant challenge [13].

The NIH is the method most widely used and recommended by the WHO. This test was developed in the USA in 1953 and is used in unmodified form till this day [3, 11, 16].

On the one hand, this method is used by nearly all production and control laboratories in the all countries [3, 9, 11], it allows you to define the protective effect of the drug on experimental animals and has a positive effect on immune prophylaxis of rabies, since the immunological response to a certain extent dependent on the activity rabies vaccine.

On the other hand, many researchers [1–3, 7, 9] noted drawbacks to this method: **a)** intracerebral infection does not follow the natural route of transmission of the rabies virus [5], **b)** there is an insufficient correlation between the test and the level of neutralizing NIH antibodies [3, 15], **c)** for intracerebral administration uses a fixed strain CVS, while its pathogenesis and neurotropism significantly differs from most field strains [5], **d)** a traditional method involves the use of 2 injections at day 0 and day 7, and a second booster injection, the experiment results are masked, resulting in weaker activity of the vaccine will correspond to the strong [5, 7, 14], and **e)** determining the correlations when there is low activity of the vaccine by NIH, obtained from different strains [17]. Moreover, the test requires a large amount of mice, prolonged (28 days) and involves the use of infectious virus. In addition there is a large (factor varying from 1 to 10) variation of test results not only between different laboratories, but also within the same. Many researchers believe that the main reasons for the wide variety and not enough high level of reproducibility of the results is: – the type of vaccine and the production strain [3] – the use of different reference drugs [10] – the use of different strains of mice – the nature of the method (intraperitoneal immunization and intracerebral challenge) – Subjective assessment – the use of different levels of individual passages of the virus (CVS) for infection [3, 10].

The problems associated with determining the activity of the test vaccines using NIH, is discussed at many meetings of the WHO [3, 6, 7, 10]. Due to the fact that as the control strain of infection with the test formulation recommended NIH strain CVS, a vaccine produce from viruses, non-strain Pasteur, under the control mice infection, may be less active than expected [3, 10] .

Experiments of some authors showed that the study of protective and immunogenic activity against rabies vaccine produced from strains of PM, SAD, Flury LEP, Vnukovo 32, Moscow and use for homologous challenge strains, vaccine potency was higher when using homologous strain [3, 8, 10, 15].

In addition, test conditions for the control NIH vaccines differ from the conditions of the target animals vaccinated in practice, even the same drug, leading to

significant errors in the assessment of the activity of vaccines [2, 17].

Based the **objective** of our work a critical analysis and an experimental solution of some of the shortcomings of the test NIH by selecting the number of vaccinations, and a method of vaccination and allowing infection.

MATERIALS AND METHODS

The NIH test. The Immunogenic activity of rabies vaccines studied classical method National Institutes of Health (NIH). For the assay, groups of 16 mice were formed, weighing 12–14 g, where administered intraperitoneally at 0,5 cm^3, vaccines in dilutions of 1:5, 1:25, 1:125, 1:625 within an interval of 7 days. At the expiration of 14 days after the first vaccine injection, spent intracerebral challenge of a standardized virus strain CVS, at a dose of 50–100 MLD_{50}/0,03 cm^3. The next 14 days spent observing the condition of the animals. The effective immunizing dose was calculated by the Reed and Menche, which are expressed in a relative to the national reference vaccine in IU.

Vaccines. The culture inactivated rabies vaccine – Rabizin (Russian Federation) – a vaccine produced from a strain Schyolkovo-51 (lots 023–025);

In the experiments, a national reference of immunogenicity of rabies vaccines was used, calibrated relative to the 4th international standard, with an index of 1,2 IU/ cm^3 immunogenicity.

Studies. *Trial 1.* This experiment included **a single intraperitoneally** injection 0,5 cm^3, into white mice, weighing 12–14 g, in standard dilutions. At the expiration of 14 days spent challenge intracerebral injection of the fixed rabies virus, strain CVS, at a dose of 50–100 MLD_{50}/0,03 cm^3. The next 14 days spent observing the condition of the animals.

Trial 2. This was carried out in an experiment which confirms the activity of vaccines under test and the NIH «peripheral test». Of modification was as follows: The trial and the reference vaccines were administered in dilutions of 1:5, 1:25, 1:125, 1:625 **intramuscularly** into white mice, weighing 12–14 grams, in volume – 0,15 cm^3. On day 21 after the vaccination, mice were challenged in the **subcutaneous** tissue of the upper lip fixed rabies virus strain «CVS» in a dose of 10–50 MLD_{50}/0,15 cm^3, and observed for 14 days. Then calculated ED_{50} and conducted a comparative analysis of the results obtained by the traditional method of NIH.

RESULTS

1. *Modified method of NIH due a single injection.* The results of comparative testing of vaccines traditional and modified NIH test are shown in Tab. 1.

Table 1 – **The results of comparative testing of anti-rabies vaccine test NIH single- and double immunization (n=3)**

Lots of vaccines	Multiple injections	lg ED_{50}	Potency, IU/cm^3*
023	2-fold	2,71±0,15	1,82
	1-fold	1,59±0,20	0,93
024	2-fold	2,12±0,18	0,47
	1-fold	1,43±0,10	0,64
025	2-fold	2,63±0,21	1,52
	1-fold	1,57±0,13	0,89
Reference	2-fold	2,45±0,15	1
	1-fold	1,62±0,20	1

The data show that the ED_{50} figure in a single injection (modification NIH) vaccine preparation was lower than twice (test NIH). Relative immunogenicity of the second lot was higher with a single injection. Potency of first and second lots of vaccine was below the minimum requirements for inactivated rabies vaccines **(1 IU).**

In both cases (modified and classic NIH), in accordance with the protocol of the test vaccine, used singly and doubly standard sample. In order to determine the level of compliance and results obtained by modification of the traditional test NIH spent correlation analysis (r= 0,99).

2. *Modification of the method by using the NIH «peripheral test».* The research results are shown in Tab. 2.

The data show that the ED-50 figure in a single injection (modification NIH) vaccine preparation was lower than twice (test NIH). Relative immunogenicity of the second lot was higher with a single injection. Potency of first and second lots of vaccine was below the minimum requirements for inactivated rabies vaccines **(1 IU).**

The data show that the immunogenicity of the third lots of vaccine, proven «peripheral» test, was 0,89 IU/cm^3, while in testing activity by NIH – 1,52 IU/cm^3. The immunogenicity of 1 and 2 lots did not differ. The correlation of results by «peripheral» test and the traditional method of NIH was 0,90.

DISCUSSION

The key to the success of rabies control is specific prevention, for which highly immunogenic inactivated rabies vaccine is used. Justification of NIH test improvements in the experiments that are represented by us is that:

1. At the traditional NIH testing, that includes twice repeated immunization – the second vaccine injection is a booster injection (shielding), which enhances the immune response to vaccines activity disproportionately and on this basis the final drug activity is overvalued.

2. At the testing there is a mismatch of conditions of practical vaccine application (intramuscular) and of potential route at bites in nature (there is no intracerebral injection in nature) and of control conditions of rabies vaccine immunogenicity (mice are immunized intraperitoneally, and definitive infection – intracerebral). This fact indicates discrepancy between

Table 2 – **The results of the potency of vaccine by «peripheral» and by NIH tests (n=3)**

Lots of vaccine	Test NIH*		«Peripheral» test*	
	log ED_{50}	potency, IU/cm^3	log ED_{50}	potency, IU/cm^3
023	2,71	1,82	2,25	1,4
024	2,12	0,47	1,87	0,58
025	2,63	1,52	2,05	0,89
Reference	2,45	1	2,1	1

delivery of viral antigen at vaccination and its control.

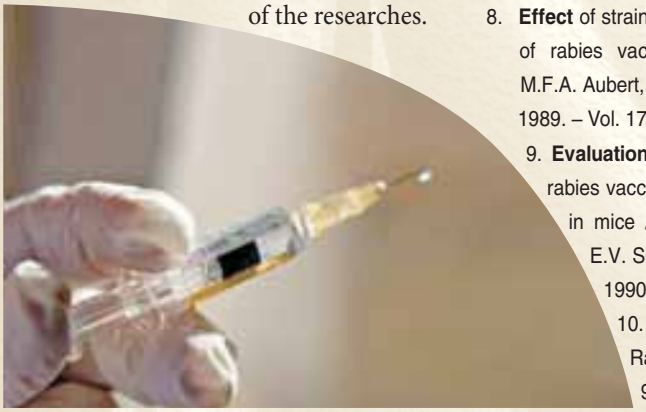
In analyses of the foregoing traditional NIH method improvement was held by using a single immunization of mice, instead of the traditional twofold. In experiments using a single immunization of mice it was established that ED_{50} for a single injection (NIH modification) was lower than at twice repeated which is natural. Nevertheless, the relative immunogenicity at the comparison of both methods was almost unchanged, due to the fact that standard absolute activity, with respect to which the vaccine activity is calculated in the IU, proportionally decreased at reducing of absolute vaccines activity.

We believe such improvement eliminates the disadvantages of the NIH test, especially as the analysis of the results showed that the correlation coefficient between the modified and conventional methods NIH was 0,99.

In the second experiment intramuscular immunization of mice and the subcutaneous tissue of the upper lip infection was used «peripheral test». The result shows a high correlation with the NIH-test (r = 0,90).

The advantage of this modification is that the method practically repeats the natural administration route of vaccine antigen and the penetration of street virus at animals infecting in nature.

Despite the small amount of drugs studied with the help of system of NIH test improving, the test shortcomings and way to eliminate them were clearly shown. Moreover, the analysis of the literature indicates the correctness of our point of view, and the authenticity of the direction of the researches.



Thus, there are shown theoretical and experimental possibilities of traditional NIH test replacing by modification which is proposed, and has a theoretical basis and can be used in practice since after the large-scale tests.

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Критичний аналіз методу NIH для оцінки активності інактивованих антирабічних вакцин. *В.В. Недосєков*

Представлено критичний аналіз методу NIH для оцінки активності антирабічних вакцин і вказано на низку суттєвих недоліків.

Проведено теоретичне обґрунтування й експериментальне вирішення двох модифікацій цього методу, показано можливість суттєвого вдосконалення даного тесту при високому рівні відповідності (кореляція 0,90–0,99).

Критический анализ метода NIH для оценки активности инактивированных антирабических вакцин. *В.В. Недосеков*

Представлен критический анализ метода NIH для оценки активности антирабических вакцин и указан ряд существенных недостатков.

Проведено теоретическое обоснование и экспериментальное решение двух модификаций этого метода и показана возможность совершенствования данного теста при высоком уровне соответствия (корреляция 0,90–0,99). ◌