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Meeting Report

WHO Working Group Meeting to Discuss the Revision of the WHO Recommendations for OPV: TRS No. 904 and 910

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ABSTRACT

Oral poliomyelitis vaccine (OPV) is a critical part of the polio eradication programme. A high number of doses are administered each year with an impact on billions of citizens worldwide. It is therefore essential that written standards concerning OPV are up to date and widely available. The World Health Organization (WHO) publishes technical guidance on the quality, safety and efficacy of vaccines intended to assist national regulatory authorities (NRAs), national control laboratories (NCLs) and manufacturers. As part of its programme, on 20-22 July 2010 WHO convened a working group meeting to initiate the revision of the WHO recommendations on the production and control of OPV as presently outlined in the Technical Reports Series (TRS) issues No. 904 and 910 (1, 2). The attendees included experts from academia, NRAs/NCLs and industry involved in the study, manufacture, and authorization and testing/release of OPV from countries around the world including representatives from China, the European Union, Indonesia, Japan, Mexico, and the USA. The objective was to review the state of knowledge concerning production and control of OPV, with a focus on neurovirulence testing, to determine how the existing guidelines should be updated and what recommendations should be made for the future. The outcomes of this meeting will be taken into consideration in future revision of the WHO TRS.

INTRODUCTION

The new global standard should reflect new developments in areas related to OPV including advanced scientific knowledge, the availability of novel laboratory techniques and the use of new vaccine formulations such as monovalent/bivalent OPV or inactivated polio vaccines (IPVs) based on Sabin seeds. Following current WHO policy, the new recommendations will also include guidance on nonclinical and clinical evaluation of vaccines.

The meeting was structured in a way that participants first discussed the current scientific knowledge related to the molecular biology, neurovirulence and pathogenesis of poliovirus in humans and various animal models as well as the process of development and characterization of live-attenuated vaccine strains. The establishment of tests to measure the neurovirulence of OPV preparations and how these tests are used within the regulatory framework during the batch release process were then described and discussed in detail.

Lastly, both NRAs and manufacturers reviewed and compared the use of these tests in their institutions.

SUMMARY OF DISCUSSIONS

Scientific background

Despite years of study there are still numerous gaps in current knowledge of poliovirus pathogenesis. For example, it is not clear how and through what cells the virus enters the system during natural infection or how the virus invades the central nervous system (CNS) and localizes to the lower motor neurons. While theories exist to explain some of these phenomena, there are as yet no clear answers.

Sabin vaccine strains of all three serotypes are attenuated when given to humans. Relatively few mutations contribute significantly to the attenuated phenotypes of the three vaccine strains with those in domain V of the 5' non-coding region (NCR) playing a key role. Reversion (or suppression) of some of these mutations is associated with an increased virulence in animal models and is believed to be a factor of pathogenicity in humans. Multiple independent properties of poliovirus appear to determine its neurovirulence phenotype. These properties might vary significantly from strain-to-strain as well as serotype-to-serotype. For the recipient, they include determinants of viral pathogenesis such as replication at point of entry, replication in lymph nodes, titre and duration of viraemia, ability to cross the blood-brain barrier, ability to replicate in anterior horn cells and spread within the CNS; and virus molecular properties such as reversion rates during vaccine formulation, reversion rates during infection, etc. There are also determinants for the community related to viral transmissibility and pathogenicity such as the amount and duration of viral excretion, the infectivity of excreted virus and the pathogenicity of excreted virus. It is evident that no single test can address all of these determinants so there are limits to the interpretations possible from any approach.

From a large number of studies in several laboratories, it can be concluded that the attenuating effects of particular mutations in animal models can depend on species, genetic background and route of inoculation, and therefore make extrapolation to humans quite uncertain.

The monkey neurovirulence test (MNVT) model

During the development of live-attenuated Sabin vaccines, monkeys were used to assess virulence and to aid the selection process during the serial passages *in vivo* and *in vitro* and plaque purification procedures that led to the preparation of vaccine candidates. It was soon obvious that a test to measure the virulence of poliovirus would be critical to assure consistent production of safe vaccines. The MNVT was developed in the 1950s and required several years of optimization by fine-tuning different aspects of the test such as the monkey species to be used, the route of inoculation, the amount of virus and number of animals to be inoculated and the laboratory, clinical and statistical analyses to be performed. The introduction of a standardized procedure by WHO including the use of a reference virus for the test was critical for the establishment of a very sensitive neurovirulence test that also allowed comparison across laboratories and countries. The current test uses a single virus dilution and requires a minimum number of 11, 11 and 18 animals for serotypes 1, 2 and 3, respectively, to account for the different neurovirulence showed by the three vaccine serotypes. The size of the groups is based on the arbitrary requirement that the test should be able to detect a 2-fold difference in neurovirulence as compared to the reference virus. Inoculated monkeys are analysed both clinically and histologically and a detailed statistical analysis that includes historical data is conducted to determine whether the test vaccine is significantly more virulent than the corresponding reference virus. The MNVT has been used successfully for more than 40 years and is considered as a key contribution to the availability of a very safe vaccine that has helped to reduce the number of polio cases worldwide by more than 99%. The MNVT has benefitted from international workshops and collaborative studies towards international standardization and is well executed; giving consistent results for all laboratories involved although some aspects still require further harmonization.

The transgenic mouse neurovirulence test (TgmNVT) model

More recently, a neurovirulence test for poliovirus using transgenic mice has been developed. Transgenic mice expressing the human poliovirus receptor were first developed in the late 1980s. Despite several efforts, as with models in monkeys, it has still not been possible to develop a suitable transgenic mouse model that resembles infection by the oral route in humans. The TgmNVT was first developed in the early 1990's by Dragunsky *et al* (3) and

was demonstrated to be a suitable model for all three OPV serotypes by a number of research and collaborative studies.

The TgmNVT was approved as an alternative to the MNVT for all three types of OPV for vaccine bulks at the Expert Committee for Biological Standardization (ECBS) meeting in October 2000 and was subsequently included in the WHO TRS 904 (for type 3) and TRS 910 (for type 1, 2 or 3) issued in 2002 and in the European Pharmacopoeia monograph for the testing of OPV monovalent bulks as a release assay since January 2006.

In 1993, based on preliminary data obtained with type 3 OPV WHO launched an international collaborative study aiming development of transgenic mouse model to replace monkey neurovirulence test for control of OPV neurovirulence. In total, the collaborative studies spanned 8 years in 5 phases, involved the performance of 206 assays on 94 different samples in 11 laboratories. The TgmNVT also required fine-tuning during the research and development phases. The test uses a particular strain of transgenic mouse (TgPVR21) and is carried out at 2 separate doses for each sample (4). Five microlitres are injected intraspinally into each mouse which is a technique that requires great dexterity and a lot of training. The test results are based on clinical observations to determine whether the test vaccine is not significantly more virulent than the test reference. The main difference with the MNVT is there is no histological examination. As with the MNVT, the statistical analysis makes use of historical data to establish the pass/fail criteria. Most OPV manufacturers in Europe have started to move from the MNVT to the TgmNVT because of price, ethical and logistic issues. However, switching to the TgmNVT is not a straightforward process and all of the steps along the way are subject to extensive training and validation. As with the MNVT, the TgmNVT has benefitted from regular workshops and collaborative studies and is well executed, giving consistent results for all laboratories. In fact, the TgmNVT is much better standardized than the MNVT in some aspects such as the animal species/strain and standard procedures used, the implementation and validation processes and the levels of control to ensure consistency and prevent a drift in clinical scoring results. These include independent observation by the responsible NRA, repeat testing annually at NIBSC of 10% of the vaccine batches produced in Europe, and the availability of independent training and proficiency testing using DVDs. It can be completed in 2 weeks for the mouse test compared to 1.5-2 months for the monkey test. The TgmNVT has been extensively used by manufacturers as part of the batch release process for OPV for serotypes 1 and 3 and to a lesser extent for

serotype 2 vaccines. However, unlike the primate test, the TgmNVT does not provide a permanent record of the test in the form of histological slides which can be independently assessed or reassessed in the future, and there is less experience with the effect of mutations in the vaccine virus on the test outcome. In both the TgmNVT and MNVT the correct placement of inoculum is documented by a characteristic limb jerking during the inoculation process. However, there is no internal control for the correct placement of the inoculum in the TgmNVT whereas in the MNVT histological damage of some degree must be seen for the animal to be included as valid.

In the existing WHO guideline, the TgmNVT is accepted for release of monovalent bulks at the discretion of the NRA, but the MNVT is regarded as the gold standard, to be used in the event of disagreements or the development of new working or master seeds. One objective of the meeting was to evaluate whether the two tests could justifiably be considered interchangeable for regulatory purposes.

The Mutation Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) assay

The MAPREC assay is a molecular method developed by Chumakov et al. (5) used to determine the proportion of single base mutations experimentally associated with attenuation at a given point within the viral RNA. If the calculated value of the mutation in Sabin vaccine at this site is greater than acceptable values, the vaccine will fail the MAPREC test. For each serotype, specific PCR primers are used to amplify a short segment of the genome containing the base to be quantified. Mutations in domain V of the internal ribosome entry site (IRES) are the target for this test. One of the primers contains modifications required to create a unique restriction site for enzyme digestion. The acceptable level of mutant content from a batch of vaccine determined by MAPREC is currently defined only for type 3 in the WHO TRS 904. Reference reagents used for the MAPREC test were established by WHO collaborative studies and adopted by ECBS in stages: Type 3 in 1996 and 1997, Type 2 in 2004 and Type 1 in 2009. They consist of an International Standard (DNA), a high mutant reference virus, and a low mutant reference virus, as well as a DNA containing 100% of the mutation of interest, to control enzyme digestion.

In type 3 vaccines the level of reversion is directly related to the Mean Lesion Score (MLS) in monkeys, such that if the level of reversion from T to C at base 472 is above 1%, the

vaccine fails the MNVT. As the passage level of the vaccine increases, so does the 472-C content. The Sabin Original (SO) vaccine has very low 472-C content and MLS, as does a new seed derived from a SO+5 RNA and designated RSO1. As the passage level increases the failure rate in the MNVT increases, as does the MLS and the %472-C. The available seed virus for Sabin poliovirus type 3 is limited and producing vaccine from additionally passaged working seed results in an increased percentage levels of 472-C, although below the maximum permitted. For types 1 and 2 vaccines, the percentage of mutations at which a virus sample reliably fails the MNVT is much higher (about 10-fold) than the range observed in commercially produced vaccines. Therefore within this range there is no correlation between the percentage of mutations and neurovirulence. The availability of the reference preparations means that tests for type 1 and type 2 could be included in the revised guidelines to monitor consistency.

MAPREC has been used by manufacturers and NCLs for a number of years to assess serotype 3. At the time of this meeting, MAPREC for serotype 1 and 2 had not been validated for use as a quality control test of monovalent bulks. The experience from laboratories is that it is a very laborious test and the fact that requires the use of radio isotopes adds to its technical difficulty. Different laboratories appear to have problems in different aspects of the test. However, consistency data has been derived from all laboratories.

Recent advances in molecular technologies promise to address limitations and shortcomings of the MAPREC assay. The new approaches, particularly massively parallel sequencing, may eventually result in introduction of methods for detailed quantitative characterization of mutations in the entire viral genome, complementing existing neurovirulence tests and making them less critical. However, validation of such techniques might take several years.

TgmNVT and MAPREC as alternatives for MNVT

The MAPREC test is based on the analysis of a single nucleotide mutation. For type 3 vaccines there is a strong correlation between the proportion of the target mutation and the neurovirulence phenotype for commercial batches of vaccine from existing manufacturers. It has limited scope to predict the neurovirulence of poliovirus in general which may be determined by mutations at different sites in the viral genome. In addition, types 1 and 2 vaccines rarely, if ever, fail the MNVT, and therefore it was not possible to determine

whether the percentage of mutations analysed by MAPREC is a good predictor of the vaccine neurovirulence. MAPREC could be used as a consistency test to complement neurovirulence testing at different stages during vaccine production.

The monkey and mouse models for poliovirus infection are known to differ in some respects of pathogenesis. In turn both differ from the chimpanzee model and humans, suggesting that they cannot be used directly to predict clinical outcomes during vaccination, and therefore provide only information about consistency of vaccine batches. Both models appear to also have different sensitivity to certain viral mutations in terms of the neurovirulence phenotype shown after virus inoculation. It is not clear if these differences matter for virus delivered intraspinally. All known vaccine lots that failed the MNVT were tested in mice during the development of TgmNVT, and the results showed an excellent agreement. European manufacturers' experience accumulated since TgmNVT was approved for lot release is that vaccine lots that pass the MNVT also pass the mouse TgmNVT. However no additional batches that failed the MNVT were reported at the meeting. Therefore no more data were available to test whether batches that failed the MNVT will also always fail the TgmNVT.

Although only limited information is available, there are mutations that have a different effect on virulence when using the MNVT or the TgmNVT. The TgmNVT was validated using type 3 vaccines produced from WHO-accepted RSO seed virus and the WHO-validated SO+2 neurovirulence reference was also used for MNVT. Some manufacturers produce OPV from other derivative strains containing complete amino acid substitutions with respect to the consensus of Sabin original stock, most notably Thr->Ile at amino acid 6 of VP1 (nucleotide 2493). This mutation does not affect either clinical safety of the vaccine or monkey neurovirulence, but appears to slightly increase neurovirulence in TgmNVT. During the development of the TgmNVT, two type 3 vaccines failed and one marginally passed the TgmNVT while all three clearly passed the MNVT. These three batches contained low levels of the 472-C mutation associated with virulence in both models and would therefore be predicted to pass. However, they were made from a seed strain that contained 100% 2493-U. If this accounts for the failure of the batches it seems reasonable to conclude that the TgmNVT is more sensitive with respect to detection of this mutation than the MNVT. It also suggests that when tested against the current TgmNVT reference prepared from SO, other vaccines made from alternative seeds containing 2493-U may fail the test in Tg mice, while still being acceptable based on monkey test results. Therefore TgmNVT in combination with

the current type 3 reference could be used for lot release of vaccine batches made from RSO Sabin 3 seeds, while vaccines made from strains with different nucleotide sequence should be tested either in MNVT, or in TgmNVT with a strain-specific reference. No such validated reference exists at the moment. It is likely that new OPV manufacturers (if any) will use the WHO-provided RSO strain to produce working seeds, and therefore final bulks will be suitable for testing in TgmNVT. The use of the TgmNVT for other vaccines produced from other seeds will require specific validation including the use of appropriate references.

The current design of TgmNVT is aimed at reaching simple pass/fail decision during the lot release process, and does not allow for a detailed quantitative comparison of the neurovirulence between different poliovirus isolates. However, there is another version of the test that includes more dilutions to allow for the quantitative determination of the 50% paralytic dose.

Demonstrating an association between two variable test methods requires testing of samples over a wide range of possible test values. However, commercial vaccines that had failed the MNVT were only available for use in the original collaborative studies for type 3, so experimental vaccines prepared in the laboratory were used for types 1 and 2 during the development of the TgmNVT. As a consequence, only vaccines with a clear pass or a clear fail in the MNVT were included in the studies for types 1 and 2. A single vaccine with a marginal fail result in the MNVT was available for type 3. This vaccine was used in repeated tests in several laboratories demonstrating that the TgmNVT is at least as sensitive as the MNVT in detecting this vaccine as neurovirulent. However, no other marginal vaccines have been identified. No vaccine lots have failed either the MNVT or the TgmNVT test since the TgmNVT was established and the spread of responses in passed batches is very narrow. Repeat testing of samples of a similar value can only demonstrate the variability of both tests but cannot provide any accurate information on the comparison between the tests.

The MNVT produces quantitative results that allow comparison of the effect of different mutations on the neurovirulence of poliovirus. It is a very sensitive test that, for serotype 3 vaccines, can detect small increases in virulence between successive passage levels from the vaccine master seed. Type 1 and type 2 vaccine strains appear to be more stable in the hands of established manufacturers and so the level of virulence does not increase significantly during successive passages under normal growth conditions.

In summary, since the introduction of the TgmNVT in 2000, there has not been sufficient additional scientific evidence that proves that the MNVT and TgmNVT are equivalent in their response to all mutations known to attenuate virulence. Many participants felt strongly that the degree of equivalence that had been demonstrated was sufficient for all practical purposes. They argued that the TgmNVT is as good as the MNVT to assess the neurovirulence of commercial OPV batches as shown by research and collaborative studies and several years of experience with the TgmNVT for the batch release of OPV. In their opinion, equivalence between the models did not need to be demonstrated in order to give the TgmNVT and the MNVT equal status for neurovirulence testing of OPV. Others considered that this was not appropriate at present. While there is evidence that the two models respond in a similar way to the mutations routinely detected by MAPREC their response to other mutations is not clear and there is at least one other mutation where their responses are apparently different. The two tests may be suitable for showing consistency of production for bulks produced at the same passage level under GMP as stated in the current WHO and European documents. However they may not be equivalent if comparing stocks with significantly different mutational composition, for instance comparing products from different manufacturers. The collaborative study had demonstrated that MNVT and TgmNVT are equivalent for testing vaccines prepared from RSO Sabin 3 seeds but TgmNVT may fail otherwise acceptable (by MNVT) lots prepared from derivative strains containing additional mutations. Therefore the TgmNVT can be used as a replacement of MNVT for vaccines made from Sabin 3 RSO seeds, while MNVT may need to be used for other type 3 vaccines until the TgmNVT is appropriately validated, including the development of an appropriate homologous reference if necessary.

Other matters

Mono and bivalent oral polio vaccines

New monovalent type 1, monovalent type 3 and bivalent type 1 + type 3 OPVs have been recently developed and successfully used to interrupt circulation of wild poliovirus in endemic areas and polio-free areas with re-established circulation. These new OPV formulations should be mentioned in the current TRS for the production and quality control of OPV.

Thermostability

The significance of thermostability testing for OPV was discussed following some out of specification results in this test leading to vaccine failures, particularly for type 1 monovalent OPV. The group agreed that thermostability is a product-specific characteristic and should be used as a consistency test and not as predictive of real time stability of the vaccine. However, any revision of the current specification criteria should be scientifically justified.

Potency specification (for type 3)

In October 1990, based on data from the Region of the Americas, the EPI Global Advisory Group recommended to increase the minimum titre of type 3 virus from 5.5 to 5.8 log₁₀ CCID₅₀ /dose for OPV formulation. This is still the recommendation for OPV for WHO program use which is not mentioned in the current TRS for the production and quality control of OPV.

Sabin IPV vaccine seed

As stated in the Addendum to the Recommendations for the Production and Quality Control of Poliomyelitis Vaccine (Inactivated) [WHO TRS, No. 926, 2004], “attenuated poliovirus strains (such as Sabin strains) that have been approved by the NRA in the country of manufacture for use as an OPV, when used for manufacturing IPV, do not require containment in BSL-3/IPV facilities provided they are produced under conditions that would make them suitable for oral vaccine use. If conditions other than those approved for production of OPV are used (e.g. different multiplicity of infection or fermentation temperatures), it must be verified that the virus so produced poses no greater medical risk than that accepted for OPV, or BSL-3/IPV containment must be instituted”. Institution of BSL-3/IPV containment is virtually impossible in developing countries and therefore Sabin vaccine seed for IPV production would need to be evaluated for OPV equivalence in terms of neurovirulence testing. There was a long debate on how this could be accomplished with some participants arguing that the TgmNVT should be more than sufficient for this purpose. Other participants insisted that if the use of authentic OPV is to be ensured, the same requirements as those necessary for the production of OPV should be applied.

CONCLUSIONS

This meeting set up the basis for the future revision of the WHO recommendations for the production and control of OPV as presently outlined in the TRS No. 904 and 910. WHO will establish a drafting group to prepare revision drafts and then consult experts for review and comments until submission to ECBS for final approval. The following issues identified in this meeting will be taken into consideration in the revision:

1. If the process of OPV production is proven to be consistent and batches are already released by the TgmNVT, the TgmNVT can be used alone; for example in preparing a new working seed from an existing master seed in which case monkeys need not be used.
2. If the manufacturing process of OPV production is new or a new master seed is used, or major changes occur in vaccine production that leads to a significantly altered mutational composition, full characterization will be required and detailed guidance will be provided in the revised TRS.
3. More data are required to assess the equivalence between the MNVT and the TgmNVT: comparison of results from MNVT and TgmNVT at different passage levels (master seed lots, working seed lots, bulks) and comparison of specific poliovirus variants by the intraspinal route. NIBSC should review relevant data provided by manufacturers and the FDA. NIBSC should assess the virulence in the TgmNVT of poliovirus vaccines at different passage levels and variants with known phenotype in the MNVT.
4. It would be advisable that a reference laboratory or laboratories should be identified by WHO to maintain global capability on the MNVT.
5. It is proposed that, although the acceptable level of mutant content from a batch of vaccine determined by MAPREC should be agreed with the NRA, it should not exceed the following values when normalised against the international reference for the specific reaction:
Type 1 Sabin 2.0% (for the sum of both mutations 480-A, 525-C)
Type 2 Sabin 1.5% (481-G)
Type 3 Sabin 1.0% (472-C)

6. Regular workshops and proficiency test studies should be held for MAPREC to improve agreement between laboratories.
7. Non radioactive methods for MAPREC should be validated and introduced as soon as possible. Utility of new sequencing technologies for quality control of OPV should also be explored.
8. Clarify in the recommendations that thermostability is a product-specific characteristic which is used to assess consistency of production. Indicate that changes in the specification for the OPV thermostability test should be scientifically justified. The WHO Working Group should review available data particularly for new monovalent and bivalent OPVs.
9. Clarify that the use of sub-passage of the master seed to produce the working seed for type 3 is necessary considering that there is a limited amount of the Pfizer RSO master seed lot available for future production.
10. Mono and bivalent OPV formulations to be included in the general introduction and Part A of the revised TRS for the production and quality control of OPV.
11. Potency specification (for type 3) to be brought into line with the programmatic specifications in the new TRS for the production and quality control of OPV.
12. Discussions on how to control the neurovirulence of Sabin vaccine seeds for the production of IPV should continue.
13. Revision of the current WHO recommendations will include guidance on non-clinical and clinical evaluation.

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Appendix. List of participants (Alphabetical by Country)

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