Annex 1

Recommendations for the production and control of poliomyelitis vaccine (oral) (Addendum 2000)

Introduction

The Recommendations (formerly Requirements) for Poliomyelitis Vaccine (Oral) were last revised in full in 1999 (1). At that time, new quality control tests were introduced for the vaccine. One such test was a neurovirulence test for poliovirus vaccine in the TgPVR21 transgenic mouse line. The test in TgPVR21 mice was shown in WHO-supported studies to be a suitable alternative to the monkey neurovirulence test for poliovirus type 3. At its fiftieth meeting, in 1999, the Committee was informed of the excellent progress with TgPVR21 neurovirulence tests for poliovirus types 1 and 2 and encouraged completion of these studies as soon as possible.

The studies with poliovirus types 1 and 2 in TgPVR21 mice were completed by June 2000 and a Working Group met to review the data. The Working Group concluded that the data validated the neurovirulence test in TgPVR21 mice for poliovirus types 1 and 2. They advised that the Recommendations for the Production and Control of Poliomyelitis Vaccine (Oral) be amended to include the neurovirulence test in TgPVR21 mice as an alternative to the neurovirulence test in monkeys for all three poliovirus serotypes.

The 1999 Recommendations state that to qualify as competent to perform the mouse neurovirulence test, laboratories should complete a standard implementation process. The Working Group defined the details of a standard implementation process. This is incorporated into a revision of the Standard Operating Procedure for the mouse neurovirulence procedure.

The report of the Working Group is available as a separate document from WHO.1

The proposed amendments to the 1999 Recommendations are given below:

A.4.4.5.3 Neurovirulence test in transgenic (TgPVR21) mice for poliovirus type 1, 2 or 3

The TgPVR21 transgenic mouse model provides a suitable alternative to the monkey neurovirulence test for the neurovirulence testing

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of type 1, 2 or 3 vaccines once a laboratory qualifies as competent to perform the test as specified below and experience is gained to the satisfaction of the national control authority. Experience in a national control laboratory should be assessed by WHO. The test should be performed according to the standard operating procedure, “WHO neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus”, available from WHO.¹

Although the murine model may be used for neurovirulence testing of filtered bulk suspensions, the monkey neurovirulence test should remain as the definitive reference test to requalify vaccine production, for example to evaluate any new virus seed materials or vaccines produced on a new substrate and lots prepared to establish consistency from the new seed or substrate.

To qualify as competent to perform the mouse neurovirulence test, laboratories should complete a standard implementation process. Details of the process are included in the standard operating procedure “WHO neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccines (oral) in transgenic mice susceptible to poliovirus”, available from WHO.¹ The process should be fully documented. Once qualified as competent, each laboratory should continue to monitor their competence to perform the test. Laboratories are requested to submit the results of their tests both during the implementation phase and subsequent routine use to WHO. These data should be evaluated by WHO in order to monitor globally how the new test performs in practice.

1) TgPVR21 mice

Mice used for the neurovirulence test must be aged 6–8 weeks at the time of inoculation and should be from a source defined in the standard operating procedure “WHO neurovirulence test of type 1, 2 or 3 live poliomyelitis vaccine (oral) in transgenic mice susceptible to poliovirus”. Mice should be allowed to recover from shipping for at least 7 days before inoculation. Procedures and standards for maintenance of TgPVR21 mice should follow the WHO Guidelines (2).

2) Number of mice

A vaccine and appropriate homotypic reference virus should be tested concurrently. Equal numbers of animals, with equal numbers of males and females, should be inoculated with the reference virus and the vaccine being tested. Mice should be allocated
to vaccine or reference virus and to particular cages using a randomization procedure (defined in the standard operating procedure); 32 mice per test dose should be used for evaluation of the vaccine and 32 mice per test dose for evaluation of the reference. More than one vaccine may be tested with the same homotypic reference virus at the same time. If a test is done on two working days, equal numbers of mice should be inoculated with the vaccine and the reference virus on each working day.

(3) Virus content of vaccines and reference virus inoculated

The virus content of the vaccines and reference preparations should be determined with a precision of $\pm 0.3 \log_{10}$ cell culture infections doses 50 (CCID$_{50}$) or better, and normalized against titration standards, as described in the standard operating procedure. Groups of mice should be inoculated with two test doses of vaccine and reference. For poliovirus type 1 vaccines tested against the WHO(SO+2)/I reference virus, the doses are 1.75 and 2.75$\log_{10}$ CCID$_{50}$ in 5µl. For poliovirus type 2 vaccines tested against the WHO (SO+2)/II reference virus, the doses are 5.0 and 6.0$\log_{10}$ CCID$_{50}$ in 5µl. For poliovirus type 3 vaccines tested against the WHO(SO+2)/III reference virus, the doses are 3.5 and 4.5$\log_{10}$ CCID$_{50}$ in 5µl.

If other reference viruses are used, the doses should be determined by the paralysis proportions of the reference: at the high dose <0.95, at the low dose >0.05.

Mice should be sedated appropriately and inoculated into the lumbar region of the spinal cord as described in the standard operating procedure.

(4) Observation of mice

Mice should be observed for occurrence of paresis or paralysis daily for 2 weeks after inoculation. Paralysed mice should be humanely killed as soon as paralysis is confirmed. Other mice should be humanely killed on day 14 after inoculation. Data should be recorded on a standard form (see the standard operating procedure). Mice with traumatic paralysis (appearing 24 hours or less after inoculation and not progressing) and those that die from causes other than poliomyelitis should be excluded from evaluation.

(5) Evaluation of the neurovirulence test

Comparison of the virus neurovirulence activity in the vaccine(s) and reference preparations should be based on the numbers of animals with paresis or paralysed animals in both groups of mice,
inoculated with two test doses of the vaccine and reference preparation. Validity criteria for each test must be met and are specified in the standard operating procedure.

The filtered bulk suspension passes the test if the numbers of animals with paresis or paralysed mice in the groups inoculated with vaccine are not significantly greater than the numbers in the groups inoculated with the reference material. Detailed statistical criteria for acceptance of vaccines after neurovirulence testing in TgPVR21 mice are given in the standard operating procedure.

References
