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Scientific and Regulatory Considerations for Controlling Residual Neurovirulence of Viral Vaccines

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Neurovirulence assessment of live viral vaccines

- Some of the most successful vaccines against human viral diseases are live viral vaccines → It is critical to ensure that the live viruses present in vaccines are adequately attenuated and do not cause serious adverse reactions.
- At the preclinical stage, neurovirulence tests are performed if residual neurovirulence of the production strain is suspected, for example:
  - The wild-type virus, from which the production strain is derived, is known to have neurovirulence properties (e.g., poliovirus, mumps virus), or
  - The production strain is attenuated through serial passage in tissues of the central nervous system (CNS).
- During routine manufacturing, neurovirulence tests are performed in cases of unacceptable lot-to-lot variability in residual neurovirulence
  - Some attenuated production strains can undergo mutations (reversions) that increase their neurovirulence.
- Monkey neurovirulence test (MNVT) has played an important role in ensuring the safety of several live viral vaccines (e.g., YFV, OPV) since the early 1900s.

Summary of requirements for neurovirulence testing specified in WHO documents published prior to 2025

Live attenuated viral vaccine	Neurovirulence testing recommended by WHO	
	Virus seed lots	Routine manufacture
Sabin Oral Poliomyelitis Vaccine	Test using HTS[1] or in vivo (monkeys or transgenic mice).	Test each monovalent bulk using HTS or in vivo (monkeys or transgenic mice).
Novel Oral Poliomyelitis Vaccine	Test using HTS or in vivo (monkeys or transgenic mice).	Test each monovalent bulk using HTS or in vivo (monkeys or transgenic mice).
Japanese Encephalitis Vaccine	Test in mice and monkeys[2].	Test each final bulk or final product in mice.
Dengue	1) Not for dengue virus. 2) Test if using yellow fever virus as vector.	No.
Measles, Mumps, Rubella, Yellow fever, Varicella	Test in monkeys.	No.
Smallpox[3]	Test in mice (may be in rabbits).	Test each bulk suspension (same test as virus seed).

[1]High Throughput Sequencing.
[2]For SA14-14-2: Test in monkey may be replaced by an alternative method approved by the NRA.
[3]Live, but not attenuated, vaccine.

Yellow fever vaccine – residual neurovirulence associated with commercial lots

- In 1941, cases of encephalitis were reported in individuals who received YFV vaccine prepared from a 17D-derived substrain (Fox *et al.*, 1942).
- Studies (Fox *et al.,* 1943) in monkeys using intracerebral inoculation identified
  - The ability to produce encephalitis varies between different 17D-derived substrains.
- As a result of this incident, the seed-lot system of production strains was introduced to improve lot-to-lot consistency.
- Despite the use of neurovirulence testing of virus seed lots, YFV is still associated with neurotropic disease (YEL-AND), a rare adverse event with no clear mechanism.

MNVT can detect various levels of residual neurovirulence of YFV but cannot eliminate YFV-associated adverse reactions.

OPV - limitations of monkey neurovirulence test

- **Experience with a new attenuated type 3 poliovirus developed by the Institute of Sera and Vaccines in Prague:**
  - **The new OPV3 strain passed MNVT and seemed to be safer than Sabin type 3 strain.**
  - **However, the clinical study of this new strain led to an extensive outbreak of vaccine-related poliomyelitis in 1968.**
- **A review of data from 80 Sabin type 3 lots manufactured between 1964 and 1983 in the US:**
  - **Type 3 OPV is less neurovirulent than type 1 OPV, based on MNVT results.**
  - **However, in field use, type 3 OPV is associated with vaccine-related poliomyelitis more frequently than type 1 OPV.**
- **Partially attenuated polioviruses, as determined by intracerebral and spinal inoculation in monkeys, are not paralytic at maximal doses by the spinal route in chimpanzees (Sabin et al, 1957).**

MNVT results are not always predictive of residual neurovirulence in humans.

Mumps vaccine - limitations of monkey neurovirulence test

- **Mumps virus is a major cause of aseptic meningitis in unvaccinated populations.**
- **MNVT failed to detect residual neurovirulence of an attenuated strain (Urabe Am9) used by several manufacturers to produce live mumps vaccines.**
  - **A number of aseptic meningitis cases associated with Urabe Am9-derived live vaccine were reported in Canada, Japan, and Europe in the late 1980s.**
  - **Urabe Am9 derived live mumps vaccines are no longer marketed.**
- **A more sensitive test using neonatal rats has been developed, which can differentiate attenuated strains with varying levels of residual neurovirulence.**
- **MNVT is not suitable for the control of residual neurovirulence of live attenuated measles, mumps and rubella → the neuropathological manifestations of these viruses in monkeys do not correlate with the strains' known neurovirulence in humans.**

Overall experience with monkey neurovirulence test

- **MNVT played an important role in ensuring safety of several live-attenuated viral vaccines developed in the first half of the 1900s (e.g., YFV, OPV).**
  - **Limited understanding concerning the mechanisms and genetic basis of viral attenuations at that time and no other suitable tests.**
- **The interpretation of MNVT results is largely based on the correlation between the neuropathological manifestations of the virus in monkeys and the strains' known neurovirulence in humans.**
  - **MNVT is not suitable for the control of several live attenuated viral vaccines, such as measles, mumps.**
  - **MNVT alone can't eliminate the rare neurological adverse reactions associated with YFV and OPV, which are characteristics of the vaccines.**
- **Advances in molecular biology have led to new methods for neurovirulence assessment, based on:**
  - **Improved understanding of mechanisms and genetic basis of attenuation.**
  - **More sensitive technologies (e.g. Polymerase Chain Reaction (PCR), nucleic acid sequencing)**

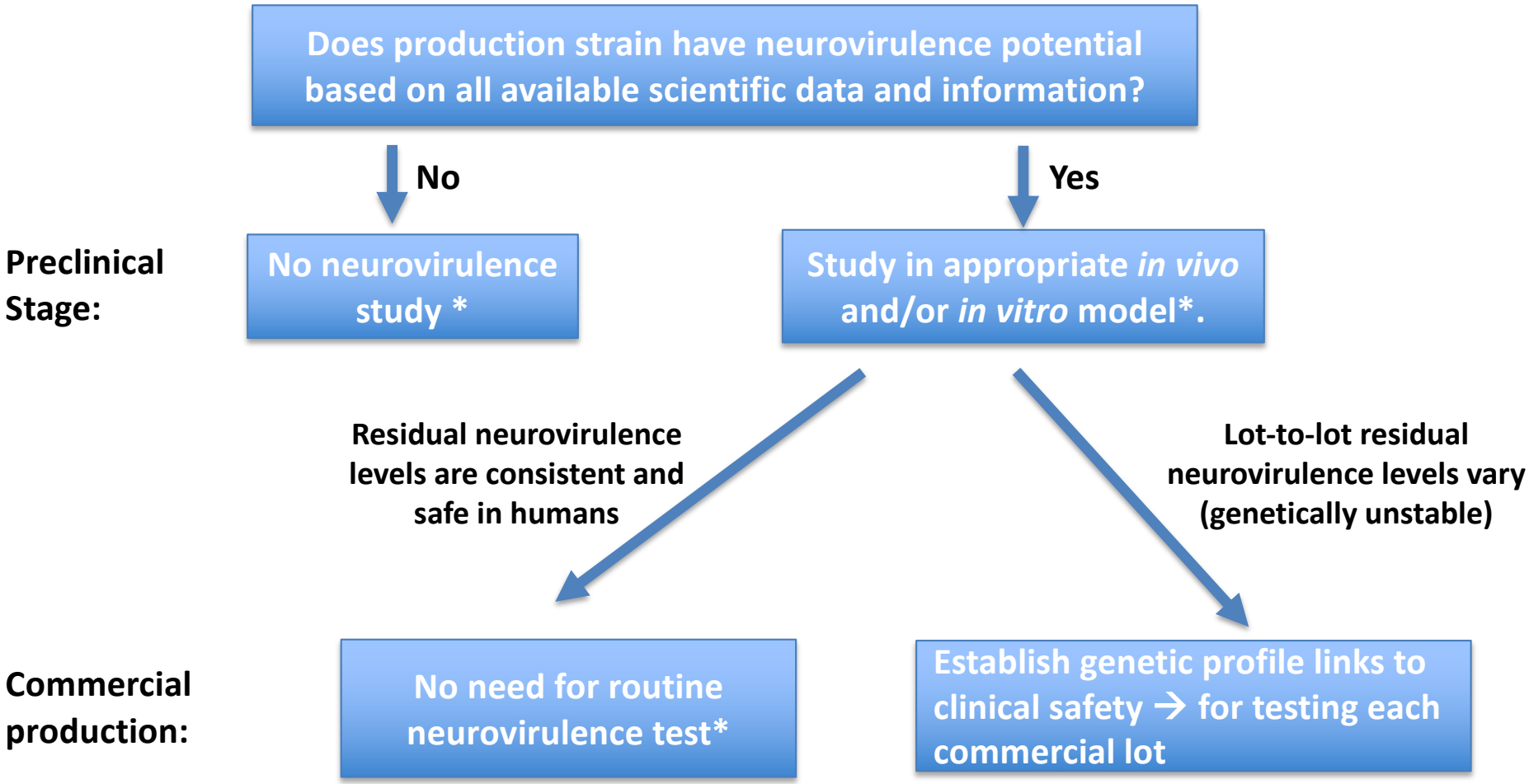
Advances in science lead to new tests for OPV control

- **Identification of genetic basis of attenuations due to advances in molecular biology.**
  - **Identification of principal nucleotides for the attenuation of Sabin OPV (all 3 types)**
  - **Identification of mechanisms of action for attenuations and reversions.**
- **In 1990s, a mutant analysis by PCR and restriction enzyme cleavage (MAPREC) assay was developed by CBER (US FDA).**
- **In 1990s, a neurovirulence test (TgmNVTs) was developed based on behavioral observation ("normal" vs "paralysed") of transgenic mice following intraspinal inoculation.**
- **High-Throughput Sequencing (HTS) → a massively parallel sequencing technology**
  - **HTS has the capacity to measure the level of polymorphisms at each genome nucleotide.**
  - **A validated whole genome HTS assay can be used to replace the in vivo neurovirulence test for routine manufacturing control.**

Consistency approach to ensure safety and effectiveness of commercial vaccine lots

- **Regulatory expectation: commercial vaccine lots retain the safety profile and efficacy/effectiveness demonstrated in clinical studies.**
- **Principles of quality control of commercial vaccines lots**
  - **Consistency approach: Quality attributes of commercial lots, throughout product lifecycle, are comparable to those of lots shown to be safe and effective (efficacious) in clinical studies.**
- **The quality of commercial vaccine lots is built into the manufacturing process through:**
  - **Stringent adherence to Good Manufacturing Practices (GMP).**
  - **Adequate control of starting and raw materials.**
  - **Use of validated manufacturing process.**
  - **Implementation of robust and appropriate quality control tests.**
  - **Implementation of appropriate quality specifications.**

Proposed strategy for neurovirulence assessment when developing a new live viral vaccine



* Genome sequencing is recommended for the control of virus seeds.

New WHO guidelines on the replacement or removal of animal tests for the quality control of biological products

- **Current status**
  - **Final public consultation was completed on 19 September 2025**
  - **The new guidance document was adopted at the 81st meeting of the WHO Expert Committee on Biological Standardization (ECBS), 13-16 October 2025.**
- **General considerations**
  - **recognize the scientific limitations of many animal assays particularly in the context of routine batch QC testing (e.g., variability)**
  - **recommend the use of scientifically relevant in vitro methods, over in vivo methods, for routine quality control of biological products.**
- **Recommendations provided in each of the main sections of the new WHO guidelines are intended to supersede any corresponding quality control requirements concerning in vivo assays specified in WHO guidance documents published prior to 2025.**

New WHO Guidelines – specific considerations on neurovirulence testing

- **At nonclinical (preclinical) stage**
  - **The potential neurovirulence of a new attenuated virus strain should be evaluated based on all available scientific data and information.**
  - **Neurovirulence testing in a suitable animal model should be considered in cases where the wild-type virus is neurovirulent or was passaged through tissues of the CNS – or where a chimeric virus has components that may be neurovirulent.**
  - **It is noted that all neurovirulence tests in experimental animals (such as monkeys, mice and rats) were established based on an assumed correlation between the neuropathological manifestations of the attenuated virus strain in animals and the safety profile of the same strain in humans established through clinical studies.**
  - **It is acknowledged that emerging technologies may lead to the development of suitable in vitro models, which could replace nonclinical neurovirulence testing.**

New WHO Guidelines – specific considerations on neurovirulence testing (cont.)

- **At clinical development stage**
  - **Each virus seed and all vaccine lots used in clinical studies should be characterized by full-length genome sequencing, in part to facilitate the subsequent use of molecular methods for quality control of commercial product at appropriate manufacturing steps (e.g., virus seed lots).**
- **For commercial production**
  - **residual neurovirulence is controlled at appropriate manufacturing steps, e.g., virus seeds, each commercial lot only in case of lot-to-lot variability (production strain is genetically unstable).**
  - **Use of an appropriate molecular method (e.g., HTS) based on consistency approach.**

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