



International Alliance for
Biological Standardization

Animal Testing Replacement for Vaccines. A One Health View: Global Outlook and Future Strategy

December 2-4, 2025
Bangkok, Thailand

Next generation sequencing as an alternative to neurovirulence tests in animals for the quality control of live-attenuated viral vaccines

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This work explores next generation sequencing (NGS) as a replacement for animal neurovirulence tests (NVTs) in the quality control of live-attenuated viral vaccines, with a primary focus on oral poliovirus vaccine (OPV) and extension to other vaccines such as yellow fever. Genetic attenuation of Sabin OPV strains is maintained by a limited set of key mutations, and current safety control relies on in vivo NVTs in monkeys or transgenic mice, complemented by MAPREC (Mutation Analysis by PCR and Restriction Enzyme Cleavage) assays targeting hot-spot mutations in the 5' non-translated region. These approaches are ethically challenging, costly, and of limited throughput.

High-throughput NGS enables deep, quantitative measurement of single nucleotide polymorphisms (SNPs) across the whole viral genome, generating a detailed "molecular fingerprint" for each vaccine seed and lot. We conducted stepwise studies to (Phase 1) evaluate NGS as a replacement for MAPREC in quantifying critical 5'-NTR revertant mutations in OPV1 and OPV3, and (Phase 2) assess whether whole-genome SNP profiles can serve as surrogate markers for neurovirulence and overall molecular consistency, ultimately enabling removal of routine animal NVTs from lot release.

Multi-laboratory collaborative studies showed strong concordance between independent NGS pipelines and strong correlation with established MAPREC readouts. Whole-genome analysis demonstrated highly consistent SNP profiles for most OPV products from the same manufacturer, while distinguishing products derived from different seeds or cell substrates. On this basis, candidate WHO reference reagents for OPV1 and OPV3 have been characterized with defined mutation frequencies suitable for assay calibration and validation. We propose a future control paradigm in which historical NGS data from consistency lots define acceptable genomic variability, allowing routine lot release based on conformity to predefined SNP profiles, with animal NVTs reserved only for out-of-trend investigations. This NGS-based framework offers a scientifically robust, animal-free approach aligned with One Health and 3Rs principles for global vaccine quality control.

