International Meeting on Quality Control Assays for Polio Vaccines

Standardizing oral polio vaccine high throughput sequencing and Sabin-inactivated polio vaccine *in vivo* potency assays and assessing hyper-attenuated S19 strains for IPV production and serology testing

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PATH

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Contents

Acknowledgments

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Abbreviations

BPOM	Indonesian regulatory authority
CAG	Containment Advisory Group
cDNA	complementary DNA
cVDPV2	circulating vaccine-derived poliovirus type 2
CNS	central nervous system
ECBS	Expert Committee on Biological Standardization
ELISA	enzyme-linked immunosorbent standardization assay
GAP-IV	WHO Global Action Plan for Poliovirus Containment, 4th edition
GMP	good manufacturing practice
GPEI	Global Polio Eradication Initiative
HTS	high throughput sequencing
INDELs	insertions and deletions
IPV	inactivated polio vaccine
IS	international standards
MAPREC	mutant analysis by PCR and restriction enzyme cleavage
mAbs	monoclonal antibodies
MTA	material transfer agreement
MVNT	monkey neurovirulence test
nOPV	novel oral polio vaccine
nOPV1	novel oral polio vaccine type 1
nOPV2	novel oral polio vaccine type 2
nOPV3	novel oral polio vaccine type 3
NHP	non-human primateQC quality control
SAGE	Strategic Advisory Group of Experts on immunization
SAM	Subgroup of PRAG on Modelling
SNP	single nucleotide polymorphisms
TgmNVT	transgenic mouse neurovirulence test
TRS	Technical Report Series
VAPP	vaccine associated paralytic poliomyelitis
VHH	variable domain of heavy-chain-only antibody

VLP	vaccine-like particle
VOI	variants of interest

Executive summary

PATH, along with the Medicines and Healthcare products Regulatory Agency, UK (MHRA), Gates Foundation and the World Health Organization (WHO) organized an international meeting on Quality Control Assays for polio vaccines in Bangkok, Thailand, for manufacturers and regulatory testing laboratories. Presenters provided updates on evolving guidance for the implementation of quality control assays for polio vaccines. This included the dissemination of results from a recent WHO collaborative study supporting the replacement of animal models with high-throughput sequencing (HTS) for routine vaccine lot release. Other topics included the latest WHO collaborative study results on the suitability of the Sabin inactivated polio vaccine (sIPV) International Standard (IS) for *in vivo* rat potency testing; the availability of WHO universal reagents for polio vaccine potency assay; and the importance of harmonizing in-house assay standards and reagents with international standards and the WHO guidance. Finally, presenters discussed the potential of next-generation products, such as S19 virus based IPV, that can be developed currently without biocontainment requirements.

Key meeting highlights:

- Presenters disseminated results from a recent WHO collaborative study that supports the replacement of animal models for neurovirulence testing with high-throughput sequencing (HTS) for routine vaccine lot release. The findings will be assembled as a draft report and submitted to the World Health Organization Expert Committee on Biological Standardization (WHO ECBS) in Q4 2025 for review and endorsement at the next WHO ECBS meeting in spring 2026. A technical standard operating procedure (SOP) will need to be developed to provide detailed procedure and explanations, this may have to be as a standalone WHO document. Following endorsement by WHO ECBS, the technical SOP will be referred to in the WHO's Technical Report Series 993 Annex 3 (TRS) when it comes up for review in 2026. In addition, an effort will be made to publish the SOP as a manuscript for open community access.
- A collaborative study to develop CCID50 potency assay and HTS reference reagents for novel oral polio vaccines (nOPVs) is planned for 2026.
- IPV vaccine manufacturers must use product-specific International Reference Standards (IS) for potency testing of Salk or Sabin inactivated polio vaccines (IPVs), but universal reagents can be used for both products and are now available: three type-specific and one cross-reactive human monoclonal antibody.
- WHO collaborative study results found the current sIPV IS (17/160) is suitable for immunogenicity testing in the *in vivo* rat potency animal model. The study report will be submitted to the WHO ECBS in Q4 2025 for review and endorsement at the spring 2026 meeting, along with results from a forthcoming study to develop a new IS for Salk IPV (to replace the current one on expiry).
- Sabin virus S19 strains uses can be expanded to other techniques, such as cell sensitivity, standard for direct detection assays, testing antiviral compounds, and testing virucidal agents. The Containment Advisory Group (CAG) lists its current approved uses in its June 6, 2023, report. MHRA has guidance documents for ordering the S19 strains for use in production of IPV or use in neutralization testing. For neutralization assays, MHRA can provide technical assistance on these viruses for manufacturers on request.
- PATH and MHRA will discuss how to best streamline manufacturer requests for antibodies and reagents.

Polio disease and vaccine landscape

Ondrej Mach, WHO, articulated the **Global Polio Eradication Initiative's (GEPI) action plan**, which will require key programmatic and operational adjustments given the new financial constraints reality.

- There are clear technical pathways, but access, financing, and delivery mechanisms are under pressure, especially in critical geographies. There is persistent wild type 1 polio transmission in Afghanistan and Pakistan as well as circulating vaccine-derived poliovirus type 2 (cVDPV2) transmission in Lake Chad basic countries, especially Nigeria.
- GPEI's approach is an "all-in strategy with no regrets" to immunize hard-to-reach children and improve access.
- Transmission is reduced during the low season, so taking advantage of this to roll out more vaccination campaigns with a mix of IPV (which boosts immunity from previously OPV-vaccinated individuals) and OPV.

Ananda Bandyopadhyay, Gates Foundation, discussed investments in next-generation polio vaccines and tools, including the following:

- **Type 2 novel oral polio vaccines (nOPV2)** have been available since 2020 and nearly 2 billion doses have been delivered in 40+ countries. nOPV2 is the result of modifications of Sabin OPV to improve genetic stability, and real-world data confirms non-inferior protection to OPV based upon the Sabin strains. nOPV2, however, still results in vaccine associated paralytic poliomyelitis (VAPP). Missed participants and reduced vaccine coverage are major risk factors for circulating vaccine-derived polioviruses to emerge. Looking ahead, the SAGE committee will discuss potential expanded use of nOPV2 beyond outbreaks.
- The Gates Foundation is also pursuing the following low-cost, **non-replicating IPV** options: hyper-attenuated S19, vaccine-like particle (VLP) vaccines, mucosal-adjuvanted IPV, and IPV-containing combination vaccines. Serum Institute of India, Pvt. Ltd.'s hexavalent vaccine was WHO prequalified in 2024, and Bio E's hexavalent product is anticipated to secure WHO prequalification in 2027. Other hexavalent formulations are in early development stages. WHO has provided guidance on use of IPV for outbreak response.
- Innovative delivery devices such as micro-needle patches and diagnostic tools for surveillance and rapid tests are additional new areas of work.

Global research mechanisms include the following:

- The WHO Polio Research Committee (PRC) is an external expert committee hosted by WHO that reviews polio eradication-related research to help identify remaining gaps in knowledge; serves as a forum for polio researchers from different countries and institutions; provides scientific review for new proposals; and makes recommendations on implementation.
- The Polio Research and Analytics Group (PRAG) provides internal GPEI research coordination. The Subgroup of PRAG on Modelling (SAM) coordinates modelling support to ensure that research/modelling responds to the needs of the program and to ensure synergy and no overlap in research activities among partners. PRAG co-chairs: Ondrej Mach (WHO) and Ananda Bandyopadhyay (Gates Foundation)

High-throughput sequencing (HTS)

Background

Kostya Chumakov, formerly of the US Food and Drug Administration, gave a historical overview of assays used to ensure consistency between polio vaccine lot testing. He gave context for tools like the animal neurovirulence test, mutant analysis by polymerase chain reaction and restriction enzyme cleavage (MAPREC), and HTS, starting with the early observation that different isolates had different pathogenicity and that virulent virus particles generate larger plaques than attenuated viruses. He also noted that attenuated virions could regain the large plaque phenotype via passage in cell culture, suggesting that this reversion needed to be controlled.

Furthermore, studies in the 1950s revealed that viruses isolated from OPV vaccine recipients were able to cause paralytic disease in animals following intracranial inoculation. This finding facilitated the requirement for manufacturers to test every batch of vaccines using the monkey neurovirulence test (nearly 200 animals needed per individual serotype lot of trivalent OPV along with controls) to measure residual virulence of the Sabin strains. Vaccine bulks pass if central nervous system (CNS) lesions are not greater than in a reference vaccine lot.

Importantly, the monkey neurovirulence test is not a safety test. It is a product consistency test. There is no direct correlation between the outcome of monkey neurovirulence test (MVNT) and vaccine safety in humans. Eventually, a transgenic mouse model expressing poliovirus receptor became available as a replacement for the use of monkeys, but the method also is laborious, time consuming and expensive.

When researchers discovered that neurovirulence (particularly of serotype 3) is determined primarily by a single mutation in domain V of the non-coding region of the poliovirus genome, the **MAPREC assay** was developed and used to test for the content of those mutants in vaccine lots. The correlation was remarkable between the content mutants and the mean histological lesion score in MVNT. A pass-fail decision for the assay was formalized and collaborative studies of MAPREC were conducted in the 1990s for all three polio serotypes. WHO recommended MAPREC in combination with an animal test for OPV QC. WHO ECBS approved MAPREC as an *in vitro* test of preference for lot release, along with transgenic mice neurovirulence test. Fifteen years ago, when HTS became available, it was compared with MAPREC and demonstrated consistency.

HTS advantages

Chumakov further described how HTS creates a molecular fingerprint for any given vaccine preparation that can be compared to similar fingerprints for other vaccine preparations (e.g., master seed versus working seed versus bulk produced from the same seed virus) as well as vaccine bulks from other manufacturers). HTS advantages include the following characteristics:

- Detecting and quantifying all single nucleotide polymorphisms (SNP) in the **entire virus genome,** not just one nucleotide.
- Multiplex testing.
- Individual molecule sequencing; accurate measurement of variant mutation frequencies, which creates a unique SNP profile.
- Lower cost compared to the animal testing per sample for large amounts of data.
- Efficiency.

- Technically simpler and more robust than MAPREC, capable of detecting low frequency variants in the linear range of raw MAPREC data.

HTS for routine lot release

MHRA will recommend the following approach to WHO ECBS based on the collaborative study results: Today's proposal for WHO ECBS consideration: test a series of consistency lots of monovalent OPV is by HTS and animal neurovirulence test to establish the range of variations in the SNP profile from the seed virus used to prepare monovalent bulks. After establishing manufacture consistency by testing the first few vaccine lots with both animal neurovirulence test and HTS, the use of animal test can be discontinued and only HTS performed for routine vaccine lot release. HTS can be used to test for molecular composition conformity for each new batch of OPV to the historical profile of mutations. Applying HTS will capture a whole genome SNP profile and obviate the need for animal neurovirulence testing, which only tells you about one property of the virus.

HTS has the following potential applications:

- Quantitative analysis of domain 5 mutations (to replace MAPREC)
    - In 2019, following a collaborative study, ECBS approved HTS as an alternative to MAPREC for OPV3. In 2022, ECBS approved HTS an alternative test to MAPREC for measuring the combined 480A + 525C and 481G content in OPV1 and OPV2 seeds and production lots for quality control and batch release purposes.
- Whole-genome SNP profiling to
    - replace animal neurovirulence testing.
    - agnostically compare SNP profiles to make pass-fail decisions.

Efforts are underway to develop HTS reference reagents for OPV and IPV that will enable whole-genome HTS analysis, support investigation of MAPREC-specific mutations by HTS assays, and, for sIPV, characterize virus bulks prior to inactivation.

HTS as a replacement of animal neurovirulence testing for OPVs

Manasi Majumdar, MHRA, presented on the interim analysis from a global WHO collaborative study on the use of HTS as a replacement for animal neurovirulence testing of OPV1 and 3 and nOPV2 blinded samples.

Comparing whole-genome SNP profiles reveals differences between batches made from different seed lots and by different manufacturers but consistency is high within a specific manufacturer and product over long periods of time. The close similarity of SNP profiles with historical data is proof of consistency and suggests that biological properties are also very similar, including neurovirulence and immunogenicity. HTS appears to be a sensitive tool to monitor consistency. Molecular profile inconsistency does not necessarily mean that a vaccine lot is unacceptable; rather, it suggests that conditions of virus growth have changes and may require further investigation.

Two working HTS reference reagent candidates for OPV 1 and 3 have been developed in a proposal to ECBS. Next steps include accumulating SNP profiles of historical vaccine lots that were successfully released and developing an algorithm to make pass-fail decisions.

Technical aspects of HTS important for manufacturers to consider will be included in an Annex to the ECBS proposal and are as follows:

- RNA extraction is a very important step in the protocol because the protocol requires intact poliovirus genomic RNA (7500 nt).
- Poor quality PCR products (double and faint bands) affect the downstream sequencing outputs (results) leading to differences in variant calling.
- Understanding the HTS platform and kit chemistry is important because artifacts can sometimes crop up when reagent kits are changed. In case of poor quality, tests must be repeated.
- Bioinformatic analyses have shown that the data for the ends of each sequence read are usually of poor quality. Setting up a stringent trimming parameter can help avoid artifacts.
- In the final output table, always check the query coverage and set a minimum coverage for the assay (e.g., a minimum of 3,000).
- In the final table, look for strand bias at the reporting position. Fifty percent (50%) is ideal, however, the limit can be set. The strong recommendation is to invalidate SNP with more than 90% strand bias.

The proposed scheme for routine OPV lot release is as follows:

- Establish a series of consistency lots of monovalent OPV using HTS to establish the range of variations of SNP profile. The lots should have known MNVT, TgmNVT, and/or MAPREC results.
- After the consistency of manufacture is established, use HTS to measure consistency of each new batch of OPV against the historical profile of mutations.
    - If the SNP profile of a new lot falls within pre-defined statistical criteria, it can be released without performing additional tests.
    - If a new lot falls outside of these criteria, an investigation is conducted, possibly including performing an animal test. If the outcome of the investigation is favorable, the historical SNP database should be updated.

Another collaborative study will soon be initiated to establish HTS reference reagents for nOPV monovalent vaccine testing for types 1, 2, and 3. This will allow use of homologous reference material and possible use in validation of existing in-house HTS methods and reassure regulators of the genetic stability of the modified regions, the overall level of variation that could increase viral fitness, and confirmation of the absence of any contaminating Sabin virus.

Whole-genome SNP profiling to monitor molecular consistency

Consistency is a cornerstone of good manufacturing practice (GMP). As such, the HTS approach being developed for polio vaccines can apply to other biomedical products in development, too. Chumakov presented a potential framework for SNP profiling using OPV3 as an example.

- SNP profiles of OPV3 lots produced from different seed viruses are different. Vaccines made by different manufacturers have unique patterns of mutations. The changes that occur during passaging depend on a lot of parameters, including multiplicity of infection, temperature, cell culture medium, and serum.
- Below is a potential framework for comparing whole-genome SNP profiles that consist of approximately 21,000 individual measurements for three possible mutations against the native nucleotide?
    - Compare the two SNP profiles add up all differences between the content of each nucleotide in two SNP profiles. Doing so enables calculations of pair-wise distances between all SNP profiles.
    - Use this information to calculate the statistical significance for each SNP that exceeds the background level (usually 1%). For SNPs that are statistically different in two profiles, evaluate

the biological significance, based on the historical data derived from analysis of previously released lots. For example, we know from experience that some differences are due to cell substrate specificity and are not biologically significant.

- Data from newly accepted vaccine lots should then be added to the historical baseline pool of information to continue to build the collective memory. Evaluating biologically significant differences that might affect vaccine quality may require animal testing.
- Pass-fail decisions should be based on the manufacturer's own product and each manufacturer should have a validated assay (bridge in-house reagents to WHO reference reagents).

In-house HTS methods for nOPV

An HTS International Standard (IS) is not yet available for nOPVs, two manufacturers outlined their in-house HTS methods.

Bio Farma, presented by Gemi Pertiwi

Bio Farma shared its approach to validating the assay for nOPV vaccine lot release. Viroclinics had conducted HTS in the past and Bio Farma used that data as a comparator for the analysis of the bridging samples.

Validation related to quantitative aspects was based on the accuracy and bridge to linearity and range of variants of interest (VOI) and non-VOIs and the closeness of bioinformatics analysis results between the two labs. First, a preliminary study bridged linearity and working range parameters between Viroclinics and Bio Farma before test validation. The similarity of the test results between the two labs helped to prove the validity of the assay.

Three variants of interest have been established for nOPV2. The HTS testing for bulk release monitored these variants of interest. It also monitored genetic stability, confirmed identity, and confirmed the absence of Sabin-2 contamination. The data were positive and the process met the validation criteria. Implementing this approach will begin when nOPV2 bulk production restarts, at which time Bio Farma will provide a recommendation to BPOM (Indonesian regulatory authority) to replace animal testing for lot release. Bio Farma will also implement the same process for nOPV1 and 3, once variants of interest and specifications have been identified for those strains.

Bio E, presented by Umakanta Mandala

Bio E developed its in-house standard using plasmid-based references. The method was both qualitative (confirming integrity of genetically modified regions) and quantitative (monitoring percentage of variants of interest). Bio E validated assay sensitivity and accuracy using the G3425A mutation.

Bio E also discussed using multiple sequencing methods for nOPV1 HTS and accurate detection and confirmation of INDELs (insertions and deletions), which do not impact safety or immunogenicity and are primarily caused by the low fidelity of poliovirus RNA-dependent RNA polymerase.

During the discussion, MHRA cautioned against the use of plasmid references because it bypasses the RNA extraction and cDNA synthesis steps, which are critical.

Indonesian regulatory readiness for HTS data assessment and vaccine lot release, presented by Yola Erwinda

Yola Erwinda, BPOM, described the Indonesian regulatory authority's development of its HTS capabilities for polio vaccines and beyond. Every vaccine batch undergoes quality assurance at BPOM to ensure standardization across the country. BPOM has experience with HTS from the COVID-19 pandemic, rotavirus microbial strain research, and nOPV2, and is continuing to deepen its expertise. BPOM digitized

the vaccine lot release system in 2024, completed HTS training, and prepared grants for updated equipment. Additional resources are being invested in method verification.

HTS is complex and the regulatory framework is evolving. Establishing clear regulations will help decrease cost through economies of scale, and BPOM plans to develop SOPs that align with WHO.

All of these activities also lay a foundation for using HTS for products other than polio vaccines, such as for adventitious agent testing; viral stability and consistency over time; support for novel vaccine platforms; early safety or efficacy issues; greater consistency/harmonization worldwide; and faster, safer, and smarter vaccine evaluation.

Day 1: meeting discussion

Meeting participants discussed the next steps for an HTS proposal to ECBS and the approach for doing so. Chumakov reinforced that animal neurovirulence testing evaluates consistency, not safety. While MAPREC is an alternative for assessing consistency, it may miss other changes to consistency that an animal test could have discovered. HTS puts these fears to rest by studying the entire genome sequence. It is superior because it is more complete than MAPREC or animal testing.

Tong Wu, Health Canada (and ECBS member), shared that the committee is implementing updates to the “three Rs” guidance on animal testing (replace, reduce, refine). The plan for the next iteration is to shift toward “remove” (for quality control testing, but not for preclinical research). The section on neurovirulence is very much in line with what Kostya articulated. We know that animal models are not predictive, while HTS offers more information. Furthermore, more information about polio is available than about other diseases and, still, even in diseases where less information is available, the ECBS still recommends HTS. The HTS proposal sets forth principles but is not a technical SOP. It will be discussed at the ECBS meeting in October 2025 and is likely to pass.

Although HTS has been in use for nOPV2 lot release, as discussed by Bio Farma and Bio E, the general consensus from the manufacturers and regulatators is that they have a lot of technical challenges to implement HTS and uptake is slow. A WHO TRS with a SOP with detailed step-wise protocol would fill an important gap for regulators and manufacturers, such as detailed explanations of how to perform the tests, define pass/fail criteria, how to assess red flags, etc.

Sabin IPV (sIPV) potency testing

sIPV IS and universal reagents

Measurement of D antigen units is a potency test for Salk and Sabin IPVs. Three ISes have been developed over the years that are traceable to vaccine lots, tested in clinical trials, and used for in-house assay calibration.

Salk and Sabin IPV were made from different strains containing different epitopes. For this reason, a Sabin-specific IS was developed. Each IS must only be used for its respective product (i.e., the Salk IS applies to Salk IPV and Sabin IS to Sabin IPV). Manufacturers having previously licensed their Sabin IPV prior to the advent of the product-specific IS can continue to do so, but harmonization is ideal.

Some ELISA reagents work for Salk but not Sabin IPV. Universal reagents are now available based on three type-specific and one cross-reactive human monoclonal antibody which works for both IPVs.

D antigen versus rat assays to assess IPV potency

The current WHO recommendations for IPV potency assessment include performing a rat i*n vivo* potency assay for each final bulk, omitting it when production consistency has been established, and using D-antigen assessment for routine lot release. New WHO guidelines for quality control are expected to be approved at ECBS meeting in October 2025. The guidelines propose eliminating animal testing in final bulk in favor of the exclusive use of *in vitro* potency methods. 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. Universal D-antigen ELISA assay advantages include the following:

- Reduced assay variability
- Faster and less resource-intensive
- Enhanced reliability in predicting vaccine-induced protective immunity in target population

sIPV IS reference reagent for in vivo potency assays

Presented by Allison Tedcastle, MHRA

A WHO collaborative study evaluated the suitability of the current sIPV IS (17/160) for immunogenicity testing in a rat i*n vivo* potency model using different references and multiple dilutions. The findings between the D antigen assay and rat i*n vivo* potency assay were comparable and results will be submitted to the ECBS for review and endorsement at the spring 2026 ECBS meeting, along with results from a forthcoming study to develop a new IS for Salk IPV.

For calibrating in-house rat i*n vivo* potency assays with the first reference reagent, Pharmacopea provides criteria and guidance for low- and middle-income country manufacturers on how to do the analysis. Manufacturers are responsible for developing pass-fail criteria. The goal is to justify drift in results by demonstrating internal consistency.

Regulatory expectations of IPV potency assays

Presented by Tong Wu (speaking in a personal capacity and not on behalf of Health Canada or ECBS)

- Quality attributes should be comparable throughout product lifecycle to vaccine lots shown to be safe and effective in clinical studies. For a i*n vivo* rat potency assay, a multi-dilution dose-response curve is the way to measure the functional immune response. More than a third of negative regulatory decisions are related to standard control.
- WHO guidance is available for establishing an in-house reference standard. New guidance is forthcoming for calibrating in-house standards with ISes.
- The goal is to ensure that D antigen units defined by the first in-house reference are comparable to subsequent in-house references. When D antigen units are redefined by a new IS, manufacturers should identify the root cause of the inconsistency, ensure the comparability among old and new in-house standards, and link the in-house standard to clinical performance of the vaccine.

S19 Sabin/Salk viruses

At the time of this meeting and writing of this summary, the hyper-attenuated S19 strains can be grown outside GAP-IV containment, which avoids huge investment in high containment laboratory/ manufacturing facilities. They have antigenic properties similar to Sabin viruses, enabling the same vaccine production methods and simple assay validation.

MHRA research and resources for working with S19 strains

Presented by Laura Stephens and Andrew Macadam, MRHA

- MHRA validation of the S19 strains as a challenge strain in neutralising antibody assays shows good correlation with Sabin/ wild-type strains.
- Rat potency results also show good correlation with S19 versus Sabin/wild-type challenge strains (final analysis pending); guidance is available.
- S19 strain uses can be expanded to other techniques, such as cell sensitivity, standard for direct detection assays, testing antiviral compounds, human immunoglobulin testing, and testing virucidal agents.
- MHRA will write a collaborative study report including validation data from all facilities involved in testing.
- MHRA will also provide a report on genetic stability of the strains. No shedding in NHPs and no paralysis in the TgmNVT was observed after giving very high doses in animal studies. These strains are regularly used for serology testing. The consistency assay protocol for quality control will apply to S19 as it does to other polio vaccines.
- Pre-GMP strains are available for vaccine production research under MTA from MHRA.

MHRA has developed an HTS-based assay for monitoring domain 5, the most concerning region for reversion to virulence. This reversion involves a base pair exchange rather than an SNP, which this assay identifies. This assay could be useful for manufacturers working with S19 viruses, and MHRA is happy to do this analysis for anyone working with these strains.

Manufacturer experience with S19: Bio E

Presented by Mallikarjuna Panchakshari, Biological E Ltd.

Bio E presented its process for identifying and addressing mutations picked up in clinical lot testing for their S19 candidate vaccine, which involved a combination of in-house and MHRA assays since some reagents do not detect all mutations. The mutations present in this example demonstrated how some epitopes worked together to affect immunogenicity in rats but not in humans, as well as why tracking these mutations was important.

New platforms and reagents

Gates Foundation priorities

Presented by Rajeshwari Adhiseshan, Gates Foundation

- Vaccine-like particle (VLP) vaccine platforms are a priority for post-eradication because cost of goods are expected to be lower than IPV. The Gates Foundation is working with CanSino and Bio E on these platforms.
- In 2024, the foundation and university of Leeds partners launched evaluations of VLP-based, IPV-containing hexavalent products using conventional and controlled release technologies.
- The foundation is also supporting the development of C antigen reactive monoclonal antibodies (mAbs) for the characterization of next-generation vaccines. On whether VLP based manufacturers should consider using an antibody cocktail for a wider range of detection, it was suggested by MHRA to start there, and then if there is C antigen present, transition to single mAbs.

CanSino experience with VLP production and characterization

Presented by Qiaoling Yan, CanSino Biologics

- CanSino selected the antigenic sites for each serotype based on the evaluation of VLP immunogenicity, stability, and yield.
- The production process is at clinical lot scale and will be ready for commercial scale in 2026. At 120 million doses per year, the process is high yield and cost-effective. All batches have consistently demonstrated high purity levels. A comprehensive characterization of attributes and methods show consistency with theoretical values.
- The production process for these VLP-polio vaccine candidates (utilizing an insect cell system) has been successfully scaled up to 300 L while maintaining consistent product quality.
- Comprehensive analytical characterization confirmed that the polio VLPs exhibit structural, physicochemical, and conformational properties consistent with theoretical expectations.
- D-specific and C-specific mAbs have been identified as critical reagents for the quality control and characterization of the VLPs.
- The replication defective pseudo-polio virus-based neutralization assay showed strong correlation with the conventional live virus assay. Replacing the live poliovirus-based test with the pseudovirus neutralization assay can further minimize the risk of live poliovirus exposure and transmission.
- The VLP candidate was found to be safe and immunogenic in a first-in-human study completed in Australia. A Phase 1/2 study in Indonesia began at the end of 2024 and interim results will be available in early 2026.

During the discussion, MHRA commented that bridging studies should occur between Sabin viruses and pseudoviruses for neutralization assay testing.

CanSino emphasized the importance of developing an IS early for VLPs. Whether current standards and reagents will suffice is an open question and depends on the antigen sequence and whether the reagents

are D antigen-specific. MHRA noted that universal reagents will help, which are designed for both Sabin and Salk IPV and are D antigen specific, so they may be worth investigating for VLPs.

VHH antibodies against polio types 1, 2, and 3

Presented by Anna Shishova, Chumakov Federal Research Center

Generation of polyclonal antibodies against D and C antigens for ELISA tests, using a nanobody platform, is stable, easy to produce in high quantities, and easy to manipulate. A few clones for each Sabin type are under patenting. Singular antibody and a set of type-specific antibodies will likely be available as a set to use in a panel. Now that the process is streamlined, turnaround time (from the point of immunizing animals) is two months.

Capture and detection antibodies are different from each other. The Chumakov Federal Resaerch Center is working on combining clones for capture and detection.

On the potential for the Variable Heavy chain (VHH) antibodies to be used as therapeutics, they are too small on their own but have been fused with Fc fragments for therapeutic application against COVID-19. Animal testing would be the first step to see if this is possible with polio.

Manufacturer perspectives

At the close of the meeting, all manufacturers had the opportunity to share where they'd like additional support. Participants voiced the need for technical support for the following activities:

- Characterization of C antigen antibodies
- Establishment of HTS in-house; assistance validating in-house methods with reference standards.
- Critical reagents
- New assays based on pseudoviruses (given limitations of type 2 use)
- Sustained availability of materials from MHRA, such as reagents
- Availability of an IS for HTS
- Immunogenicity evaluation
- Information on long-term stability of IS for regulatory purposes
- Guidance on positive control for IS
- Participation in collaborative studies
- Preparation techniques for sequencing vaccine lots

MHRA noted that if manufacturers have an in-house HTS platform, having MHRA to come and conduct a training would be best.

Remaining questions

Some gaps remain in the understanding of the S19 live viruses. At some point, these hyper-attenuated strains may come under GAP-IV containment, so preparing for that outcome is important. Another open question is whether to use pseudovirus or something else for neutralization assays. Additionally, mucosal immunity remains a challenge and VLPs and oral vaccines offer potential solution.

Photo credit: PATH. Participants at the International Meeting on Quality Control Assays for Polio Vaccines, held in Bangkok, Thailand, September 2025.

Appendices

Presentations

Presentations that have been approved for dissemination are linked in an external folder accessible by this link: https://path.box.com/s/dk9wp6sw9mk8dvpdbxlh5b06nduic8ly

Agenda

Day 1: Wednesday, September 3, 2025

8:00AM – 8:30AM: Check In & Registration

8:30AM – 9:30AM: Welcome & Introductions

Time	Topic	Presenter(s)
8:30AM-8:45AM	Welcome & Introductions by PATH	Kutub Mahmood PATH
8:45AM-9:00AM	Introduction of participants	Facilitator: Kutub Mahmood
9:00AM-9:30AM (remote)	*Update by Gates Foundation on polio vaccines & next generation vaccines*	*Ananda Bandyopadhyay The Gates Foundation*

9:30AM – 9:45AM: Coffee Break

9:45AM – 1:00 PM: Polio HTS – Session 1 (Chair: Kostya Chumakov)

Time	Topic	Presenter(s)
9:45AM-10:15AM	Historical work on animal based NVT testing: Use of MAPREC as non-animal based alternative testing for vaccine lots, and the utility of HTS for vaccine seeds and production lots testing	Kostya Chumakov George Washington University
10:15AM-10:50AM	Summary of the Collaborative Study Results for the use of HTS in vaccine testing	Manasi Majumdar MHRA
10:50AM-11:25AM	Genome-SNP profiling for monitoring molecular consistency of nOPV	Kostya Chumakov George Washington University
11:25AM-12:00PM	Use of HTS in nOPVs vaccine lot release testing	Gemi Pertiwi Bio Farma
12:00PM-1:00PM (remote)	*Use of High Throughput Sequencing as alternate to animal NVT testing for vaccine lots*	*Javier Martin MHRA*

1:00PM – 2:00PM: Lunch Break

2:00PM – 3:30PM: Polio HTS – Session 2 (Chair: Amy Rosenfeld)

Time	Topic	Presenter(s)
2:00PM-2:45PM (remote)	*Update of global polio eradication*	*Ondrej Mach WHO*
2:45PM-3:30PM	HTS for Quality Control of Next-Generation Polio Vaccines (S19 and nOPV)	Umakanta Mandala Biological E Ltd.

3:30PM – 3:45PM: Coffee Break

3:45PM – 5:00PM: Polio HTS – Session 2 (Chair: Amy Rosenfeld)

Time	Topic	Presenter(s)
3:45PM-4:15PM	Regulatory Readiness for HTS data assessment and vaccine lot release	Yola Eka Erwinda BPOM
4:15PM-5:00PM	DISCUSSION WHO PQ requirements for use of HTS as alternate to the animal NVT testing Regulatory readiness for evaluation of HTS data for vaccine lot release Revision of guidance documents (pharmacopeia, WHO TRS, regulatory guidance) Any other related topic	Facilitator: Amy Rosenfeld

7:00PM WELCOME DINNER

Volti Restaurant at the Shangri-La Hotel

Day 2: Thursday, September 4, 2025

9:00AM – 10:00AM: Sabin IPV Potency – Session 1 (Chair: Tong Wu)

Time	Topic	Presenter(s)
9:00AM-9:45AM	Overview of potency testing for Sabin IPV	Kostya Chumakov George Wasington University
9:45AM-10:45AM	Use of in vivo vs in vitro assay for routine lot release and reference standards used for potency assay	Tong Wu Health Canada

10:45AM – 11:00AM: Coffee Break

11:00AM – 12:00PM: Sabin IPV Potency – Session 1 (Chair: Tong Wu)

Time	Topic	Presenter(s)
11:00AM-11:30AM	Collaborative Study results summary for the Rat potency testing with sIPV products	Alison Tedcastle MHRA
11:30AM-12:00PM	DISCUSSION: Sabin IPV Use of in vivo vs in vitro assay for routine lot release. Management of reference standards used for potency assay. Any other related topic	Facilitator: Tong Wu

12:00PM – 1:00PM: Lunch Break

1:00PM – 2:15PM: S19 Related Activities – Session 1 (Chair: Laura Stephens)

Time	Topic	Presenter(s)
1:00PM-1:30PM (remote)	*S19 viruses use in production and testing*	*Andrew Macadam MHRA*
1:30PM-1:45PM	S19 IPV development and challenges	Mallikarjun Panchakshari Biological E Ltd.
1:45PM-2:15PM	S19 strains as useful tools under poliovirus containment	Laura Stephens MHRA

2:15PM – 2:30PM: Coffee Break

2:30PM – 3:30PM: S19 Related Activities – Session 1 (Chair: Laura Stephens)

Time	Topic	Presenter(s)
2:30PM-3:30PM	Discussion on S19 and Containment Gaps in Understanding Safety Concerns with live S19	Facilitator: Laura Stephens

Day 3: Friday September 5, 2025

9:00AM – 10:45AM: Session 1 (Chair: Kutub Mahmood)

Time	Topic	Presenter(s)
9:00AM-9:30AM (remote)	*Critical Reagents for Polio vaccines: Gates Foundation Update*	*Rajeshwari Adhiseshan The Gates Foundation*
9:30AM-10:15AM	VLPs Production & Characterization & Reagents requirements and challenges	Qiaoling Yan CanSino

10:15AM – 10:30AM: Coffee Break

10:30AM – 12:00PM: Session 2 (Chair: Kutub Mahmood)

Time	Topic	Presenter(s)
10:30AM-10:45AM	Developing VHH antibodies against poliovirus types 1, 2, and 3	Anna Shishova Chumakov Federal Research Center
10:45AM-11:45PM	Round Table Discussion on Critical Reagents & Standards Each organization representative to share summary of the polio vaccine products licensed or in development and any specific future support needs. Supply of critical reagents and standards for use in vitro assay for routine lot release. Any other critical reagents which do not exist and needs development	Facilitator: Kutub Mahmood
11:45PM-12:00PM	Closing Remarks	Kutub Mahmood PATH

12:00PM: Lunch Break

Close of Meeting

List of attendees

First Name	Last Name	Organization
Alison	Tedcastle	MHRA
Amy	Rosenfeld	Tulane University
Anantha Balaji	Kanduri	PATH
Andrew	Malkin	MP Chumakov Institute of Poliomyelitis
Anna	Shishova	MP Chumakov Institute of Poliomyelitis
Ashwin	Kharndare	Bharat Biotech
Bhanupriya	Kilari	Bharat Biotech
Bochao	Wei	CanSino Biologics
Chen	Xiaoling	Wuhan Institute of Biological Products
Chunlin	Xin	CanSino Biologics
Dan	Yu	Sinovac
Dongmei	Yan	China CDC
Gembong	Nugroho	Bio Farma
Gemi	Pertiwi	Bio Farma
Harsh	Jogdand	Biological E Ltd.
Hope	Randall	PATH
Istianti	Nurisa	Bio Farma
Jeroen	Strating	Cerba Research
Jie	Song	IMBCAMS
Jun	Li	Sinovac
Kalpana	Chandel	Biological E Ltd.
Konstantin	Chumakov	George Washington University
Kumar	Gaurav	Panacea Biotech
Kutub	Mahmood	PATH
Laura	Stephens	MHRA
Lavit	Jambu	Panacea Biotech
Luo	Min	Wuhan Institute of Biological Products
Mallikarjuna	Panchakshari	Biological E Ltd.
Manasi	Majumdar	MHRA

Meaghan	Murphy	PATH
Megan	McIntosh	PATH
Na	Liu	China CDC
Niharika	Pentakota	Bharat Biotech
Nolan	Meyer	PATH
Qiang	Sun	China CDC
Qiaolong	Yan	CanSino Biologics
Rama Devudu	Puligedda	OCMS Bio, LLC
Ruijie	Wang	CanSino Biologics
Sai Kumar	Vanaparthi Tirummal Shiva	Biological E Ltd.
Shuangli	Zhu	China CDC
Susumu	Ochiai	BIKEN
Tianjiao	Ji	China CDC
Ting	Zhao	IMBCAMS
Tong	Wu	Health Canada
Uma	Madala	Biological E Ltd.
Usha	Maddula	Bharat Biotech
Varun	Datar	Serum Institute
Xiujuan	Zhu	BIBP
Yadong	Li	IMBCAMS
Yaru	Quan	NIFDC
Yola Eka	Erwinda	Indonesian FDA
Yuan	Yuan	PATH
Zheng	Jiang	NIFDC
Wiwik	Ambarwati	Indonesian FDA
Sreenivasulu Reddy	B	GCBC Vaccines Pvt Ltd
Yujia	Chen	Cansino Biologics Inc.