Final Report

IABS Scientific Workshop on Neurovirulence Tests For Live Virus Vaccines

WHO, Geneva, Switzerland
31 January - 1 February 2005
Introduction
This report reflects the discussion and conclusions of a group of experts on regulatory aspects of vaccines from national and international regulatory agencies and scientists involved in vaccine manufacture and development, who attended an informal scientific workshop on neurovirulence testing of live attenuated viral vaccines in Geneva on 31 Jan - 1 Feb 2005. The workshop was jointly organized by the International Association for Biologicals, the European Pharmacopoeia and the World Health Organization. The background to the workshop was the desire to clarify and obtain an international consensus on the need for neurovirulence testing of live viral vaccines. Such testing currently is a common recommendation or requirement of regulatory guidelines for many live vaccines. There is concern that in several cases there is an absence of scientific data justifying such testing, and often no clear definition of criteria for assessment of the results of tests or the absence of the use of appropriate reference vaccines for use in the tests. In addition, since the testing method and its variability are not well established, the interpretation of the test can result in problems and false conclusions in both directions.

Regulatory considerations
The testing of live attenuated viral vaccines for neurovirulence has been a requirement of many regulatory authorities for a considerable time. The rationale was originally based on the need to test viruses with known neurovirulent properties, such as polio and yellow fever. The neurovirulence tests for these viruses are based on detailed knowledge of their neurotropic behaviour. The polio and yellow fever neurovirulence tests vary substantially from each other, reflecting known differences in neurotropism. For other live viral vaccines a generic neurovirulence test is described by some regulatory authorities, as exemplified in the general chapter of the European Pharmacopoeia (Ph.Eur.). For some live virus vaccines a test is specified by the World Health Organisation (WHO), the European Pharmacopoeia (Ph.Eur.), the US Food and Drug Agency (FDA) and the Japanese National Institute of Infectious Diseases (NIID) and discrepancies may exist amongst these various requirements with respect to the performance of the test. For example, in some cases the master seed, the working seed or manufacturing lots can be tested, whereas other requirements specify that all seeds are to be assessed. Also, some requirements vary with respect to the target for histological sections to be studied.

Some of these points are being addressed. The European Department for the Quality of Medicines (EDQM) is proposing to revise the Ph.Eur. requirements for measles, mumps, rubella and varicella vaccines to be in line with WHO requirements. New EU regulations for live polio vaccine will come into place in January 2006, again bringing the Ph.Eur. into line with WHO requirements. The WHO is looking to provide greater clarity in some of their requirements whilst in the USA, the requirement specified by their Code of Federal Register has been revoked and vaccines are currently assessed on a case-by-case basis relying on the revoked CFR as guidance. The US Centers for Biological Evaluation and Research (CBER) has developed an algorithm in their approach to new vaccines and neurotoxicity, although industry feels that working through the algorithm could take up valuable time in vaccine development.
General Aspects of Neurovirulence Testing

In some cases, there is proven value in the neurovirulence test (NVT), for example for polio vaccine for which the test is part of routine batch release procedures. However, there have been significant advances in the application of alternative tests for assessing polio neurovirulence. A test based on a transgenic mouse model has been validated for polio vaccine. However, the use of a small laboratory animal in place of non-human primates does not wholly eliminate ethical concerns, especially since much larger numbers are likely to be required. Furthermore, the use of transgenic animals (mice) has considerations of access to and supply of animals, and training in their use. A molecular approach, the MAPREC assay, which complements animal tests in predicting neurovirulence, has been fully validated and is in routine use for type 3 poliovirus. Studies on the MAPREC assay for poliovirus types 1 and 2 are in progress.

The neurovirulence test for yellow fever vaccine has been designed to allow quantitative assessment of the effects of the virus by examination of specific areas following directed inoculation. No reference is used and it has not been demonstrated that vaccines that pass the test are more acceptable than those that fail. The performance of the different available seeds of yellow fever vaccine in the test has not been investigated by a collaborative study. While the test measures a parameter which is expected to be relevant to the safety of a seed virus or a batch of vaccine produced from it, this has not been demonstrated and there are no objective criteria of pass or fail as there are for the polio neurovirulence test. Nonetheless, a vaccine which has high activity in the neurovirulence test would clearly be unacceptable.

There are several cases where the currently configured neurovirulence test is questionable. Firstly, in some cases, e.g. for measles, rubella and varicella vaccine, it is not apparent that the neurovirulence test in its present form provides scientific data of demonstrated relevance to vaccine safety. However, in the absence of any alternative model, it is probable that the test will continue for new vaccine strains, since the wild type viruses are known to be neurovirulent and new vaccine candidates could have an unexpected degree of neurovirulence.

The workshop concluded that there is little to be gained however in applying the test for current vaccine strains, especially for measles and rubella, since there are considerable clinical data demonstrating the safety of these vaccines which have been used widely and safely for many years. Here, the application of the neurovirulence test is currently required to qualify new seed preparations. Where there is a history of successful introduction of new seed preparations without change to the clinical safety profile then the need for neurovirulence testing is difficult to justify, particularly if only minor changes have been made to the vaccine seeds employed.

Secondly, in the case of mumps vaccine (mumps vaccines have, in some cases, been found to be neurovirulent in children), a collaborative study between two major centres demonstrated the futility of the current neurovirulence test model. Both sites were unable to distinguish attenuated vaccine virus from neurovirulent wild type viruses or viruses from vaccine associated adverse events at a statistically significant level. It was considered that the current monkey neurovirulence test did not have value in discriminating between attenuated and safe vaccine virus and virulent wild type strains. The use of more appropriate reference viruses may only complicate matters, for example a fully non-neurovirulent reference strain might make vaccine strains appear worse, which otherwise have proven field safety records. Two alternate
models under development are the neonate rat and the marmoset. The same two centres have evaluated the former model and both sites were able to distinguish different vaccines from each other and from wild type virus at a statistical level. This model needs to be fully validated although the predicted neurovirulent properties of most strains correlated well with clinical histories. In contrast, the marmoset model provided preliminary positive results for the discrimination but is still under examination.

Thirdly, for new vaccines it is not necessarily clear that a neurovirulence test is required and, if it is, what is the method of choice. What are the criteria for classifying a virus as neurotropic? Evidence for neurotropism (or its absence) of the wild type virus from which the vaccine is derived can be gleaned from the scientific literature. Recently, the European Union’s Committee for Medicinal Products for Human Use (CHMP) determined that there was no scientific reason to perform neurovirulence testing of a rotavirus vaccine. Similarly, the WHO guidance on non-clinical evaluation of vaccines states that the need for a neurovirulence test should be based on evidence either that the natural infection is neurotropic or that selection for neurotropism could have occurred during the passage history of the vaccine candidates. For example, this may occur if the attenuation process involved passage through CNS tissue. Furthermore, one can expect that, if a neurovirulence test is indicated, that any test specified is able to distinguish reliably between acceptable and unacceptable preparations.

This principle has been applied to new WHO guidelines for candidate live attenuated dengue vaccines. Since dengue viruses are not regarded as encephalitic, a neurovirulence test for each batch is not justified, nor is there a need to test each working seed. However, to illustrate the complexity of providing general guidance that fits all situations, some dengue candidate vaccines are being developed as chimeras where one component of the chimera is derived from a virus with neurovirulence potential, such as dengue-yellow fever constructs. In this case neurovirulence testing is required.

In some cases, there may be a paucity of data with respect to the neurovirulence of a virus for which a novel vaccine is being developed. In such cases, or in cases where neurotropism or neurovirulence is apparent, the new vaccine needs to be tested as part of the pre-clinical testing programme to provide some assurance on its safety. The identification of the most appropriate animal model remains an important issue in these cases. Efforts towards characterising the vaccine virus as much as possible by alternative tests, especially involving the use of non-primate models, should continue as a high priority. Only if such tests do not provide enough assurance of safety should the monkey neurovirulence test be performed.

However, if a novel vaccine has been attenuated by passage in neuronal tissue, it was agreed that it should be tested for neurovirulence.

**Application of tests and controls**

Guidelines and recommendations vary in their requirements as to whether neurovirulence tests should be applied to master seed lots, working seed lots or production lots; or two or all three of the above. The validity of the model applied should be crucial, but in many cases the MNVT is likely to be applied despite absence of good data qualifying its use.
There is a need to distinguish between what testing should be applied for an entirely new vaccine compared to a change in the seed virus or a change in production methodology. In some circumstances, this can be decided on a case-by-case basis. The need to perform tests to assure production consistency should be distinct from pre-clinical safety testing.

The Ph.Eur. general requirement stipulates the use of four (non-vaccinated) control animals in each neurovirulence test. Given that lesions have never been observed in control animals, the number of animals could be reduced. The WHO reference virus for yellow fever vaccine is a vaccine seed virus; however, it is not an ideal reference in neurovirulent tests because it has a very low neurovirulent activity. There is no positive control for rubella vaccine neurovirulence testing.

**Maintaining expertise**

Participants in the workshop were concerned that since the number of neurovirulence tests being performed is reducing (e.g. for MMR), expertise in this area is waning. There is a need to maintain capability to perform neurovirulence testing; the monkey test is probably best although training in the use of transgenic mice is also important. However, training is expensive.

A WHO set of histological slides for training purposes is available internationally in relation to polio neurovirulence tests in monkeys and this approach could be used for other vaccine types. Assuring competence is becoming difficult as fewer tests are performed and it is not ethical to employ significant numbers of animals simply for training purposes.

**Specific vaccines**

**Polio**

For lot release testing of live polio vaccine, the MNVT remains highly relevant; but it requires monkeys and expertise in performing the test. The transgenic mouse model has been validated for polio vaccines of types 1, 2 and 3 and the molecular MAPREC test (for screening before an animal test) has been validated for poliovirus type 3 with studies underway for poliovirus types 1 and 2. The MAPREC test does not assure safety but provides good evidence for the consistency of manufacture. Ethical issues remain for the mouse model, which also requires highly skilled operators. For animal testing, reading and scoring could be performed jointly between the vaccine manufacturer and the National Control Laboratory (called OMCL in Europe). All current and new live polio vaccine strains require neurovirulence testing. The monkey neurovirulence test is regarded as the ‘gold standard’ and should be applied to any new polio vaccines.

**Measles**

Current strains of measles vaccine have a good safety record and minimal change to seed lots or to manufacture should not require re-performance of neurovirulence tests. Although the value of the MNVT for measles has not been specifically determined, there was general opinion that seed and production lots of new strains of measles vaccine should be tested in the MNVT as part of pre-clinical studies, even although measles virus is only rarely neurovirulent.
Mumps
Some strains of mumps vaccine have a good safety record and minimal change to seed lots or to manufacture should not require re-performance of neurovirulence tests assuming comparability of appropriate quality parameters is demonstrated. Other mumps vaccines have been found to be neurovirulent. The MNVT has been shown to be of little value in assessing the neurovirulence of mumps vaccine strains. A neonate rat model shows promising results and full validation of this model would be important. Standardisation and validation of the rat model would be preferable to re-defining the monkey model.

Rubella
As for measles.

Varicella
As for measles.

Yellow Fever
Encephalitis is reproducible in monkeys and seed lots should be assessed by the MNVT. The WHO seed virus has a very low score and consequently is not a good control virus. Also, the WHO requirements on test procedures could be clarified.

Yellow Fever virus as a vector
Seed and production lots of 17D Yellow Fever vaccine expressing heterologous envelope proteins (e.g. JE and WNV) have been tested for safety in the infant mouse and by MNVT. The application of the MNVT followed WHO recommendations with additional procedures and made use of commercial 17D vaccine as a control.

Rota
There is no scientific reason to perform neurovirulence testing for a live rotavirus vaccine.

Dengue
Performing neurovirulence testing for Dengue vaccine would not appear to be justified, but it might be prudent to do so.

Influenza
The EU CHMP guideline on live attenuated influenza vaccine requires that absence of neurovirulence be demonstrated. However, it is questionable whether this justifies the use of a monkey neurovirulence test and other models should be considered.

Smallpox
The WHO and EU CHMP guidelines on smallpox vaccine require testing for neurovirulence; the EU guidance includes assessing the ability to cross the blood brain barrier versus the ability to replicate in the brain (once across). However, the relevance of this when there is no viraemia is questionable.
Future considerations

- A review of current regulatory requirements in respect of neurovirulence testing of live viral vaccines should be undertaken as a matter of high priority by the appropriate international and national authorities.
- A case-by-case approach should be enforced, considering the clinical pattern of the wild virus, the clinical vaccine history and experience, the *in vitro* data (e.g. molecular data) and the data from non-primate models before considering an MNVT.
- The use of animal models as alternatives to nonhuman primates needs to be encouraged and validated.
- There is a need to maintain expertise in neurovirulence testing methods.
- There is a need for more clearly defined reference materials and criteria for assessment of neurovirulence test methods.
- A European reference centre would be valuable; this could perform all tests on any appropriate model and provide OMCLs and vaccine manufacturers with the necessary expertise in performing these studies.