



**STANDARD OPERATING PROCEDURE**

**NEUROVIRULENCE TEST OF TYPES 1, 2 OR 3 LIVE ATTENUATED**

**POLIOMYELITIS VACCINES (ORAL)**

**IN MONKEYS**

Version, 2012

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Certificate of compliance with the recommendations for the neurovirulence testing of monovalent OPV bulks in monkeys

### **Authors**

### **References**

## **Part 1. Description of the test**

Monkeys used for neurovirulence tests should satisfy the relevant sections of the recommendations in Part E, section E.4.1.1 of the Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral) (1), and weigh not less than 1.5 kg.

The pathogenicity of the filtered monovalent bulk or seed for *Macaca* or *Cercopithecus* monkeys should be tested in comparison with that of a reference virus preparation for neurovirulence testing (see Part A, section A.1.3 of the Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral) (1)) by inoculation into the lumbar region of the central nervous system. A pre-injection serum sample obtained from each monkey should be shown not to contain any neutralizing antibody in a dilution of 1:4 when tested against no more than 1000 CCID<sub>50</sub> of each of the three types of poliovirus.

If only the manufacturer performs the neurovirulence test, the histological sections should be made available to the national regulatory authority for evaluation. All steps of the test must be performed by competent operators who have been trained by performing the test under supervision of experienced operators. Implementation and maintenance of competence should be documented.

### **1.1 Number of monkeys**

It is recommended that a vaccine and the appropriate homotypic reference virus should, whenever possible, be tested concurrently in a single group of monkeys. Equal numbers of animals should be inoculated with the reference virus and the vaccine being tested. Monkeys should be allocated to vaccine or reference virus and to particular cages using a randomization procedure.

The number of monkeys inoculated should be such that at least 11 positive monkeys are included in the evaluation of the vaccine and at least 11 positive monkeys are included for the reference preparation for virus types 1 and 2. For virus type 3, there should be at least 18 positive monkeys for the reference preparation and a further 18 positive monkeys for the vaccine. A “positive” monkey is one in which neuronal lesions characteristic of poliovirus are

seen in the central nervous system. A monkey with neuronal lesions in the sections but which shows no needle tract should be regarded as positive. A monkey showing a needle tract in the sections but no neuronal lesions should not be regarded as positive. All positive monkeys should be analyzed and included in the calculations. More than one vaccine lot may be tested with the same homotypic reference. The monkeys should, when possible, be from the same quarantine group and should be allocated randomly to the preparations. If it is not possible to use monkeys from the same quarantine group for both the homotypic reference and the test vaccine, monkeys from two quarantine groups should undergo tests with each of the preparations (with as close to equal numbers as possible from each quarantine group). If a test is done on two working days, equal numbers of monkeys should be inoculated with the vaccine and the homotypic reference on each working day.

In order to obtain 11 and 18 positive monkeys, it is usual to inoculate 12 and 20 monkeys, respectively.

The monkeys are sedated with ketamine hydrochloride or any other substance that has been shown to be suitable.

## **1.2 Virus content of vaccines and reference preparations inoculated**

The virus contents of the vaccine and the homotypic reference preparation should be adjusted to be as similar as possible and should be between 5.5 and 6.5  $\log_{10}/0.1\text{ml}$ , based on the virus concentration determined as described in Part A, section A.4.4.4 of the Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral) (1). A target titer of 6.0  $\log_{10}/0.1\text{ml}$  should be prepared. Monkeys should be inoculated with only one concentration of virus. A back titration of the inoculum should be done after the inoculation step is completed. The MNVT is valid if the back titration result is within  $\pm 0.5$  of 6.0  $\log_{10}/0.1\text{ml}$ , i.e. if it is between 5.5 and 6.5  $\log_{10}/0.1\text{ml}$ .

If the result of the back-titration is found to be out of specification, a repeat of the back-titration is possible and the results of both titrations could be pooled and reassessed in accordance with the standard retest policy. If the titre of any of the vaccine doses used is found to be outside of the required limits after retesting, a statistical review of the data should be performed to decide whether the MNVT needs repeating and new dilutions prepared.

### **1.3 Observation of monkeys**

All monkeys should be observed for 17—22 days for symptoms suggestive of poliomyelitis or other virus infection. Monkeys that survive the first 24 hours but die before the 11th day after inoculation should be autopsied to determine whether poliomyelitis was the cause of death. Those that have died from causes other than poliomyelitis should be excluded from the evaluation.

Animals that become moribund or are severely paralysed should be humanely killed and autopsied.

All monkeys that survive the observation period should be euthanized and processed for analysis.

For the test to be valid, no more than 20% of the animals in each group should show signs of a concurrent infection during the observation period.

### **1.4 Number of sections examined**

The lumbar cord, the cervical cord, the lower and upper medulla oblongata, the mesencephalon, the thalamus, and the motor cortex of each monkey, as a minimum, should be subjected to histological examination.

Sections should be cut at a thickness of 15µm and stained with galloxyanin. If adequately justified, sections may be cut at a thickness of 8-15 µm, and Nissl staining may be used as an alternative to galloxyanin.

The minimum number of sections examined should be as follows:

- 12 sections representative of the whole of the lumbar enlargement
- 10 sections representative of the whole of the cervical enlargement
- 2 sections from the medulla oblongata
- 1 section from the pons and cerebellum
- 1 section from the midbrain
- 1 section each from the left and the right of the thalamus and cerebral cortex.

## **1.5 Scoring of virus activity**

In the evaluation of virus activity in the hemisections of the spinal cord and brain stem, a method of scoring the severity of the lesions should be used. Since the type of damage, whether cellular infiltration or destruction of neurons, is important, the lesions should be scored as follows:

### *Score*

Grade 1: Cellular infiltration only e.g. perivascular cuffing; low, moderate or severe cellular infiltration without evidence of neuronal damage (this is not sufficient for the monkey to be considered as positive).

Grade 2: Cellular infiltration with minimal neuronal lesions

Grade 3: Cellular infiltration with extensive neuronal damage

Grade 4: Massive neuronal damage with or without cellular infiltration.

A panel of slides is available for training purposes through WHO.

The scores obtained should be recorded on a standard form (see Part 3).

A monkey with neuronal lesions in the sections but which shows no needle tract should be regarded as positive.

A monkey showing a needle tract in the sections but no neuronal lesions should not be regarded as positive.

A section that shows damage due to trauma but no specific virus lesion is not included in the score.

Severity scores are based on hemisection readings of the lumbar (L), cervical (C), and brain (B) histological sections. The lesion score (LS) for each positive monkey is calculated as follows:

$$LS = \left\{ \left[ \frac{\sum \text{ of L scores}}{\text{No. of hemisections}} \right] + \left[ \frac{\sum \text{ of C scores}}{\text{No. of hemisections}} \right] + \left[ \frac{\sum \text{ of B scores}}{\text{No. of hemisections}} \right] \right\} \div 3$$

A mean lesion score is calculated for each group of positive monkeys.

## **1.6 Evaluation of neurovirulence test**

The comparison of the virus activity in the vaccine and the reference preparation should be based on the activity in the lumbar enlargement of the cord and the degree of spread of activity from this region to the cervical enlargement and the brain.

The acceptance or rejection of the vaccine should be based on the total score of all the positive test animals. Individual animals showing unusually high activity, either in the lumbar region or as the result of spread from this region, should also be taken into consideration in the final evaluation.

The filtered monovalent bulk passes the test if the required number of animals is positive and if none of the clinical and histopathological examinations shows a significant difference in pathogenicity between the vaccine virus and the reference material.

Criteria for the acceptance of vaccines after neurovirulence testing are given in Part 2.

## **Part 2. Criteria for the acceptance of vaccines after neurovirulence testing**

When implementing the test, it is recommended that each laboratory should perform a minimum of four neurovirulence tests (referred to here as “qualifying” tests) on each reference vaccine (Types 1, 2, and 3) to provide sufficient data on the activity of such reference vaccines for the development of criteria for the acceptability of test vaccines. On practical grounds, each of these tests may include a homotypic lot of production vaccine tested concurrently with the reference so that the results of the tests may be used in assessing vaccines in addition to providing information on the reference. The minimum number of animals in each of these tests is as specified in section 1.1 for each poliovirus type. The overall mean Lesion Score (M) for the replicate tests on each reference virus is calculated together with the pooled estimate ( $s^2$ ) of the within-test variance and the within-test deviation (s).

Criteria for the validity of the results of a test of a reference preparation can be determined by each laboratory only on the basis of the data accumulated after the four qualifying tests. No generally applicable criteria can therefore be given. For laboratories with limited experience with neurovirulence testing, the following empirical method of establishing acceptable limits for the mean Lesion Score for the reference ( $\bar{X}_{ref}$ ) may be helpful:

	<i>Lower Limit</i>	<i>Upper Limit</i>
For Types 1 and 2	$M - s$	$M + s$
For Type 3	$M - \frac{s}{2}$	$M + s$

A neurovirulence test in which the mean Lesion Score for the reference ( $\bar{X}_{ref}$ ) is not consistent with previous experience (within the acceptable limits referred to above) should not be used for assessing a test vaccine.

If the test is valid, the mean Lesion Score for the test vaccine ( $\bar{X}_{test}$ ) is compared with that of the homotypic reference vaccine ( $\bar{X}_{ref}$ ) as follows ( $C_1$ ,  $C_2$  and  $C_3$  are constants defined below).

The vaccine is not acceptable if:

$$\bar{X}_{test} - \bar{X}_{ref} > C_1$$

The vaccine may be retested once at the discretion of the national regulatory authority if:

$$C_1 < \bar{X}_{test} - \bar{X}_{ref} < C_2$$

If the vaccine is retested, the means of the Lesion scores for the test and reference vaccines are recalculated, combining the data from both tests, and the vaccine is rejected if:



$$\bar{X}_{test1+test2} - \bar{X}_{ref1+ref2} > C_3$$

The constants  $C_1$ ,  $C_2$  and  $C_3$  are calculated as follows:

$$C_1 = 2.3 \sqrt{\frac{2s^2}{N_1}}$$

$$C_2 = 2.6 \sqrt{\frac{2s^2}{N_1}}$$

$$C_3 = 1.6 \sqrt{\frac{2s^2}{N_2}}$$

Where  $N_1$  = number of positive monkeys per group per vaccine test,

$N_2$  = number of positive monkeys per group for the two tests combined,

2.3 = normal deviate at the 1% level,

2.6 = normal deviate at the 0.5% level,

1.6 = normal deviate at the 5% level.

In some countries, the national regulatory authority may permit an experienced manufacturer to accumulate data on the qualifying tests of the Types 1 and 2 international preparations for neurovirulence as serial batches of vaccine are tested and released, rather than wait until the data are available from the four qualifying tests before the release of any future vaccine.

It is assumed that the values of the constants  $C_1$ ,  $C_2$ , and  $C_3$  will be calculated by each laboratory for each reference vaccine. As experience with the reference accumulates, it is recommended that laboratories should recalculate the values of  $s^2$  and  $M$  using data from their last 10 tests only, and update their values of  $C_1$ ,  $C_2$ , and  $C_3$  accordingly.

Estimates of the probability that test vaccines with a true Lesion Score double that of the reference vaccine will be rejected, are given in Table 1 for different coefficients of variation.

The number of animals needed for the test have been chosen so that, for a single test, the analysis recommended above will result in the rejection of approximately 1% of test vaccines that are identical to the homotypic reference, on the assumption that, in each laboratory, the within-test variation is similar to that observed in the qualifying tests with that reference.

**Table 1**

**Estimated probability that test vaccines with a true Lesion Score double that of the reference vaccine will be rejected for different coefficients of variation.**

Total number of positive animals per test	Coefficient of Variation <sup>a</sup>					
	0.4	0.6	0.8	1.0	1.2	1.4
24 <sup>b</sup>	99%	96%	77%	55%	39%	28%
40	99%	99%	95%	80%	62%	47%

<sup>a</sup> The coefficient of variation is defined as the within-test standard deviation divided by the mean Lesion Score. Boxes show acceptable coefficients of variation.

<sup>b</sup> Divided equally between tests on Type 1 and 2 vaccines.

<sup>c</sup> Corresponds to tests on Type 1 and 2 vaccines.

<sup>d</sup> Corresponds to tests on Type 3 vaccines.

In tests on vaccines which satisfy the above criteria of acceptability, individual animals may occasionally develop extremely high Lesion Scores. Such findings should be taken into consideration in evaluating the acceptability of vaccines, but precise criteria for use in making a decision are difficult to define.

Sample calculations are shown in Tables 2 and 3.

**Table 2. Sample calculations of results of qualifying tests of reference**

Basic data		Type 1	Type 2	Type 3
Overall mean Lesion Score ( $M$ ) (initial four tests)		1.110	0.878	1.043
Within-test pooled variance ( $s^2$ ) of $M$		0.444	0.236	0.686
Within-test pooled standard deviation ( $s$ ) (square root of $s^2$ )		0.666	0.486	0.828
Coefficient of variation (CV) ( $CV = s/M$ )		$\frac{0.666}{1.110} = 0.60$	$\frac{0.486}{0.878} = 0.55$	$\frac{0.828}{1.043} = 0.79$
Upper and lower limits for satisfactory test result. Mean Lesion Score of reference vaccine ( $\bar{X}_{ref}$ )		$M + s = 1.110 + 0.666 = 1.776$ $M - s = 1.110 - 0.666 = 0.444$	$M + s = 0.878 + 0.486 = 1.364$ $M - s = 0.878 - 0.486 = 0.392$	$M + s = 1.043 + 0.828 = 1.871$ $M - \frac{s}{2} = 1.043 - \frac{0.828}{2} = 0.629$
Constants for assessing acceptability of difference between mean Lesion Score of test vaccine ( $\bar{X}_{test}$ ) and mean Lesion score of reference vaccine ( $\bar{X}_{ref}$ )	$C_1 = 2.3 \sqrt{\frac{2s^2}{N_1}}$  $C_2 = 2.6 \sqrt{\frac{2s^2}{N_1}}$  $C_3 = 1.6 \sqrt{\frac{2s^2}{N_2}}$	$2.3 \sqrt{\frac{2 \times 0.444}{12}} = 2.3\sqrt{0.074} = 0.626$  $2.6 \sqrt{\frac{2 \times 0.444}{12}} = 2.6\sqrt{0.074} = 0.707$  $1.6 \sqrt{\frac{2 \times 0.444}{24}} = 1.6\sqrt{0.037} = 0.308$	$2.3 \sqrt{\frac{2 \times 0.236}{12}} = 2.3\sqrt{0.039} = 0.456$  $2.6 \sqrt{\frac{2 \times 0.236}{12}} = 2.6\sqrt{0.039} = 0.516$  $1.6 \sqrt{\frac{2 \times 0.236}{24}} = 1.6\sqrt{0.020} = 0.224$	$2.3 \sqrt{\frac{2 \times 0.686}{20}} = 2.3\sqrt{0.069} = 0.602$  $2.6 \sqrt{\frac{2 \times 0.686}{20}} = 2.6\sqrt{0.069} = 0.681$  $1.6 \sqrt{\frac{2 \times 0.686}{40}} = 1.6\sqrt{0.034} = 0.296$

**Table 3**  
**Examples of tests with Type 1 reference and vaccine**

Example	Mean Lesion Scores		Difference between vaccine and reference  ( $\bar{X}_{test} - \bar{X}_{ref}$ )	Conclusion/Action <sup>a</sup>
	Reference ( $\bar{X}_{ref}$ )	Reference ( $\bar{X}_{test}$ )		
One test	0.826	1.188	0.362	Less than $C_1$ : vaccine acceptable
Two tests 1 <sup>st</sup>	0.826	1.493	0.667	More than $C_1$ but less than $C_2$ : Vaccine unacceptable: retest is permissible
2 <sup>nd</sup>	1.285	1.209	-0.076	-
Combined tests	1.056	1.351	0.295	Less than $C_3$ : vaccine acceptable
Two tests 1 <sup>st</sup>	0.826	1.493	0.667	More than $C_1$ but less than $C_2$ : Vaccine unacceptable: retest is permissible
2 <sup>nd</sup>	1.285	1.405	0.120	-
Combined tests	1.056	1.449	0.393	More than $C_3$ : vaccine fails

<sup>a</sup> For values of  $C_1$ ,  $C_2$  and  $C_3$  see Table 2

### **Part 3. Form on which to report the score of virus activity for each histological section from all monkeys included in the neurovirulence test**

Although the test requires that at least 11 monkeys should be positive after inoculation with the vaccine and reference virus for Types 1 and Types 2, provision is made on the following form (pp. 14-15) for recording results for 12 monkeys that may be inoculated and survive the test. A separate form to record the lesions in 20 monkeys will be required for Type 3.

On the forms, the method of scoring the lesions used for all sections from all areas is that already indicated in Part 1.5, namely:

Grade 1: Cellular infiltration only e.g.: perivascular cuffing; low, moderate or severe cellular infiltration without evidence of neuronal damage. (this is not sufficient for the monkey to be considered as positive)

Grade 2: Cellular infiltration with minimal neuronal lesions

Grade 3: Cellular infiltration with extensive neuronal damage

Grade 4: Massive neuronal damage with or without cellular infiltration.

A model of the certificate of compliance with the international requirements for the neurovirulence testing of monovalent OPV bulks in monkeys is given below.

### **Certificate of compliance with the recommendations for the neurovirulence testing of monovalent OPV bulks in monkeys**

Monovalent bulk or seed number. \_\_\_\_\_

Date of certification \_\_\_\_\_

I certify that the above monovalent bulk or seed complies with the recommendations for tests in monkeys for neurovirulence published in WHO Technical Report Series, No. xxx. A.4.4.5.2

Signature \_\_\_\_\_

Name (typed) \_\_\_\_\_

Date \_\_\_\_\_

All data from all monkeys must be recorded, which may require a larger form.

Clinical signs of paralysis must be recorded on a separate form.

Test no. \_\_\_\_\_ Type \_\_\_\_\_ Monovalent bulk no. \_\_\_\_\_ Monkey Species \_\_\_\_\_

Post-inoculation (Back titration) titre: log<sub>10</sub> CCID<sub>50</sub>/ml \_\_\_\_\_ Dilution \_\_\_\_\_

Date of inoculation \_\_\_\_\_ Date of end of test \_\_\_\_\_

N	V		Histological lesions due to poliomyelitis																												Lesion score																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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	1222	1223	1224	1225	1226	1227	1228	1229	1230	1231	1232	1233	1234	1235	1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246	1247	1248	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260	1261	1262	1263	1264	1265	1266	1267	1268	1269	1270	1271	1272	1273	1274	1275	1276	1277	1278	1279	1280	1281	1282	1283	1284	1285	1286	1287	1288	1289	1290	1291	1292	1293	1294	1295	1296	1297	1298	1299	1300	1301	1302	1303	1304	1305	1306	1307	1308	1309	1310	1311	1312	1313	1314	1315	1316	1317	1318	1319	1320	1321	1322	1323	1324	1325	1326	1327	1328	1329	1330	1331	1332	1333	1334	1335	1336	1337	1338	1339	1340	1341	1342	1343	1344	1345	1346	1347	1348	1349	1350	1351	1352	1353	1354	1355	1356	1357	1358	1359	1360	1361	1362	1363	1364	1365	1366	1367	1368	1369	1370	1371	1372	1373	1374	1375	1376	1377	1378	1379	1380	1381	1382	1383	1384	1385	1386	1387	1388	1389	1390	1391	1392	1393	1394	1395	1396	1397	1398	1399	1400	1401	1402	1403	1404	1405	1406	1407	1408	1409	1410	1411	1412	1413	1414	1415	1416	1417	1418	1419	1420	1421	1422	1423	1424	1425	1426	1427	1428	1429	1430	1431	1432	1433	1434	1435	1436	1437	1438	1439	1440	1441	1442	1443	1444	1445	1446	1447	1448	1449	1450	1451	1452	1453	1454	1455	1456	1457	1458	1459	1460	1461	1462	1463	1464	1465	1466	1467	1468	1469	1470	1471	1472	1473	1474	1475	1476	1477	1478	1479	1480	1481	1482	1483	1484	1485	1486	1487	1488</

(continued on next page)

N	V		Histological lesions due to poliomyelitis																																Lesion score
			Lumbar enlargement																Cervical enlargement										Brain						
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
			0	1	2																														
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		R																																	
		L																																	
		R																																	
Mean lesion score																																			

KEY

N = Monkey number  
V = Valid  
L = Left  
R = Right  
Av = Average  
Med = Medulla  
Cb = Cerebellum  
P = Pons  
M = Midbrain  
T = Thalamus  
Co = Cortex

Signature \_\_\_\_\_ Date \_\_\_\_\_

Name (typed) \_\_\_\_\_



## Authors

The first version of the protocol for the Monkey Neurovirulence Test which is based on the text and appendices from WHO TRS 904 (Annex 1) was prepared by Dr Morag Ferguson, Horning, UK, Dr Javier Martin, Ms Glynis Dunn and Mr Alan Heath, National Institute of Biological Standards and Control, South Mimms, UK with input from Dr Ghazi Auda, National Institute of Biological Standards and Control, South Mimms, UK; Dr Laetitia Agostini Bigger who compiled comments from IFPMA Vaccines Committee, Geneva, Switzerland; Dr Iin Susanti Budiharto, Bio Farma, Bandung, Indonesia; Dr Emmanuelle Coppens, Sanofi Pasteur, France; Dr. Amando Meneses Vionet. Laboratorios de Biológicos y Reactivos de México, México; Ms Virginie Pithon, Agence Française de Sécurité Sanitaire des Produits de Santé, Lyon, France; Dr Anna Laura Salvati, Istituto Superiore di Sanità, Roma, Italy; Dr. Minerva J. Uribe Serralde, Laboratorios de Biológicos y Reactivos de México, México; Dr Hui Wang, Beijing TianTan Biological Products Co.,Ltd Beijing, P.R. of China; Dr Geneviève Waeterloos, Scientific Institute of Public Health, Brussels, Belgium and Dr TieQun Zhou, Quality, Safety and Standards/Immunization, Vaccines and Biologicals /Family, Women's and Children's Health, World Health Organization, Geneva, Switzerland.

The first version was reviewed and comments taken into consideration at a WHO consultation held on 27-29 March 2012 attended by: Dr Shinobu Abe, Japan Poliomyelitis Research Institute, (JPRI), Tokyo, Japan; Dr Maria Baca-Estrada, Biologics and Genetic Therapies Directorate, Ottawa, Canada; Dr Wilfried A.M. Bakker, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; Dr Jacqueline Fournier-Caruana, Quality, Safety, and Standards, Immunization, Vaccines, and Biologicals, Family, Women's and Children's Health, World Health Organization, Geneva, Switzerland; Mr Bhupender Singh Chauhan, Bharat Biotech International Limited, Hyderabad, India; Dr Konstantin Chumakov, Office of Vaccine Research and Review, Food & Drug Administration Bethesda, USA; Dr Emmanuelle Coppens, Sanofi Pasteur, Marcy L'Etoile, France; Dr Michel Duchêne, GSK Biologicals, Wavre, Belgium; Ms Glynis Dunn, National Institute for Biological Standards and Control, South Mimms, UK; Dr Diana Felnerova, Crucell, Berne, Switzerland; Dr Lucia Fiore, Istituto Superiore di Sanità, Roma, Italy; Mr José Bugarín González, Laboratorios de Biológicos y Reactivos de México S.A. de C.V. México D.F., Mexico; Dr Morag Ferguson (Lennon), Horning, United Kingdom; Dr Martha Ayala Gonzalez, Federal Commission for the Protection from Sanitary Risks (COFEPRIS), Ministry of Health, Mexico; Prof Victor Grachev, Russian Academy of Medical Sciences (RAMS), Moscow Region, Russian Federation; Ms Wang Hui, Tiantan Biological Products Co., Ltd, Beijing, China; Mrs Teeranart Jivapaisarnpong, Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand; Dr Ivana Knezevic, Quality, Safety, and Standards, Immunization, Vaccines, and Biologicals, Family, Women's and Children's Health, World Health Organization, Geneva, Switzerland; Dr Hiromasa Okayasu, Research, Policy and Product Development, World Health Organization, Geneva, Switzerland; Dr Dede Kusmiaty, National Quality Control Laboratory of Drug and Food, Ministry of Health, Jakarta, Indonesia; Dr Kazuhiko Katayama, National Institute of Infectious Diseases (NIID), Tokyo, Japan; Dr Changgui Li, National Institutes for Food and Drug Control (NIFDC), Beijing, P.R. China; Dr Javier Martin, National Institute for Biological Standards and Control (NIBSC), South Mimms, UK; Dr Catherine Milne, European Directorate for the Quality of Medicines and HealthCare (EDQM), Strasbourg, France; Dr Rajiv Modi, Cadila Pharmaceuticals Limited, Ahmedabad, India; Ms Elisabeth Niogret, Sanofi Pasteur, Marcy L'Etoile, France; Dr Le Van Phung, National Institute for Control of Vaccine and Biologicals, Hanoi, Vietnam; Dr Virginie Pithon, Agence Française

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Further changes were made, taking into consideration comments received during the public consultation along with the WHO/BS/2012.2185, and following the review by the 63<sup>rd</sup> Expert Committee on Biological Standardization, 15-19 October 2012, resulting in the present document.

## Reference

1. Recommendations to Assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine (oral). Proposed replacement of: TRS 904, Annex 1 and Addendum TRS 910, Annex 1. *Expert Committee on Biological Standardization* 2012. World Health Organization.