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REPORT OF WHO CONSULTATION ON POTENCY TESTS  
FOR RABIES VACCINES

Geneva, 7-8 December 1987

1. Overview of the present situation

A consultation on the potency testing of rabies vaccine was held in Geneva from 7-8 December 1987. The reports of previous WHO meetings on rabies vaccine potency testing held in 1982<sup>1</sup> and 1985<sup>2</sup> were reviewed. A common goal of these meetings was to determine if the NIH test or European Pharmacopoeia test should be modified, complemented, or replaced because of poor reproducibility and a lack of correlation to host animal immunogenicity. This latter problem is especially true when vaccines of different strains are compared. If a replacement test is established, it should be one that could be applied worldwide and one in which vaccine potency is expressed in international units (I.U.) or equivalent units (E.U.).

2. Status of present vaccine testing

To obtain information on the acceptability of the NIH test as a means of measuring the potency of inactivated rabies vaccine, a questionnaire was sent to six national control laboratories, eight vaccine producers, and three scientific institutions. The questions asked were directed at defining the test protocol used in each laboratory and at determining the acceptability of the test in general. The responses indicated that the test is generally accepted as the test of choice but only half of the respondents were satisfied with the reproducibility of the test. There was disagreement in the number of vaccinations that should be utilized, and five laboratories were using only one vaccination as recommended by the European Pharmacopoeia. The general opinion of the respondents to the questionnaire was that the test should not be abolished but should be modified to reduce the variability and to more accurately measure the potency of the different vaccine types.

<sup>1</sup> Report of Discussions on Improvement of Potency Tests for all Types of Rabies Vaccines and of Potency Requirements for Animal Rabies Vaccines, Geneva, 23-24 September 1982, Document WHO/Rab.Res./82.17.

<sup>2</sup> Report of a WHO Workshop on NIH Potency Test for Rabies Vaccines, Geneva, 4-6 November 1985, Document VPH/86.63.

In addition to the results of the questionnaire, the participants at the consultation provided data that addressed possible sources of test variability. These include: (1) age of mice (four-week old mice were recommended regardless of weight); (2) strain of mice; (3) consistency of mouse brain challenge material; (4) interval between time of vaccine reconstitution and time of dilution; (5) interval between time of challenge preparation and mouse inoculation, and most importantly (6) strain of challenge virus used.

### 3. Methods available and being developed

3.1 In vivo. The European Pharmacopoeia test was discussed. It was felt that this test has a higher degree of variability than the 2-dose NIH test and as a consequence the correlation of the potency values observed with the two tests is poor. This is especially true of vaccine with low relative potencies. Suggestions for improving the tests include:

1. Calculating the ED<sub>50</sub> of the reference preparation by Bayesian analysis.
2. Improving the stability of the reference by requiring that the next reference be at least one year old at the time of evaluation. This would eliminate the initial drop in potency.
3. Standardize the preparation of the CVS challenge virus.
4. Using additional lower dilutions of the reference and vaccine to more accurately estimate the ED<sub>50</sub>.
5. Prepare the next reference in larger quantities of an antigenic value greater than or equal to the present reference.

The last two suggestions apply only to the European Pharmacopoeia test.

A simplified NIH test which utilizes only one dilution and 10 mice was also discussed. It is a qualitative test that was shown to be a reliable predictor of vaccine with potency values of  $\geq 1$  I.U. The simplified test has been used for five years in France, during which time over 90% of the vaccine tested had relative potency of  $\geq 1$  I.U.

The last in vivo test system discussed was that of the Centers for Disease Control (CDC).

The results of this test were shown to correlate better than those of the NIH with the immunogenicity as conferred by the different vaccine types in the host animal. This may be due to the ability of the CDC test to measure adjuvant effect while the NIH test cannot. However, the CDC test possesses a high degree of variability and may have to be modified before it will be useful for the routine measurement of vaccine potency.

3.2 In vitro test. Direct enzyme immunoassays have been developed to quantify the rabies virus glycoprotein in viral suspensions and in vaccines. They can be used to establish the potency value of a vaccine and for the "in process" control of vaccine production. These tests are conducted with either an anti-glycoprotein polyclonal sera (Essen) prepared with purified glycoprotein of different vaccine strains (ERA, LEP and PV) or murine neutralizing monoclonal antibody (Paris) specific for PV, ERA and HEP. Both tests are highly reproducible and accurate and the results are obtainable within five hours. They can detect at least 0.5 mcg/ml of glycoprotein and 0.01 IU/ml.

A reference vaccine of known relative potency by the NIH test is included in each test so that the glycoprotein content can be expressed in equivalent units by graphic or computer analysis. Both veterinary vaccines (with or without adjuvant) and human vaccines can be quantitated by at least one method. The correlation between the potency values obtained by either of the direct enzyme-immunoassay tests and those obtained by the NIH potency test are satisfactory.

#### 4. Specific problems linked with the challenge strain

Antigenic differences between rabies virus, namely challenge virus standard (CV SAD, and Flury (LEP or HEP) strains were studied by cross-challenge tests of vaccine produced from these strains, and by cross-neutralization tests performed on sera of mice immunized with these vaccines. A wild (fox) virus strain was also used as control. Changing the virus strain affected the potency values obtained in either the standard NIH test or the European Pharmacopoeia test. The effect of changing the challenge virus was not as great in the cross-neutralization tests conducted on the mouse sera.

#### 5. Recommendations for the correlation between potency value of vaccine and its immunogenicity in animals and man

5.1 In animals. The actual protective value of any vaccine to be licensed should be established on the basis of a vaccination challenge test in the target species. These results should show that at least 18 of 20 vaccinated animals are protected against a street rabies challenge which kills at least 80% of the controls. The challenge should be done at the end of the period of immunity to be claimed by the vaccine producer.

The potency value of the batch of vaccine used in the target animal immunogenicity test shall be assessed by either the NIH test or the European Pharmacopoeia test. The value obtained shall become the minimum value for all subsequent serials of vaccine except that it can be no lower than the WHO recommendation of 0.3 IU/ml by the NIH test.

If an in vitro test is approved for serial release, the minimum equivalent value required by this test shall be correlated to the established minimum NIH test value as established above.

5.2 In humans. There are three major problems concerning the testing of individual batches of human rabies vaccines:

- a) classical types of brain vaccines which are still widely produced in developing countries are often not subjected to the NIH or Habel potency tests because of the lack of mouse colonies;
- b) suckling mouse brain, modern tissue culture, and purified duck embryo vaccines often call for a more accurate test than the NIH test in which the results may vary within a range 1 log 10;
- c) vaccines produced from virus strains other than PM or CVS virus derived seed material appear to be discriminated against by the intracerebral challenge with CVS strain utilized in the NIH test.

For example, a study conducted with homologous and heterologous challenge systems indicated challenge with the CVS strain resulting in a lower antigenic value of LEP origin vaccine. However, ELISA, SRD, and antibody binding tests are not affected as much by heterogeneity within their test systems.

Apart from the disadvantages of the NIH test, it represents the only standardized protection test in laboratory animals and it is the only test by which the International Unit of antirabies vaccine for humans is presently defined

In the development of new rabies vaccines and schedules of vaccine application new approaches have become common practice. Within the last decade, modern tissue culture vaccines have set new standards for the rapidity and duration of the immunity. In spite of research data on the role of cellular immunity, the evaluation of the antibody profile in human patients has become common practice for vaccine comparison.

From the above observations, the following recommendations could be made for the establishment of human vaccines and the routine testing of vaccine batches:

- a) A newly developed vaccine should prove that it induces in human subjects an antibody response which is not less in rapidity, level, and duration than that induced by vaccines proven to be effective in post-exposure treatment by the 2.1.1 schedule.
- b) Once the antibody profile is established as being adequate, the vaccine potency should be compared with that of the international or national reference vaccine by the use of the NIH test as well as an approved in vitro test. For this purpose, the ELISA test should soon be standardized internationally. The antibody binding test could also be applied but it needs a tissue culture system and infective virus. The radial diffusion test is an alternative though the ELISA test is simpler, quicker, and more sensitive. The minimum potency of the vaccine in the term of equivalent units should be specified by the above test.
- c) The same test system should be applied for each new production batch of the vaccine and the potency should be equal to or greater than the value established in (b) above.

NOTE: Antibody profiles as stipulated under (a) above should be examined in a test system which employs the same indicator virus for both the reference vaccine and the vaccine under test. Limited data indicate that the virus strain utilized does not appear to play a significant role in the result obtained.

Similarly, the antibody used in the in vitro potency test of the vaccine is not of great significance but it should be ensured that at least one of the tests (antibody profile or vaccine potency) employs a Pasteur-derived reagent.

## 6. Needs for future research

### 6.1 Future research on vaccines for veterinary use

It has been established that when all strains of vaccine are considered, the correlation between the NIH test results or the European Pharmacopoeia test results and host animal immunogenicity is affected by the strain of challenge virus utilized. The CVS virus which is presently approved appears to result in the lowest level of correlation. The optimal challenge strain should be determined. A host animal protection test with vaccine produced from the various strains should be conducted and the relative immunogenicity of the vaccines established by serological monitoring and challenge. Potency test results in mice using different vaccine challenge strains should be obtained by either the NIH or the European Pharmacopoeia test. Consideration should be given to changing the challenge virus from the CVS to the strain which provides the best correlation with host animal immunogenicity. In this way, the strain bias that is inherent in the present potency tests could be reduced if not eliminated.

### 6.2 Future research on vaccine for human use

Until now the only way to evaluate the efficiency of immunization of humans is to measure the level of neutralizing antibodies. However,  $T_1$  lymphocyte response is definitely induced by vaccination, and some researchers suggested that the  $T_1$  lymphocyte response would be a good indicator of immunity. Therefore, it would be of great interest to research the level and quality of  $T_1$  lymphocyte response following vaccination. Another area of further research should be directed towards establishing the level of immune response elicited by virus nucleocapsid.

Rabies kills approximately 50 000 people per year in the developing world. In these countries, due to other infections and under-nourishment, the immune response may be lower than in the previous studies which were mainly conducted in Europe in young and healthy adults. It may be of interest to study the antibody profile in the developing countries after vaccination and compare it with the results obtained in the studies in Europe.

ANNEX I

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