Transgenic mice as an alternative to monkeys for neurovirulence testing of live oral poliovirus vaccine: validation by a WHO collaborative study

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Objective Extensive WHO collaborative studies were performed to evaluate the suitability of transgenic mice susceptible to poliovirus (TgPVR mice, strain 21, bred and provided by the Central Institute for Experimental Animals, Japan) as an alternative to monkeys in the neurovirulence test (NVT) of oral poliovirus vaccine (OPV).

Methods Nine laboratories participated in the collaborative study on testing neurovirulence of 94 preparations of OPV and vaccine derivatives of all three serotypes in TgPVR21 mice.

Findings Statistical analysis of the data demonstrated that the TgPVR21 mouse NVT was of comparable sensitivity and reproducibility to the conventional WHO NVT in simians. A statistical model for acceptance/rejection of OPV lots in the mouse test was developed, validated, and shown to be suitable for all three vaccine types. The assessment of the transgenic mouse NVT is based on clinical evaluation of paralysed mice. Unlike the monkey NVT, histological examination of central nervous system tissue of each mouse offered no advantage over careful and detailed clinical observation.

Conclusions Based on data from the collaborative studies the WHO Expert Committee for Biological Standardization approved the mouse NVT as an alternative to the monkey test for all three OPV types and defined a standard implementation process for laboratories that wish to use the test. This represents the first successful introduction of transgenic animals into control of biologicals.

Keywords Poliovirus vaccine, Oral/toxicity; Mice, Transgenic/physiology; Macaca mulatta; Nervous system/virology; Virulence; Sensitivity and specificity; Reproducibility of results; World Health Organization; Comparative study; Validation studies (*source: MeSH, NLM*).

Mots clés Vaccin antipoliomyélitique Sabin/toxicité; Souris transgéniques/physiologie; Macaca mulatta; Système nerveux/virologie; Virulence; Sensibilité et spécificité (Epidémiologie); Reproductibilité des résultats; Organisation mondiale de la Santé; Etude comparative; Etude validation (*source: MeSH, INSERM*).

Palabras clave Vacuna antipolio oral/toxicidad; Ratones transgénicos/fisiología; Macaca mulatta; Sistema nervioso/virología; Virulencia; Sensibilidad y especificidad; Reproducibilidad de resultados; Organización Mundial de la Salud; Estudio comparativo; Estudios de validación (*fuente: DeCS, BIREME*).

الكلمات المفتاحية: اللقاح الفموي لشلل الأطفال، سمية لقاح شلل الأطفال، الفأر المتغاير الأجناس، فيزيولوجيا الفئران، المكاك الملاتي، الجهاز العصبي، فيزيولوجيا الجهاز العصبي، الفوعة، الحساسية والنوعية، إمكانية تكرار النتائج، منظمة الصحة العالمية، دراسة مقارنة، دراسات توثيق المصدوقية (*المصدر: رؤوس الموضوعات الطبية، إقليم شرق المتوسط*).

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Introduction

The neurovirulence test (NVT) for oral poliovirus vaccine (OPV) is a key test for monitoring the consistency of vaccine production (*1*), and following WHO guidelines is required for each monovalent bulk lot of OPV produced. The WHO NVT (*2*) is a standardized procedure. If consecutive lots of monovalent bulks consistently meet the specifications of the

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WHO test, there is a high level of assurance that the vaccines will be safe when used for human immunizations (*3, 4*). So far, the test for neurovirulence safety of OPV has been performed using monkeys, because only primates are naturally susceptible to poliovirus. In 1990–91, two laboratories (*5, 6*) with the support of WHO, established lines of transgenic mice carrying a human receptor to poliovirus. In 1992, WHO recommended that a comparison be made of the sensitivity of TgPVR mice (*7*) with that of monkeys for type-3 poliovirus strains with different degrees of neurovirulence, and an evaluation of TgPVR mice as a possible alternative to monkeys for the neurovirulence testing of OPV (*8*). Initial experiments were performed in Japan and the USA that were aimed at selecting the most suitable TgPVR mouse line and route of inoculation, developing basic test methodology, and accumulating initial data. The results obtained with TgPVR21 mice (*9, 10*) indicated the capacity of the test to discriminate between acceptable batches of OPV and preparations of high neurovirulence. A collaborative study was therefore launched by WHO in 1993 (*11*) to investigate in more detail the suitability of the method for batch release of bulk OPV. Investigators at the Central Institute for Experimental Animals (CIEA, Japan) succeeded in developing TgPVR21 mice from a limited research tool into a reliable supply of standard animals available in large numbers (*12, 13*). Eleven institutions from Asia, Europe, and the USA participated in the study.[a]

The study started with type-3 OPV, the least stable strain in terms of its neurovirulence, and was completed for all three serotypes in October 2000. The results of the collaborative study up to 1999 have recently been published (*14*). The present paper presents the final results of the collaborative study and validation of the mouse NVT. A statistical model was developed for acceptance or rejection of OPV batches in the mouse test. It has previously been shown that the WHO monkey NVT was a reproducible and sensitive assay, ensuring the safety of OPV (*3, 4*). Numerous data, obtained in this collaborative study, have proven that the mouse NVT is as reliable as the WHO monkey NVT for OPV. In 1999 the WHO Expert Committee on Biological Standardization therefore approved the mouse NVT as an alternative to the monkey test for poliovirus type-3 (*15*) and in 2000 for poliovirus type-1 and type-2 (*16*).

Materials and methods

Vaccines

The type-1, type-2, and type-3 OPV virus samples used in the study had been tested previously using the monkey NVT according to the WHO requirements for OPV (*2*) by six manufacturers and three national control authorities. Vaccines of all three types produced in each of the three currently permissible cell substrates (primary monkey kidney, Vero monkey kidney, and human diploid cells) were obtained from nine manufacturers, including six UNICEF suppliers. In all, 75 commercial samples and one experimental sample that passed the monkey NVT were evaluated in mice. In addition, the following vaccine virus samples that failed the monkey NVT were used in this study: nine type-3 commercially produced vaccines and three samples of each serotype that were either experimental vaccines or derivatives of commercially produced vaccines additionally passaged in African green monkey kidney (AGMK) or Vero cells at 37–38 °C (a temperature favouring reversion to neurovirulence). The experimental samples of type-1 generated by passage of vaccine lots at elevated temperature were used as surrogates for commercially produced vaccines that consistently failed the monkey NVT, samples of which could not be located despite intensive worldwide searches. This is a limitation of the study design. Experimental samples of type-3 increased the number of preparations that failed the monkey NVT.

Mice

Two of several mouse lines, TgPVR1 and TgPVR21, derived in Dr A. Nomoto's laboratory (*6, 7*), were evaluated in the investigative stage of the study. TgPVR1 mice contained more copies of the poliovirus receptor (PVR) and were more sensitive to poliovirus, whereas TgPVR21 mice with a lower PVR copy number were less sensitive. After initial experiments (*17, 18*), the TgPVR21 mouse line and the intraspinal route of inoculation were selected as the most suitable combination for evaluation of all three poliovirus serotypes. TgPVR21 mice were monitored at the CIEA for freedom from 22 specified pathogens and for generational stability of genetic background and the introduced gene. Maintenance, containment, and transport of mice were conducted in accordance with recommendations of the WHO Memorandum on transgenic mice susceptible to human viruses (*19*). Each laboratory animal facility that participated was approved by the CIEA before entering the study.

Inoculation procedure

Sixteen 6-to-7-week-old mice of each gender in each dose group were inoculated with the test vaccine and the same number of animals with the reference vaccine, resulting in 128 mice per test. A technique for intraspinal inoculation of mice described previously (*17, 18*) was scrupulously optimized, standardized (*20*), and used in the study. The US Food and Drug Administration (FDA) developed a multi-step system to train investigators in the technique of intraspinal inoculation of mice and in evaluation of clinical signs. All the participants received training at FDA or the Japanese Poliomyelitis Research Institute. The mouse test methodology is fully described and illustrated in a standard operating procedure (SOP) available from WHO.[b]

Statistical methodology

The key components of the statistical design and analysis are outlined below.

1. A test vaccine was tested concurrently with the WHO reference vaccine in a randomized experiment.
2. The test vaccine and the concurrently tested reference vaccine were tested at two doses: 3.5 and 4.5 $\log_{10}TCID_{50}$/5 µl (5.8 and 6.8 $\log_{10}$ $TCID_{50}$/ml) for type-3, 1.75 and 2.75 $\log_{10}TCID_{50}$/5 µl (4.05 and 5.05 $\log_{10}$ $TCID_{50}$/ml) for type-1 and 5.0 and 6.0 $\log_{10}TCID_{50}$/5 µl (7.3 and 8.3 $\log_{10}$ $TCID_{50}$/ml) for type-2. The need to use different doses for different virus types was determined in the investigative phase of the study (see below).
3. Each dose was inoculated intraspinally into 16 male and 16 female mice.

[a] Names of participating investigators and institutions are given in the Annex 1, part III (see online version at: www.who.int/bulletin).

[b] Available from Dr E. Griffiths, Coordinator, QSB, World Health Organization, 1211 Geneva 27, Switzerland (email: griffithse@who.int).

Table 1. **Summary of results of the WHO collaborative study of TgPVR21 mice with type-3 oral poliovirus vaccine lot 93/636**[a]

Laboratory	Test	Vaccine	No. of mice/dose[b]	Proportion paralysed										Statistical analysis (*P*)
				Dose (log_{10} $TCID_{50}$)[c]										
				1	2	3	4	5	1.5	2.5	3.5	4.5	5.5	
C	1	WHO/III[d]	5, 6	0	0.2	0.5	0.4	1	ND	ND	ND	ND	ND	NA
		93/636	5, 6	0	0.333	0.667	1	1	ND	ND	ND	ND	ND	0.029[e]
	2	WHO/III	10	ND	ND	ND	ND	ND	ND	0.1	0.1	0.6	ND	NA
		93/636	10	ND	ND	ND	ND	ND	ND	0.1	0.6	0.9	ND	0.004 [e]
	3	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.4	0.733	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.2	0.867	ND	0.380[f]
	4	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.2	0.467	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.6	0.867	ND	<0.001[e]
	5	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.067	0.4	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.467	0.933	ND	<0.001[e]
	6	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.143	0.467	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.933	0.933	ND	<0.001[e]
	7	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.067	0.6	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.467	0.8	ND	<0.001[e]
A	1	WHO/III	10	ND	0	0.6	0.8	0.9	ND	ND	ND	ND	ND	NA
		93/636	5–10	ND	0.444	1	1	0.9	ND	ND	ND	ND	ND	0.004[e]
	2	WHO/III	9	ND	0	0.1	ND	ND	ND	ND	ND	ND	ND	NA
		93/636	10	ND	0.222	0.556	ND	ND	ND	ND	ND	ND	ND	0.003[e]
B	1	WHO/III	10	ND	0	0.1	0.2	1	ND	ND	ND	ND	ND	NA
		93/636	10	ND	0	0.5	0.9	0.9	ND	ND	ND	ND	ND	<0.001[e]
	2	WHO/III	12	ND	0	0.1	0	0.7	ND	ND	ND	ND	ND	NA
		93/636	12	ND	0	0	0.833	1	ND	ND	ND	ND	ND	<0.001[e]
D	1	WHO/III	15	ND	ND	ND	ND	ND	0	0	0	0.333	0.733	NA
		93/636	15	ND	ND	ND	ND	ND	0.67	0.53	0.867	0.933	0.867	<0.001[e]
	2	WHO/III	10	ND	ND	ND	ND	ND	ND	0	0.2	0.3	0.9	NA
		93/636	10	ND	ND	ND	ND	ND	ND	0.2	0.9	0.9	1	<0.001[e]
E	1	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.467	0.467	ND	NA
		93/636	15	ND	ND	ND	ND	ND	ND	ND	0.8	1	ND	<0.001[e]
I	1	WHO/III	16	ND	ND	ND	ND	ND	ND	ND	0.375	ND	ND	NA
		93/636	14	ND	ND	ND	ND	ND	ND	ND	0.929	ND	ND	<0.001[e]
F	1	WHO/III	16	ND	ND	ND	ND	ND	ND	ND	0.25	0.813	ND	NA
		93/636	16	ND	ND	ND	ND	ND	ND	ND	0.938	1000	ND	<0.001[e]
G	1	WHO/III	15	ND	ND	ND	ND	ND	ND	ND	0.4	0.667	ND	NA
		93/636	14, 15	ND	ND	ND	ND	ND	ND	ND	0.714	1000	ND	<0.001[e]
H	1	WHO/III	7–10	ND	ND	ND	ND	ND	0	0	0.5	0.6	ND	NA
		93/636	10	ND	ND	ND	ND	ND	0	0.1	0.5	0.9	ND	0.112[f]
	2	WHO/III	10	ND	ND	ND	ND	ND	0	0	0.1	0.3	ND	NA
		93/636	9, 10	ND	ND	ND	ND	ND	0	0	0.8	0.6	ND	<0.001[e]

[a] Lot 93/636 failed monkey neurovirulence test and contains 3% 472-C revertants.
[b] Equal numbers of mice were given at each dose unless otherwise stated.
[c] TCID = tissue culture infectious dose; NA= not applicable; ND = not determined.
[d] WHO/III = reference vaccine for type-3.
[e] Statistically significant: more neurovirulent than reference vaccine ($P<0.05$).
[f] Statistically insignificant: not more neurovirulent than reference vaccine ($P>0.05$).

4. Mice were randomized to cages, doses, and vaccines. Randomization, which protects against possible inadvertent biases, was also applied to cage location and order of inoculation.
5. Clinical observations of mice and recording of specific neurological signs, such as paresis and paralysis, were performed daily. Paralysis was taken as the primary indicator of degree of neurovirulence and the log odds ratio (LOR) was used as a measure of the neurovirulence of the test vaccine relative to that of the reference vaccine.
6. Estimates and tests of significance were based on logistic regression analysis of the proportions of paralysed mice.
7. Validity criteria that were applied to ensure that each experiment has adequate power to differentiate between

Table 2. **Summary of results of the WHO collaborative study of TgPVR21 mice with type-3 oral poliovirus vaccine lot 95/526[a]**

Laboratory	Test	Vaccine	No. of mice/dose[b]	Paralysis rate		Statistical analysis (*P*)
				3.5 $\log_{10}$ $TCID_{50}$[c]	4.5 $\log_{10}$ $TCID_{50}$[c]	
C	1	WHO/III[d]	20	0.4	0.7	NA[e]
		95/526	20	0.3	0.85	0.400[f]
	2	WHO/III	20	0.15	0.6	NA
		95/526	20	0.45	0.9	<0.001[g]
	3	WHO/III	30	0.033	0.467	NA
		95/526	30	0.533	0.933	<0.001[g]
	4	WHO/III	30	0.333	0.633	NA
		95/526	30	0.6	0.967	<0.001[g]
	5	WHO/III	30	0.133	0.267	NA
		95/526	30	0.167	0.767	<0.001[g]
D	1	WHO/III	30, 29	0.233	0.379	NA
		95/526	21, 24	0.381	0.75	0.001[g]
A	1	WHO/III	30	0.133	0.633	NA
		95/526	30	0.2	0.9	0.010[g]
	2	WHO/III	30	0.333	0.533	NA
		95/526	30	0.467	0.967	<0.001[g]
E+I	1	WHO/III	30	0.567	0.767	NA
		95/526	29, 30	0.483	1	0.162[f]
F	1	WHO/III	28, 30	0.429	0.967	NA
		95/526	30	0.8	0.967	0.003[g]
	2	WHO/III	30	0.4	0.833	NA
		95/526	30	0.667	0.967	0.002[g]
G	1	WHO/III	31, 30	0.226	0.567	NA
		95/526	30	0.452	0.839	0.007[g]
	2	WHO/III	29, 28	0.241	0.607	NA
		95/526	29, 30	0.552	0.833	0.007[g]
B	1	WHO/III	29	0.276	0.483	NA
		95/526	28, 30	0.321	0.7	0.061[f]
	2	WHO/III	29, 30	0.172	0.567	NA
		95/526	30, 29	0.433	0.828	0.008[g]
	3	WHO/III	26, 30	0.231	0.533	NA
		95/526	30	0.4	0.8	0.005[g]

[a] Lot 95/526 failed monkey neurovirulence test and contains 1.7% 472-C revertants.
[b] Equal numbers of mice were given at each dose unless otherwise stated.
[c] TCID = tissue culture infectious dose.
[d] WHO/III = reference vaccine for type-3.
[e] NA = not applicable.
[f] Statistically insignificant: not more neurovirulent than reference vaccine ($P>0.05$).
[g] Statistically significant: more neurovirulent than reference vaccine ($P<0.05$).

good and bad vaccines included the following:

(a) the combined (male plus female) paralysis rates for the reference vaccine must be $\leqslant 0.95$ at the high dose and $\geqslant 0.05$ at the low dose;
(b) dose effect must be significant; if it is not significant, the vaccine effect must be significant; and
(c) no significant vaccine-by-dose interaction.

The decision rule, i.e. the specific criteria for accepting or rejecting a vaccine lot, requires comparison of the LOR with limits, L1 and L2, derived from historical data for the reference vaccine. A test vaccine passes if the LOR $\leqslant$ L1. L1 was calculated so that a test vaccine equivalent to the reference vaccine would have a 95% probability of passing. A test vaccine fails if the LOR $\geqslant$ L2 and hence L2 was calculated so that a test vaccine equivalent to the reference vaccine would have a 1% probability of failing.

The statistical decision model for acceptance/rejection of a test vaccine is presented in more detail in Annex 1, part I (see online version at: www.who.int/bulletin). It has been applied and successfully validated in the last phases of the study.

Results

Investigative stage

The studies began with a comparison of the suitability of TgPVR1 and TgPVR21 mouse strains for OPV neuroviru-

Table 3. **Results of the WHO collaborative study of TgPVR21 mice with type-3 oral poliovirus vaccine**

Laboratory	Test	Vaccine	Monkey NVT[a]	Mouse NVT[a]			Results[b]
				No. of mice/dose[c]	Paralysis rate		
					3.5 $\log_{10}$ $TCID_{50}$[d]	4.5 $\log_{10}$ $TCID_{50}$	
C	1	WHO/III[e]	Reference	32	0.188	0.469	–
		96/568	Failed	32	0.438	0.75	Failed
		93/664	Passed	32	0	0.156	Passed
	2	WHO/III	Reference	32	0.344	0.75	–
		96/568	Failed	32	0.688	0.938	Failed
		93/644	Passed	32	0	0.062	Passed
A	1	WHO/III	Reference	32, 30	0.219	0.7	–
		95/526	Failed	31, 32	0.419	0.967	Failed
		93/664	Passed	31	0.032	0.129	Passed
	2	WHO/III	Reference	32, 31	0.188	0.742	–
		96/568	Failed	31, 32	0.548	1	Failed
		93/644	Passed	32, 31	0.062	0.065	Passed
	3	WHO/III	Reference	32, 30	0.344	0.733	–
		96/568	Failed	32	0.594	0.906	Failed
		93/664	Passed	32	0.031	0.156	Passed
F	1	WHO/III	Reference	32	0.188	0.562	–
		96/568	Failed	32	0.531	0.875	Failed
		93/664	Passed	32	0. 062	0.031	Passed
	2	WHO/III	Reference	32, 31	0.188	0.742	–
		96/568	Failed	32	0.344	0.969	Failed
		93/644	Passed	32, 31	0.031	0.032	Passed
G	1	WHO/III	Reference	31, 30	0.226	0.567	–
		95/526	Failed	31	0.452	0.839	Failed
		93/658	Passed	30, 32	0.367	0.625	Passed
	2	WHO/III	Reference	32	0.281	0.594	–
		96/568	Failed	32	0.469	0.812	Failed
		93/644	Passed	30, 32	0.067	0.094	Passed
	3	WHO/III	Reference	30, 31	0.267	0.581	–
		96/568	Failed	31, 32	0.419	0.688	Passed[f]
		93/664	Passed	29, 30	0.103	0.167	Passed

[a] NVT = neurovirulence test.
[b] Results were analysed using the decision model.
[c] Equal numbers of mice were given each dose unless otherwise stated.
[d] TCID = tissue culture infectious dose.
[e] WHO/III = reference vaccine for type-3.
[f] Invalid test.

lence testing. Although TgPVR1 mice discriminated between wild-type poliovirus and a vaccine strain, they did not distinguish between vaccine lots that passed or failed the monkey NVT (9). Therefore, the TgPVR21 mouse line was selected for further studies (*17, 18, 20*). Preliminary experiments were conducted using these mice with all three types of polioviruses. TgPVR21 mice were able to discriminate OPV samples that passed from those that failed the monkey NVT. The data generated allowed selection of the appropriate dose range for inoculation, duration of clinical observation, identification of paresis/paralysis as the most important clinical sign for assessment of neurovirulence, and development of criteria for statistical decision-making model.

These preliminary studies also suggested that the mouse model could be based on paralysis scores, in contrast to the simian model, which is based on lesion scores from histopathological examination. A special study was therefore performed to investigate the added value of histopathological examination of the mouse central nervous system for pass/fail decisions. Unlike the situation with the monkey NVT, histological examination of the mouse central nervous system offered no advantage for discriminating vaccine batches over clinical observation alone (*21*). The mouse test thus can be completed more rapidly than the monkey test.

WHO collaborative study

The WHO collaborative study underwent five phases. The first three phases were focused on type-3 poliovirus as this is agreed to be genetically the least stable strain of OPV. Type-1 and type-2 poliovirus vaccine samples were studied in phases 4 and 5. WHO vaccine references of all three types for the monkey NVT were used in all mouse tests.

The choice of type-3 vaccine viruses was based on previous results from the monkey test and from the mutant

Table 4. **Summary of results of the WHO collaborative study of TgPVR21 mouse neurovirulence test with oral poliovirus vaccine**

Type	Samples	No. of samples	Monkey NVT[a]	Mouse NVT			
				Tests (*n*)	Laboratories (*n*)	Results	
						Pass	Fail
Type-3	Commercial batches	31	Pass	54	8	54	0
	Commercial batches	9	Fail	56[b]	10	0	53
	Experimental samples[c]	3	Fail	4	2	0	4
	Total	**43**	**NA**	**114**	**20**	**54**	**57**
Type-1	Commercial batches	20[d]	Pass	32[e]	5	29	0
	Experimental sample[c]	1	Pass	1	1	1	0
	Experimental samples[c]	3	Fail	6	2	0	6
	Total	**24**	**NA**	**39**	**8**	**30**	**6**
Type-2	Commercial batches	24	Pass	39[f]	6	36	2
	Experimental batches[g]	3	Fail	14	6	0	14
	Total	**27**	**NA**	**53**	**12**	**36**	**16**

[a] NVT = neurovirulence test; NA, not applicable.
[b] Three tests in one laboratory were invalid; improvement in technique was required.
[c] Passages of original vaccine in AGMK or Vero cells at 37–38 °C.
[d] Batches evaluated in "in-house" tests against national references are not included.
[e] Two tests were repeated because L1<LOR<L2, where LOR is log odds ratio; one test was repeated because paralysis rate at a low dose was <0.05.
[f] One test was repeated because L1<LOR<L2.
[g] Obtained from one manufacturer experimenting with production process.

analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC) test. Initial evaluations in mice used vaccine samples that failed the monkey test by a large margin and contained unusually high amounts (>3%) of neurovirulent 472-C revertants (*23*, *24*) (Table 1). The evaluation was continued with vaccine lots that failed the monkey test and which contained only slightly increased amounts (>1%) of 472-C mutants (Table 2). These first two phases of the study provided data for development of a statistical model to define pass/fail decisions. Phase 3 was designed to validate the statistical decision model in tests when previously passed and failed vaccine lots were tested simultaneously (Table 3). Altogether 43 vaccine samples were tested in 10 laboratories in 114 mouse tests (Table 4). Thirty-one commercial OPV lots that passed the monkey NVT also passed the mouse NVT. Nine vaccine lots that failed the monkey NVT also failed the mouse NVT. To increase the number of samples that failed the monkey NVT, commercially produced vaccine viruses were passaged at 37–38 °C in AGMK or Vero cells to increase their neurovirulence for monkeys. Three such vaccine derivatives were prepared by this method and they also failed both monkey and mouse NVTs.

The applicability for type-1 strain of the statistical pass/fail decision model developed for type-3 OPV was evaluated in two series of tests. Initially doses of 1.5 and 2.5 $\log_{10}$ $TCID_{50}$ were used (Table 5) but were subsequently increased to 1.75 and 2.75 $\log_{10}$ $TCID_{50}$ to achieve paralysis rates of the reference vaccine at the low dose of above 0.05 (Table 6), as required by the statistical decision model.

Since there were no commercial vaccine lots available that had failed the monkey test, original vaccines were passaged in AGMK or Vero cells at 37–38 °C. Four such experimental samples, one that passed and three that failed the monkey NVT, were tested in mice. A total of 20 type-1 commercial vaccine lots that had passed the monkey NVT were included in the study. In 39 mouse NVTs performed in five laboratories there was complete correlation of results between mice and monkeys (Table 4).

A total of 27 type-2 vaccine samples were tested in six laboratories in 53 mouse tests (Table 4). Twenty-three commercial vaccine batches that had passed the monkey test also passed the mouse test. One vaccine batch that passed the monkey test gave variable results in the mouse test, passing five times and failing twice. This suggests that the batch concerned may have been on the borderline between pass and fail in the mouse test. It is not known what the results would have been had the monkey test been repeated one or more times, and thus whether this batch was also on the borderline between pass and fail in the monkey test. This batch was the only one that gave anomalous results in the mouse and monkey tests for any of the serotypes. Three experimental batches obtained from one manufacturer, who at that time was investigating potential changes to the production process, were the only available lots that failed the monkey NVT. All three samples failed the mouse NVT in all laboratories. Thus there was close agreement between mice and monkeys in the study with serotype 2. The results obtained demonstrated that the statistical pass/fail decision model developed for type-3 polioviruses was applicable and valid for type-2 OPV (Table 7).

Conclusion

A WHO Collaborative Study on transgenic mice as an alternative to the monkey NVT has been completed with all three OPV serotypes. Eighty-four commercial vaccine batches and ten experimental vaccine samples of type-1, type-2, and type-3 were tested in 206 mouse NVTs. A limitation to the study design was the unavailability of commercially produced type-1 batches that consistently failed the monkey NVT.

Table 5. **Summary of results of the WHO collaborative study of TgPVR21 mice with type-1 oral poliovirus vaccine (initial doses of inoculum)**

Laboratory	Test	Vaccine	Paralysis rate (*n*)				LOR[a]	Results	
			Females		Males				
			Dose (log_{10} $TCID_{50}$)[b]					Mouse NVT[c]	Monkey NVT
			1.5	2.5	1.5	2.5			
A	1	I-2	0.063 (16)[d]	0.5 (16)	0 (16)	0.467 (15)	0.291	Re-test[e]	Pass
		I-5	0 (16)	0.5 (16)	0.063 (16)	0.4 (15)	0.174	Re-test[e]	Pass
		WHO/I[f]	0 (16)	0.5 (16)	0 (16)	0.375 (16)	–	Reference	Reference
	2	I-1	0 (16)	0.333 (15)	0 (16)	0.813 (16)	–1.437	Pass	Pass
		I-7	0.063 (16)	0.286 (14)	0 (16)	0.267 (15)	–0.517	Pass	Pass
		WHO/I	0.133 (15)	0.688 (16)	0.063 (16)	0.563 (16)	–	Reference	Reference
	3	I-4	0.063 (16)	0.6 (15)	0.313 (16)	0.733 (15)	0.162	Pass	Pass
		I-6	0.125 (16)	0.625 (16)	0.125 (16)	0.875 (16)	0.202	Pass	Pass
		WHO/I	0.25 (16)	0.563 (16)	0.067 (15)	0.733 (15)	–	Reference	Reference
E	1	I-10	0.2 (15)	0.313 (16)	0.067 (15)	0.25 (16)	–1.939	Pass	Pass
		I-3	0 (16)	0.063 (16)	0.063 (16)	0.188 (16)	–0.686	Pass	Pass
		WHO/I	0.063 (16)	0.625 (16)	0.133 (15)	0.438 (16)	–	Reference	Reference
F	1	I-3	0 (16)	0.063 (16)	0.063 (16)	0.063 (16)	–2.44	Pass	Pass
		I-6	0.188 (16)	0.813 (16)	0.063 (16)	0.625 (16)	0.816	Re-test[g]	Pass
		WHO/I	0.125 (16)	0.5 (16)	0 (16)	0.563 (16)	–	Reference	Reference
	2	I-4	0 (16)	0.563 (16)	0.063 (16)	0.4 (15)	–0.111	Pass	Pass
		I-9	0.125 (16)	0.625 (16)	0.063 (16)	0.313 (16)	0.039	Pass	Pass
		WHO/I	0.188 (16)	0.333 (15)	0 (15)	0.563 (16)	–	Reference	Reference
C	1	I-2	0.063 (16)	0.25 (16)	0.063 (16)	0.563 (16)	–1.17	Pass	Pass
		I-8	0.125 (16)	0.75 (16)	0.125 (16)	0.5 (16)	–0.096	Pass	Pass
		WHO/I	0 (16)	0.375 (16)	0.25 (16)	0.938 (16)	–	Reference	Reference
	2	289	0.187 (16)	0.562 (16)	0.063 (16)	0.562 (16)	–0.089	Pass	Pass
		SID 38/4[h]	0.313 (16)	0.875 (16)	0.25 (16)	0.938 (16)	1.469	Fail	Fail
		WHO/I	0 (16)	0.562 (16)	0.25 (16)	0.623 (16)	–	Reference	Reference

[a] LOR = log odds ratio; limit values for LOR used in the analysis: L1 = 0.718; L2 = 1.016.
[b] TCID = tissue culture infectious dose.
[c] NVT = neurovirulence test.
[d] Figures in parentheses indicate the number of mice.
[e] Re-test because reference paralysis rate at the 1.5 dose is 0.0.
[f] WHO/I = reference vaccine for type-1.
[g] Re-test because L1<LOR<L2.
[h] Fourth passage of original vaccine in Vero cells at 38 °C.

Table 6. **Summary of results of the WHO collaborative study of TgPVR21 mice with type-1 oral poliovirus vaccine (final doses of inoculum)**

Laboratory	Test	Vaccine	Paralysis rate (*n*)				LOR[a]	Results	
			Females		Males				
			Dose (log_{10} $TCID_{50}$)[b]					Mouse NVT[c]	Monkey NVT
			1.75	2.75	1.75	2.75			
A	1	I-10	0.063 (16)[d]	0.625 (16)	0.2 (15)	0.75 (16)	–0.696	Pass	Pass
		I-2	0.125 (16)	0.313 (16)	0.063 (16)	0.333 (15)	–1.95	Pass	Pass
		WHO/I[e]	0.125 (16)	0.875 (16)	0.133 (15)	0.875 (16)	–	Reference	Reference
	2	99-I-1	0.062 (16)	0.625 (16)	0 (16)	0.5 (16)	–0.225	Pass	Pass
		99-I-2	0.375 (16)	0.467 (15)	0.25 (16)	0.467 (15)	–0.518	Pass	Pass
		WHO/I	0.25 (16)	0.938 (16)	0 (16)	0.562 (16)	–	Reference	Reference
	3	99-I-2	0.125 (16)	0.8 (15)	0.133 (15)	0.733 (15)	0.603	Pass	Pass
		WHO/I	0.063 (16)	0.625 (16)	0.083 (12)	0.714 (14)	–	Reference	Reference
E	1	I-10	0.125 (16)	0.438 (16)	0.063 (16)	0.688 (16)	0.107	Pass	Pass
		I-11	0 (16)	0.375 (16)	0.133 (15)	0.875 (16)	0.163	Pass	Pass
		WHO/I	0.063 (16)	0.667 (15)	0.125 (16)	0.438 (16)	–	Reference	Reference
J	1	J/I/1	0.25 (16)	0.875 (16)	0.25 (16)	0.875 (16)	–0.202	Pass	Pass
		J/I/2	0.313 (16)	1 (16)	0.438 (16)	0.875 (16)	0.409	Pass	Pass
		WHO/I	0.313 (16)	0.813 (16)	0.313 (16)	0.938 (16)	–	Reference	Reference

[a] LOR = log odds ratio; limit values for LOR used in the analysis: L1= 0.734, L2 = 1.037.
[b, c, d, e] See corresponding footnotes, Table 5.

Table 7. **Summary of results of the WHO collaborative study of TgPVR21 mice with type-2 oral poliovirus vaccine**

Laboratory	Test	Vaccine	Paralysis rate (*n*)				LOR[a]	Results	
			Females		Males				
			Dose (log_{10} $TCID_{50}$)[b]					Mouse NVT[c]	Monkey NVT
			5	6	5	6			
A	1	98/690	0 (16)[d]	0.75 (16)	0.375 (16)	0.938 (16)	0.118	Pass	Pass
		98/702	0.625 (16)	1 (16)	0.938 (16)	1 (16)	3.434	Fail	Fail
		WHO/II[e]	0.063 (16)	0.625 (16)	0.375 (16)	0.938 (16)	–	Reference	Reference
	2	99-II-3	0.063 (16)	0.313 (16)	0.125 (16)	0.563 (16)	–0.47	Pass	Pass
		99-II-5	0.063 (16)	0.188 (16)	0 (16)	0.313 (16)	–1.375	Pass	Pass
		WHO/II	0.188 (16)	0.438 (16)	0.125 (16)	0.625 (16)	–	Reference	Reference
C	1	98/688	0.188 (16)	0.5 (16)	0.625 (16)	0.813 (16)	0.353	Pass	Pass
		99/II/4	0.125 (16)	0.563 (16)	0.25 (16)	0.875 (16)	–0.092	Pass	Pass
		WHO/II	0.063 (16)	0.375 (16)	0.563 (16)	0.875 (16)	–	Reference	Reference
	2	99-II-1	0 (16)	0.188 (16)	0.25 (16)	0.625 (16)	–0.756	Pass	Pass
		99-II-2	0.062 (16)	0.25 (16)	0.312 (16)	0.733 (15)	–0.29	Pass	Pass
		WHO/II	0.062 (16)	0.375 (16)	0.438 (16)	0.688 (16)	–	Reference	Reference
	3	M-2-4	0.063 (16)	0.125 (16)	0.5 (16)	0.75 (16)	0	Pass	Pass
		WHO/II	0.188 (16)	0.25 (16)	0.313 (16)	0.688 (16)	–	Reference	Reference
E	1	98/690	0.125 (16)	0.313 (16)	0.375 (16)	0.533 (15)	–1.106	Pass	Pass
		98/702	0.563 (16)	0.875 (16)	0.938 (16)	0.867 (15)	1.287	Fail	Fail
		WHO/II	0.4 (15)	0.533 (15)	0.5 (16)	0.875 (16)	–	Reference	Reference
F	1	98/688	0 (16)	0.25 (16)	0.125 (16)	0.5 (16)	–0.625	Pass	Pass
		99-II-6	0 (16)	0.375 (16)	0.125 (16)	0.438 (16)	–0.495	Pass	Pass
		WHO/II	0.062 (16)	0.562 (16)	0.125 (16)	0.5 (16)	–	Reference	Reference
	2	M-2-4	0.313 (16)	0.688 (16)	0.313 (16)	0.938 (16)	0.403	Pass	Pass
		WHO/II	0.063 (16)	0.5 (16)	0.438 (16)	1 (16)	–	Reference	Reference
	3	98/688	0.063 (16)	0.333 (15)	0.5 (16)	0.75 (16)	0.959	Fail	Pass
		98/702	0.688 (16)	0.6 (15)	0.938 (16)	0.938 (16)	3.001	Fail	Fail
		WHO/II	0 (16)	0.188 (16)	0.313 (16)	0.5 (16)	–	Reference	Reference
	4	98/688	0.063 (16)	0.438 (16)	0.5 (16)	0.688 (16)	0.984	Fail	Pass
		98/702	0.5 (16)	0.875 (16)	0.625 (16)	0.938 (16)	2.929	Fail	Fail
		WHO/II	0 (16)	0.4 (15)	0.062 (16)	0.563 (16)	–	Reference	Reference
J	1	98/702	0.875 (16)	1 (16)	0.813 (16)	1 (15)	2.433	Fail	Fail
		WHO/II	0.188 (16)	0.75 (16)	0.625 (16)	1 (16)	–	Reference	Reference
	2	J/II/2	0.25 (16)	0.667 (15)	0.188 (16)	0.75 (16)	0.226	Pass	Pass
		J/II/3	0.125 (16)	0.563 (16)	0.4 (15)	0.75 (16)	0.184	Pass	Pass
		WHO/II	0.125 (16)	0.688 (16)	0.25 (16)	0.625 (16)	–	Reference	Reference
	3	J/II/4	0.063 (16)	0.75 (16)	0.375 (16)	1 (16)	0.774	Re-test[f]	Pass
		J/II/5	0.063 (16)	0.467 (15)	0.375 (16)	0.813 (16)	–0.049	Pass	Pass
		WHO/II	0.188 (16)	0.375 (16)	0.188 (16)	1 (16)	–	Reference	Reference
	4	J/II/4	0.375 (16)	1 (16)	0.25 (16)	0.688 (16)	–0.851	Pass	Pass
		J/II/1	0.125 (16)	0.875 (16)	0.625 (16)	0.933 (15)	–0.588	Pass	Pass
		WHO/II	0.312 (16)	0.875 (16)	0.688 (16)	1 (16)	–	Reference	Reference

[a] LOR = log odds ratio; limit values for LOR used in the analysis: L1 = 0.665; L2 = 0.940.
[b, c, d] See corresponding footnotes, Table 5.
[e] WHO/II = reference vaccine for type-2.
[f] Re-test because L1<LOR<L2.

However vaccine derivatives were used as surrogates. There was good correlation between the results of the monkey and mouse NVTs for all three OPV types. Statistical analysis of the data demonstrated that the TgPVR21 mouse test is as sensitive and reliable as the monkey NVT. A statistical model for acceptance/rejection of OPV lots tested in the mouse test has been validated and proved to be suitable for all three types. Our results demonstrate the first successful introduction of transgenic animals into control of biologicals. The special line of mice with defined genetic and microbiological quality standards yielded highly uniform results, and a significantly shorter time was required for the test — 2 weeks for the mouse test instead of 1.5–2 months for the monkey test. The transgenic mouse NVT is more attractive than the monkey NVT for ethical and practical considerations since it reduces use of primates and eliminates hazards to personnel working with primates. The WHO Ethical Committee on Biological Safety has approved the mouse NVT as an alternative to the

monkey NVT for all three types of OPV (*16*). To avoid confusion however the Committee also confirmed that the test in simians remains the gold standard for evaluating the neurovirulence of OPV, and should be used to validate new virus seed lots or changes in the manufacturing process.

Laboratories cannot simply switch from using monkeys to mice. Although the transgenic mouse NVT was successfully introduced into most laboratories, others had some methodological difficulties. For example, the test requires the very precise positioning of the inoculum into the mouse spinal cord — a very small target area. Operators are therefore required to acquire this special skill during the training period. A standard implementation process has been developed by WHO to facilitate introduction of the new technique. Also, WHO has recommended that to qualify as competent to perform the mouse test, laboratories should complete the standard implementation process (see Annex 1, part II at www.who.int/bulletin) and satisfy their national control authority that they have gained sufficient experience in the test. Once qualified as competent, each laboratory should continue to monitor its continued competence to perform the test (*22*). In order to ensure the supply of such mice, two breeding stations of TgPVR21 mice have been established in Asia and Europe. Both these stations are provided with frozen embryos from the CIEA and will conduct consistent controls of the quality of animals as prescribed by the CIEA. ■

Résumé

Des souris transgéniques en remplacement des singes pour l'épreuve de neurovirulence appliquée au vaccin antipoliomyélitique oral vivant : validation par une étude collective de l'OMS

Objectif Déterminer si des souris transgéniques sensibles au poliovirus (souris TgPVR lignée 21, élevées et fournies par le Central Institute for Experimental Animals (Japon)) peuvent être utilisées en remplacement des singes dans l'épreuve de neurovirulence appliquée au vaccin antipoliomyélitique oral (VPO).

Méthodes Les données de 9 laboratoires ont été utilisées pour évaluer la neurovirulence de 94 préparations de VPO ou dérivés du vaccin contre les trois sérotypes de poliovirus testées sur la souris TgPVR21 lors d'une vaste étude collective menée par l'OMS.

Résultats L'analyse statistique des données a montré que l'épreuve de neurovirulence sur la souris TgPVR21 était de sensibilité et de reproductibilité comparables à celles de l'épreuve classique OMS sur le singe. Un modèle statistique d'acceptation ou de rejet des lots de VPO d'après les résultats de l'épreuve chez la souris a été développé et validé, et s'est révélé convenir pour les trois types de vaccin. L'évaluation de l'épreuve de neurovirulence chez la souris transgénique reposait sur l'examen clinique des souris paralysées. Contrairement à ce qui se passe dans l'épreuve de neurovirulence chez le singe, l'examen histologique du système nerveux central de chacune des souris ne présentait pas d'avantage sur un examen clinique approfondi.

Conclusion A partir de ces données, le Comité OMS d'experts de la standardisation biologique a approuvé l'épreuve de neurovirulence chez la souris en remplacement de l'épreuve sur le singe pour les trois types de vaccin VPO et a défini une procédure normalisée de mise en œuvre à l'intention des laboratoires qui souhaitent appliquer cette nouvelle épreuve. Il s'agit là de la première utilisation réussie d'animaux transgéniques dans le domaine du contrôle des produits biologiques.

Resumen

Ratones transgénicos como alternativa a los monos para la prueba de neurovirulencia de la vacuna oral viva contra el poliovirus: validación en un estudio en colaboración de la OMS

Objetivo Determinar la idoneidad del uso de ratones transgénicos sensibles al poliovirus (ratones TgPVR, cepa 21, criados y proporcionados por el Instituto Central para Animales de Laboratorio, Japón) como alternativa a los monos en la prueba de neurovirulencia (PNV) para la vacuna oral viva contra el poliovirus (OPV).

Métodos Se utilizaron los datos de nueve laboratorios para evaluar la neurovirulencia de 94 preparados de OPV o derivados vacunales contra los tres serotipos en ratones TgPVR21 en un amplio estudio en colaboración de la OMS.

Resultados El análisis estadístico de los datos demostró que la PNV aplicada a los ratones TgPVR21 era comparable, en cuanto a sensibilidad y reproducibilidad, a la prueba convencional de la OMS con monos. Se desarrolló y validó un modelo estadístico para aceptar o rechazar los lotes de OPV en la prueba con ratones, modelo que resultó adecuado para los tres tipos de vacuna. La evaluación de la PNV en ratones transgénicos se basa en la observación clínica de los ratones con parálisis. A diferencia de la PNV con monos, el examen histológico del sistema nervioso central de los ratones no reportó ninguna ventaja adicional en comparación con una observación clínica cuidadosa y detallada.

Conclusión Teniendo en cuenta estos datos, el Comité de Expertos de la OMS en Patrones Biológicos aprobó la PNV en ratones como una alternativa válida a la prueba en monos para los tres tipos de OPV, y describió un procedimiento de aplicación normalizado para los laboratorios que deseen utilizarla. Es la primera vez que se logra utilizar con éxito animales transgénicos para controlar productos biológicos.

ملخص

الفأر المتغاير الجينات كبديل للقرود في اختبار الفوعة العصبية للقاح الحي الفموي لشلل الأطفال: توثيق مصدوقية لدراسة بالتعاون مع منظمة الصحة العالمية

الغرض: أجريت دراسات واسعة بالتعاون مع منظمة الصحة العالمية لتقييم ملاءمة الفأر المتغاير الجينات المؤهب للعدوى بفيروس شلل الأطفال (وهو الفأر المتغاير الجينات TgPVR من الذرية ٢١ وقد تم تنسيله والحصول عليه من المعهد المركزي لحيوانات التجارب في اليابان) كبديل للقرود في اختبار الفوعة العصبية للقاح شلل الأطفال الفموي.

الطريقة: لقد أوضح التحليل الإحصائي للمعطيات أن اختبار الفوعة العصبية لدى الفأر المتغاير الجينات TgPVR من الذرية ٢١ له درجة من الحساسية وقابلية التكرار تضاهي ما لاختبار منظمة الصحة العالمية التقليدي للفوعة العصبية لدى القرود، وقد تم إعداد نموذج إحصائي لقبول أو رفض اللقاح الفموي لشلل الأطفال في الفئران المختبرة، وتم توثيق مصدوقية ذلك الاختبار. وقد اتضح أنه ملائم لجميع أنماط اللقاحات الثلاثة. ويرتكز تقييم اختبار الفوعة العصبية لدى الفئران المتغايرة الجينات على التقييم السريري للفأر المصاب بالشلل، ولم يقدم الفحص الهيستولوجي لنسيج الجهاز العصبي المركزي في كل فأر من الفئران المتغايرة الجينات ما يزيد على ما تقدمه الملاحظات السريرية المفصلة والدقيقة من منافع، وذلك بخلاف ما يقدمه اختبار الفوعة العصبية لدى القرود.

الاستنتاج: لقد وافقت لجنة الخبراء التابعة لمنظمة الصحة العالمية المعنية بوضع المعايير البيولوجية على اختبار الفوعة العصبية بناءً على المعطيات المجموعة من الدراسة المتعاونة مع منظمة الصحة العالمية، كبديل للاختبار الذي يجرى على القرود لجميع الأنماط الثلاثة من اللقاحات الفموية لشلل الأطفال، وحددت عملية معيارية للتنفيذ في المختبرات التي ترغب بإجراء ذلك الاختبار، وهذا ما يمثل أول إدخال ناجح للحيوانات المتغايرة الجينات في مكافحة العوامل البيولوجية.

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Annex 1

Part I. Statistical decision model for acceptance or rejection of a test vaccine

The decision model for acceptance or rejection of a test vaccine can be applied by using the six steps described below.

Step 1: Check paralysis proportions for the reference vaccine. The combined (male and female) paralysis rates for the reference vaccine must be $\leqslant 0.95$ at the high dose and $\geqslant 0.05$ at the low dose. Samples that do not meet this criterion require re-testing.

Step 2: Check estimability. If the maximum likelihood procedure fails to converge, then the pass/fail criteria are applied at each dose. The test vaccine is accepted if the vaccine passes at both doses (LOR<L1). The test vaccine fails if the vaccine fails at both doses (LOR>L2). (LOR is log odds ratio and L1 and L2 are limits 1 and 2, respectively, indicating the lower and upper limits applied in the statistical decision model).All other outcomes will require a re-test.

Step 3: Check vaccine × dose interaction by applying the maximum likelihood procedure to the logistic regression model. If the interaction is significant, the pass/fail criteria are applied to each dose. The test vaccine is accepted if the vaccine passes at both doses (LOR<L1 at each dose) and fails if LOR>L2 at both doses. If paralysis rates for the test vaccine doses are either at 0.0 or 1.0, the following decision process can be applied: if both doses of the test vaccine produce 0.0 paralysis, the vaccine is accepted; if 0.0 paralysis occurs for the low dose and at high dose the paralysis rate is lower than the corresponding reference result, use step 5; if the test vaccine has paralysis rates of 1.0 at both doses, the vaccine fails; if the test vaccine has 1.0 paralysis rate only at the high dose, then the decision process is applied to the combined results for both doses and also to the LOR for the low dose — the vaccine is required to pass the decision criteria for both the combined estimate of the LOR and the estimate at the low dose.

Step 4: Check for a significant dose effect. If the dose effect is significant then proceed to Step 5: if it is not significant, test the vaccine effect. If the vaccine effect is also not significant, the experiment must be repeated. Otherwise, proceed to Step 5.

Step 5: Calculate the LOR: if LOR $\leqslant$ L1, the vaccine passes; if L1<LOR<L2, retesting is required (go to Step 6); and if LOR $\geqslant$ L2, the vaccine fails.

Step 6: If a pass/fail decision is not reached in Steps 1–5 and a repeat experiment is required, the decision process is applied either to pooled data from the two experiments or the data from the second experiment alone. If the re-test was initiated as a result of a technical problem in the first test or because of a lack of validity of the reference profile, steps 1–5 must be repeated using the data from the second experiment alone. If the re-test was initiated because LOR was between L1 and L2, or because of a problem with the test profile, Steps 1–5 must be repeated using pooled data from both experiments.

If the experiment involved testing with more than one test vaccine, individual analysis of the data must be carried out comparing each vaccine with the concurrent WHO reference vaccine.

Details of the procedures used to calculate the limits, L1 and L2, are available in the standard operating procedure available from WHO.

Part II. Implementation process

The implementation process consists of three main components: training, evaluation, and implementation.

Training: Intraspinal inoculation of mice assumes that the inoculum will be delivered to a small target area, the anterior horns of the spinal cord lumbar segment. The laboratory is required to acquire a special skill during the training period.

(a) Initial training in the intraspinal inoculation, clinical assessment of mice, and statistical analysis procedure at the Food and Drug Administration or Japanese Poliomyelitis Research;
(b) Practising the intraspinal inoculation of conventional mice with India ink;
(c) Performing two tests on TgPVR mice with vaccine samples of each type. The samples are provided with known titres and the monkey and mouse NVT data (passed or failed vaccine).

Evaluation of precision of virus titration: The laboratory is required to use the standard WHO poliovirus titration method (*1*) and is provided with a titration reference reagent. The laboratory should perform several assays to obtain a precision for the confidence limits for the mean $\leqslant 0.3$ $\log_{10}$ (tissue culture infectious dose) $(TCID)_{50}$. The mean value obtained is compared with the assigned value for the reference to normalize titration values for the vaccine samples.

Implementation procedure:

(a) Vaccines are provided by WHO to the laboratory as a panel of coded samples.
(b) A minimum of three valid tests (from a total of no more than four tests) are required to complete the implementation process. Test results are submitted to WHO.
(c) On the basis of the obtained results, WHO will assess whether the laboratory has successfully implemented the mouse test.
(d) The implementation procedure shall be performed for each oral poliovirus vaccine type.

During the implementation process the laboratory accumulates data from five valid tests to determine its own L1 and L2 limits and will then use these for batch release purposes after the implementation has been successfully completed.

Part III. Participating investigators and institutions

Principal investigators: Dr T. Nomura, Central Institute of Experimental Animals (CIEA), Japan; Dr S. Abe, Japan Poliomyelitis Research Institute (JPRI), Japan; Dr T. Kurata, National Institute of Infectious Diseases (NIID), Japan; Dr A. Schmeel, Chiron Behring (CB), Germany; Dr Guo Ren, Central Institute of Medical Biology, China; Dr A. Deatly, Wyeth-Lederle Vaccines and Pediatrics (WLVP), USA; Dr E. Dragunsky, US Food and Drug Administration (FDA); Dr G. Karganova, Institute of Poliomyelitis and Viral Encephalitides (IPVE), Russian Federation; Dr E. Evreinova, LA Tarassevich State Research Institute for Standardization and Control of Medical and Biological Preparations, Russian Federation; Dr O. Vanloocke, GlaxoSmithKline Biologicals (GSKB), Belgium; Dr D. J. Wood, National Institute for Biological Standards and Control (NIBSC), England.

Statistical analysis of the data was performed, and the statistical model for acceptance or rejection of oral poliovirus vaccine lots in the mouse NVT was developed by Dr K. Karpinski (Canada), with participation of Dr R. Taffs, Dr A. Ivshina, Dr H. Hsu (FDA), and Dr A. Heath (NIBSC). Standard operating procedures were prepared by Dr E. Dragunsky, Dr R. Taffs, Dr D. Asher, Dr I. Levenbook (FDA), Dr K. Karpinski (Canada), and revised by Dr D. J. Wood (NIBSC). Histological examination was performed by Dr E. Dragunsky (FDA) and Mrs S. Marsden (NIBSC). The study was initiated and supported by WHO (Dr Y. Ghendon, Dr Y. Pervikov, Dr E. Griffiths) and coordinated by Dr I. Levenbook (FDA, later WHO). The consultant for the study was Dr J. Furesz (Canada).

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