WHO workshop on implementation of international standards for quality control of polio vaccines including OPV and IPV
31 Oct- 2 Nov 2023, Jakarta, Indonesia

Session I. Opening of the meeting

World Health Organization

WHO workshop on implementation of international standards for quality control of polio vaccines including OPV and IPV
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Background, objectives and expected outcomes of the workshop

Tiequn ZHOU, Scientist, WHO/MHP/HPS/TSS/NSB

World Health Organization

Background- 1

- OPV and IPV are used by the Global Polio Eradication Initiative (GPEI) and these vaccines are still playing a major role in the endgame of polio eradication and beyond.
- In response to the scientific and technological advances in polio vaccines field, and GPEI strategic need, and to align with other recently published WHO general guidance as relevant, WHO has updated **written technical standards/guidance** for polio vaccines:
  - IPV Recommendations amendment, 2019
  - Full revision of OPV Recommendations, 2022
  - Guidelines for safe production and quality control of polio vaccines, 2018 & 2020
- Meanwhile, various **international measurement standards** and new QC technologies (e.g., high throughout sequencing, HTS) for control of OPV and IPV have been developed and become available to countries.
- It is important for manufacturers and regulators to be aware of current standards and understand their proper use.

Setting norms and standards and promoting and monitoring their implementation" are WHO core functions

Background- 2

- During the process of developing those standards, issues have been identified among stakeholders and requests have been received by WHO to organize **implementation workshop** to provide additional technical support.
- These issues were selected as the main subjects of this workshop, e.g.,
  - HTS: utility, validation, bioinformatics, standardization aspects
  - Potency tests (in vitro, in vivo) and standardization issues
  - Rationale and proper use of WHO international standards and reference reagents
  - Vaccine stability monitoring considerations
  - Production consistency issues & Reference standards management
- Additional issues may be raised during the workshop by manufacturers, regulators for discussion and exchanging perspectives.

Preparations for the workshop

- Preparatory work started in early 2023; meeting approval obtained in July.
- WHO/NSB convened discussions with a small group of experts: K Chumakov (former CBER/FDA, US), J Martin (MHRA, UK), L Mallet (EDQM, France), T Wu (Health Canada) to propose and discuss the program of workshop.
- WHO/NSB convened preparatory meeting with facilitators/speakers on 25 Sep
  - Konstantin Chumakov, Teeranart Jivapaisarnpong, Juliati, Manasi Majumdar, Javier Martin, Catherine Milne, Laurent Mallet, Julia Panov, Alison Tedcastle, Tong WU, John Konz, Kutub Mahmood
  - Discussed, streamlined and optimized agenda flow and presentations, agreed on work assignments
- A pre-meeting was held on 30 Oct with above experts to prepare the workshop

Long process – technical preparations, nominations of participants, logistics including travel and meeting arrangements and so many aspects…

This workshop

- WHO had invited global manufacturers and National Control Laboratories (NCL) who are involved in OPV (including nOPV2) and/or IPV production and control
  - Willingness to participate was received from 14 NCLs (1- cancelled) and 11 companies (1- unable to attend)

- In this workshop
  - Regulators: around 26 experts from 17 countries across 6 WHO Regions
  - Manufacturers: around 18 experts from 10 companies
    International Federation of Pharmaceutical Manufacturers and Associations (IFPMA)- unavailable to attend
  - Other experts: PATH, Tauber Bioinformatics Research Center
  - WHO Polio Eradication Initiative (POL): Dr Martin EISENHAWER
  - WHO Norms and Standards (NSB) team (organizer)
  - WHO Country office, Regional office
  - BMGF: expressed interest, cancelled due to change

Workshop objectives

- Provide updates on WHO standardization of polio vaccines (OPV, IPV) including written and measurement standards
- Provide lectures, case studies, and Q&A sessions to manufacturers and regulators to elaborate on important issues related to polio vaccine production and QC, including the rationale and proper use of WHO standards
- Exchange experiences and views among experts, manufacturers and regulators, promote implementation of WHO standards into working practice, and identify future need for technical support

Expected outcomes

- This workshop is expected to **facilitate and promote the implementation of up-to-date WHO standards (written & measurement) for polio vaccines into manufacturing and regulatory practice**
  - available standards & their proper use
  - available technical resources and support to users

❖ Lectures, case studies (example of experience, group work on a given case), Q&A and discussions- **provide a forum for exchange of knowledge, experience and perspectives, and for discussion among experts, manufacturers and regulators.**

Please actively join the discussions

Important- Your feedback

We want to hear from you -

Session VI- presentations by manufacturers and NCLs

For those who do not have presentations, please be encouraged to express your views orally during the workshop.

Your feedback will help us plan future work to provide practical support to countries.

Acknowledgements

- WHO Collaborating Centers on biological standardization, worldwide laboratories and institutions including academia, industry, regulatory authorities, and international agencies (e.g., PATH) for contributing to WHO biological standardization work.
- Special thanks to Indonesian FDA for hosting this workshop, providing local support
  - Indonesian FDA colleagues devoted lot of time and work to arrange logistics, provide local support to the organization of this workshop
- K Chumakov (CBER,US FDA), J Martin (MHRA, UK), L Mallet (EDQM, France), T Wu (Health Canada) for critical valuable input to the design and preparation of this workshop.
- Facilitators and Speakers for preparing extensive lecturers/talks and sharing expertise and experience in the workshop; Chair and Rapporteurs for their hard work.
- Workshop participants for devoting time to attend this workshop and exchange perspectives.
- WHO/HQ admin support: Ms Sue Jenner
- WHO Regional office (SEARO), WHO Country office in Indonesia for support

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Thank you

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Session II. Updates on WHO standards for quality control (QC) of polio vaccines (OPV, IPV)

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Update on current WHO written standards for OPV and IPV

Tiequn ZHOU, Scientist, WHO/MHP/HPS/TSS/NSB

World Health Organization

Presentation Outline

- WHO Norms and Standards* for biologicals (written & measurement standards) - brief concept
- Current WHO written standards for OPV
- Current WHO written standards for IPV
- Overarching WHO guidance for polio vaccines (OPV and IPV)
- WHO guidance on preparation and calibration of measurement standards
- Useful web resources

** Established by the Expert Committee on Biological Standardization (ECBS)*

WHO and standards-setting

- WHO **Constitution** (1946, Article 2)- *"to develop, establish and promote international standards with respect to food, biological, pharmaceutical and similar products"*, as well as *"to standardize diagnostic procedures as necessary"…*
  - *http://www.who.int/governance/eb/who_constitution_en.pdf*
- *"Setting norms and standards and promoting and monitoring their implementation"* are WHO **core functions**

Norms & Standards for Biologicals

- WHO has played a key role for **over 70 years** in establishing the WHO **Biological Reference Materials** necessary to standardize biological products as well as developing WHO **Guidelines and Recommendations** for the production, control and licensing of biological products and technologies.

- This work is **accomplished through** WHO biological programme, WHO Collaborating Centers, and WHO Expert Committee on Biological Standardization (**ECBS**); involves close collaboration with international scientific and professional communities, regional and national regulatory authorities, manufacturers and expert laboratories worldwide.

- Published in WHO **Technical Report Series** (TRS): *https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/trs-publications-listing*

WHO written standards: Guidelines, Recommendations

- Developed based on scientific evidence and international consensus
- Technical specifications that help define safe and efficacious vaccines, provide guidance for NRAs and manufacturers on international regulatory expectations for the production and quality control, non-clinical and clinical evaluation of vaccines
- **Intended to be scientific and advisory in nature**
  - serve as a basis for setting national requirements and WHO prequalification
  - leave space for NRAs to formulate additional/ more specific requirements
- Taking into consideration guidance issued by other bodies – intention to complement them, not to create a conflict
- ***Living documents*** **-** will be updated/revised in light of future advances in scientific knowledge and experience in the field

WHO measurement standards: International Reference Preparations

WHO role- "To define an internationally agreed unit to allow comparison of biological measurements worldwide".

- Established through **scientific studies** involving participation of a large number of laboratories worldwide.

- Serve as reference sources of defined biological activity expressed in an **internationally agreed unit**.

- Basis of a **uniform reporting system**, helping physicians and scientists involved in patient care, regulatory authorities and manufacturing settings to communicate in a **common language** for designating the activity or potency of biological preparations used in prophylaxis or therapy, and ensuring the reliability of *in vitro* biological diagnostic procedures used for diagnosis of diseases and treatment monitoring.

WHO International standards (IS)

- Established by the Expert Committee on Biological Standardization (ECBS) with an assigned International Unit of biological activity.
- Standards of highest order that serve as the primary standards for **characterization/calibration** of the activity of secondary standards (regional, national, in-house working standards); calibration/validation of assays.
- Tool for **monitoring** production consistency and product quality, enable **comparison** of results across laboratories/assays globally.
- Support **regulatory convergence** in the evaluation of biological products at the global level.
- Facilitate **development** of vaccines, diagnostics and therapeutics.
- Recognized by **other international standards-setting bodies** (e.g., World Trade Organization, International Standards Organization)

Update on current WHO written standards for OPV and IPV

WHO TRS guidance for OPV

- The first WHO requirements for OPV were formulated in 1962.
- Subsequently it has been revised several times (1966, 1972, 1982, 1989, 1999, 2000, 2012) in light of the developments and advances in vaccine production and quality control technology, vaccine formulations, and global programmes (Global Polio Eradication strategies and Global Action Plan- e.g., containment).
- Since the full revision in 2012, significant progress has been made towards global polio eradication, important advances made in scientific knowledge, novel laboratory techniques (including the use of HTS), development of new non-pathogenic strains of polioviruses for use in quality control tests, and advanced development of novel OPV2, and new publication of relevant WHO guidance documents.
- Last full revision of TRS 980 (Annex 2) was made during 2021- 2022 through consultation process. Final document was adopted by ECBS in Oct 2022, published in WHO TRS No. 1045, Annex 2.

Key issues addressed in last revision (2021-22)

- Use of HTS in QC of OPV as an alternative to MAPREC assay as a preferred in vitro test;
- Analysis of whole genome mutational profiles generated by HTS as a **possible** future replacement of the MNVT and TgmNVT for routine lot release once manufacturing consistency has been established;
- Removal of rct40 test due to its insufficient sensitivity and requirement for WPVs as control strains which is not in compliance with GAPIV;
- Consideration of the design, manufacture and QC of nOPV strains;
- Use of new non-pathogenic strains for the measurement of neutralizing antibodies to polioviruses;
- Updates on international reference materials relevant to OPV manufacture and control, and inclusion of a new appendix on such materials;
- Clinical evaluation of new and safer OPV strains that may be developed;
- Aligned with other WHO recommendations published since its previous revision (i.e., 2012).

*RECAP: Current WHO written standards for OPV

- **Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated). Annex 2, TRS 1045.** (*Replacement of Annex 2 of WHO TRS 980).* **(2022).** *https://www.who.int/publications/m/item/recommendations-to-assure-the-quality--safety-and-efficacy-of-poliomyelitis-vaccines-(oral--live--attenuated)--annex-2*

- SOP: Mutant Analysis by PCR and Restriction Enzyme Cleavage (MAPREC) for Oral Poliomyelitis (Sabin) Vaccine Types 1, 2 or 3: *https://www.who.int/publications/m/item/maprec-sop-for-opv-types-1-2-or-3*

- SOP: Neurovirulence Test of Types 1, 2 or 3 Live Attenuated Poliomyelitis Vaccines (Oral) in Monkeys: *https://www.who.int/publications/m/item/neurovirulence-test-of-types-1-2-or-3-opv-in-monkeys*

- SOP: Neurovirulence Test of Types 1, 2 or 3 Live Attenuated Poliomyelitis Vaccines (Oral) in Transgenic Mice susceptible to Poliovirus: *https://www.who.int/publications/m/item/neurovirulence-test-sop-of-types-1-2-or-3-opv-in-transgenic-mice-susceptible-to-poliovirus-v8*

https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis

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WHO TRS guidance for IPV

- The Requirements for IPV were first formulated in 1959 and revised in 1965.
- Subsequently it has been updated and revised several times (1981, 1985, 2000, 2003) in light of the developments and advances in vaccine production and quality control technology, and global programmes (Global Polio Eradication strategies and Global Action Plan)
- Since then, there have been changes and developments in vaccine production, including the use of seed viruses derived from Sabin strains, which make further revision of the Recommendations necessary.
- Last full revision of IPV TRS was made during 2012-2014 through consultation process; final document was adopted by ECBS in Oct 2014, published in WHO TRS No. 993, Annex 3.

Key issues addressed in last revision (2012-14)

- Reflecting future development of IPV in accordance with global programmatic need (use of Sabin strains and strains derived by recombinant DNA technology);
- Inclusion of a new Appendix 1 to update the history of the different virus seed strains used by manufacturers for IPV production;
- Updating section on international standards and reference preparations;
- Updating section on general manufacturing recommendations and control tests;
- Updating terminology, and appendices;
- Inclusion of specific tests for sIPV and IPV made from strains derived by recombinant DNA technology; *(in light of GAPIII status at that time)*
- Inclusion of new sections on nonclinical and clinical evaluation of new IPV;
- Aligned with other WHO Recommendations published since the last revision (i.e., 2000).

2019 Amendment to IPV TRS 993

- When conducted the TRS revision in 2012-14, the situation was:
  - Recommendations were based on GAPIII status at that time (2014 version)
  - limited data/experience with sIPV (only one sIPV licensed at that time)
- In 2018, requests were received from WHO/POLIO, PQ and some sIPV manufacturers regarding difficulties in complying with TRS 993 requirements due to final GAPIII (2015) implementation etc. (e.g., restricted global laboratory capacity handling wild PV)- Proposal to develop an amendment was endorsed by ECBS in Oct 2018.
- Following consultation process, the **Amendment to Annex 3 of WHO TRS 993** was developed and adopted by ECBS in **Oct 2019;** published in **WHO TRS No. 1024, Annex 3.**

2019 Amendment to IPV TRS 993- key issues

- Modified definitions of “virus sub-master seed lot” and “virus working seed lot”;
- Updated information on available WHO international standards;
- Modified requirements for confirming the genetic stability of attenuated vaccine seeds and monovalent virus pools to provide flexibility for vaccine developers;
- Included additional cell substrates that can be used for the effective-inactivation test;
- Deleted general safety (innocuity) test in line with the decision made by ECBS in 2018 to discontinue the inclusion of this test in all WHO TRS Recommendations, Guidelines and other guidance documents for biological products;
- Updated recommendations for the evaluation of sIPV immunogenicity in nonclinical and clinical studies to provide much needed flexibility, and thus facilitate the development and licensure of new vaccines.

*RECAP: Current WHO written standards for IPV

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated). Annex 3, TRS 993. (*Replacement of Annex 2 of WHO TRS 910).* **(2014)**
https://www.who.int/publications/m/item/poliomyelitis-vaccines-inactivated-annex-3-trs-no-993

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)- Amendment to Annex 3 of WHO TRS 993. Annex 3, TRS 1024. (2019)
https://www.who.int/publications/m/item/poliomyelitis-vaccines-annex-3-trs-no-1024

https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis

Overarching WHO guidance for polio vaccines

Guidelines for safe production and quality control of poliomyelitis vaccines, Annex 4, TRS 1016. Replacement of Annex 2 of WHO TRS 926. (2018)
https://www.who.int/publications/m/item/poliomyelitis-vaccines-annex-4-trs-no-1016

Guidelines for the safe production and quality control of poliomyelitis vaccines, Annex 3, TRS 1028. Amendment to Annex 4 of WHO TRS 1016. (2020)
https://www.who.int/publications/m/item/polio-annex-3-trs-1028

- Guidelines address the containment measures needed during the production and quality control of 1) IPV produced from wild-type poliovirus strains; 2) IPV produced from the live attenuated vaccine (Sabin) strains used in the manufacture of OPV; and 3) OPV and IPV produced from novel safer strains developed by genetic manipulation.

WHO measurement standards for OPV/IPV

- **Catalogue of the WHO international reference preparations- to be updated:** *https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/catalogue*
- **NIBSC/MHRA catalogue:**
  - *https://www.nibsc.org/products/brm_product_catalogue/who_standards.aspx*
  - *https://www.nibsc.org/products/brm_product_catalogue/sub_category_listing.aspx?category=Vaccines&subcategory=Polio*

- To be presented by Dr Javier Martin, MHRA, UK

WHO Guidance: measurement standards

- **Recommendations for the preparation, characterization and establishment of international and other biological reference standards, Annex 2, TRS No. 932:** *https://www.who.int/publications/m/item/annex2-trs932*. (*Revision in pipeline)*

WHO manual for the establishment of national and other secondary standards for vaccines (2011): *https://www.who.int/publications/i/item/WHO-IVB-11.03*

Vaccine potency testing

WHO manual for the preparation of secondary reference materials for in vitro diagnostic assays designed for infectious disease nucleic acid or antigen detection: calibration to WHO International Standards, Annex 6, TRS No. 1004. (2017): *https://www.who.int/publications/m/item/annex-6-trs-no-1004*

IVD- NAT or antigen detection

WHO manual for the preparation of reference materials for use as secondary standards in antibody testing, Annex 2, WHO TRS No. 1043. (2022): *https://www.who.int/publications/i/item/9789240057081*

Antibody testing- immunoassays

Additional guidance provided in disease-specific context (vaccine-specific TRS guidance)

Useful resources

- WHO Expert Committee on Biological Standardization: https://www.who.int/groups/expert-committee-on-biological-standardization
  - Vaccine-specific standardization and general guidance: https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/vaccine-standardization/
  - Polio vaccine standardization: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization/poliomyelitis
- WHO Vaccine Position Papers: https://www.who.int/teams/immunization-vaccines-and-biologicals/policies/position-papers
- Global Polio Eradication Initiative: https://polioeradication.org/
- WHO prequalification of vaccines: https://extranet.who.int/pqweb/vaccines

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World Health Organization

Medicines & Healthcare products Regulatory Agency

Overview of available international reference materials for OPV and IPV

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The quality control of polio vaccines including OPV and IPV
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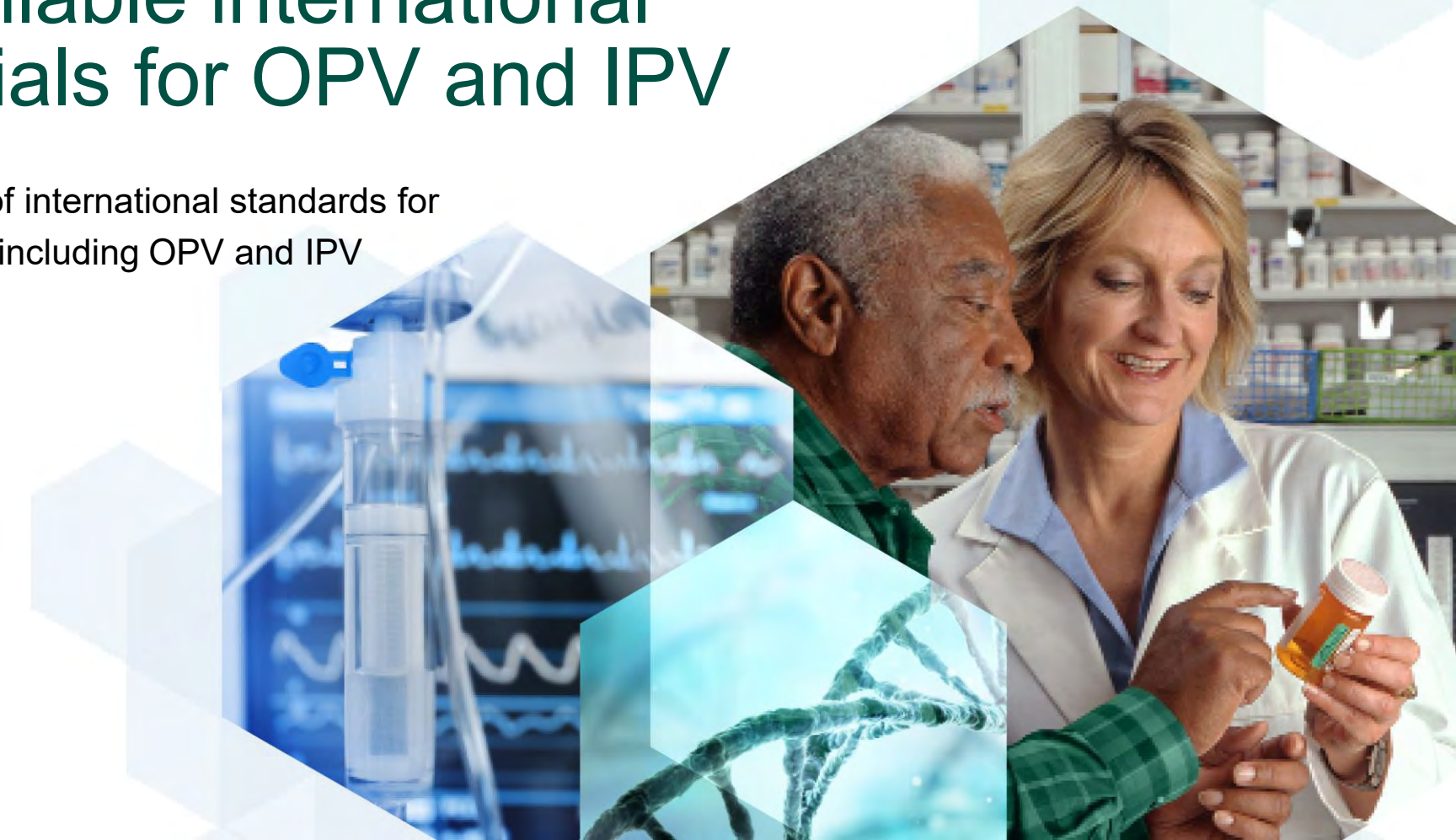
Javier Martin



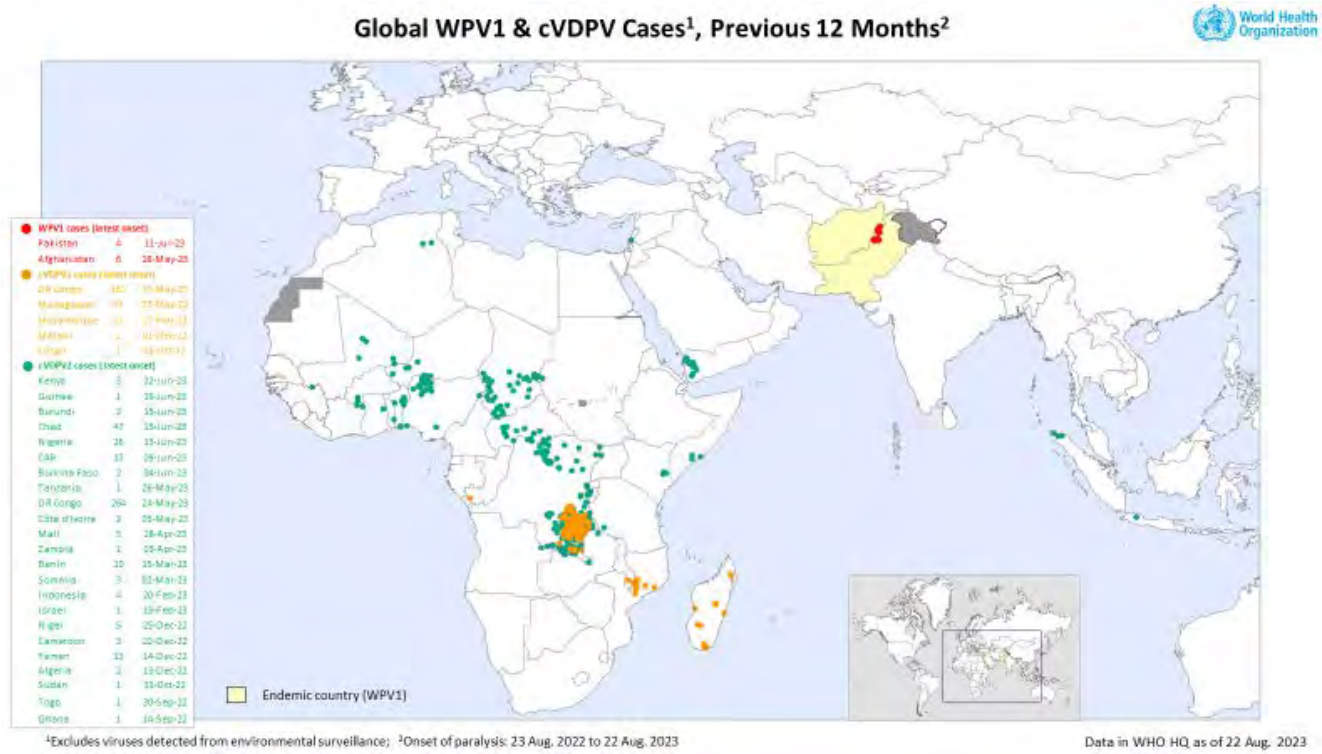
Vaccines for the Global Polio Eradication Initiative (GPEI) Endgame

GPEI facts

- More than 99% reduction in the number of cases since launched
- Wild PV Serotypes 2 and 3 eliminated
- However, circulation of WPV1 in Pakistan and Pakistan and extensive cVDPV2 outbreaks in Africa, Pakistan and Pakistan still occurring causing paralytic cases due to incomplete immunisation
- Main vaccines vaccines used include bOPV (PV types 1, 3) and IPV
- Vaccines available to respond to cVDPV2 and WPV1 outbreaks include mOPV2 and tOPV (PV types 1, 2 and 3)

New tools required for the GPEI endgame

- Novel vaccines including nOPV, Sabin-IPV and VLP



QC assays for poliovirus vaccines

QC tests for OPV

OPV prepared by serial passage of poliovirus that leads to virus attenuation

Virus identification
Virus concentration
Neurovirulence
- MNVT
- TgmNVT

Genetic markers
- Rct 40 test
- MAPREC

Thermal stability

QC tests for IPV

IPV prepared by incubation of wild poliovirus with formaldehyde leading to destruction of Infectivity

Virus identification
Virus concentration
Effective inactivation
D-Antigen content
In vivo potency
Thermal stability
Adjuvant adsorption

Measurement of Biological Medicines

- Absolute measurement is required for the quality assessment and licensing of biological products for human use to ensure safety, efficacy and consistency
- Most biological products currently not measurable by physical-chemical means; most medicines need to be measured in activity
- Biological standardization was developed as a way of assigning a numerical value to the strength of a medicine without necessarily knowing its active principle
- There is a fundamental need for reference materials to support biological standardisation; reference standards need to be robust, fit for purpose and shared widely and often get used up and need replacement
- WHO plays a global role in biological standardization developing norms and standards through WHO guidelines and recommendations to assure the quality, safety, and efficacy of biological products which includes the establishment of WHO Biological Reference Materials

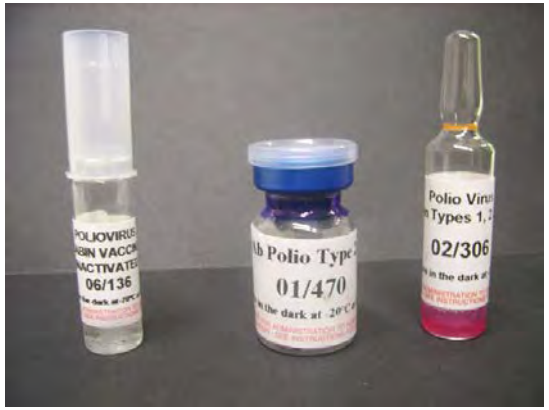
International Reference Standards

- The primary purpose of International Biological Standards and Reference Reagents is to provide a means of ensuring uniformity throughout the world in the designation of the potency, activity or specificity of human medicinal products that cannot be expressed directly in terms of chemical and physical quantities.
- International standards are intended for use in the calibration of the activity of national or working standards and for the expression of their biological activity in international units.
- International Reference Reagents Biological are established mainly for the purpose of providing reference materials of high specificity for the identification of micro-organisms or their products, as well as for other assays of a variety of biological substances.
- NIBSC produces >95% of International Standards for biological medicines.

International Reference Standards for Vaccines

- Vaccines are usually subject to multiple biological tests for quality assurance
- More than one standard often required to match specific test; some products may require many standards
- Standards needed for batch testing
  - Potency
  - Safety

  and more widely
  - Measuring anti-vaccine immune responses in humans
  - Epidemiological analysis/diagnosis
    - Vaccine failures
    - Strain prevalence



- Reference standards help establishing consistency of vaccine production
- The use reference standards allow comparison of vaccines from different manufacturers and vaccines from different batches
- Develop and characterise new vaccines

Timeline for the production of WHO International Standards

Milestone
Endorsement by ECBS
Source material obtained
Trial fills
Definitive fill and post-fill characterisation
Study design and Collaborative Study organization
Collaborative study
Statistical analysis of results and preparation of report
Submission and establishment by ECBS
Post ECBS training activities

International Standard for OPV potency

- The 2nd International Standard (02/306) was established in 2004, calibrated against the 1st International Standard 85/659. 02/306 was prepared by mixing three commercially produced and released monovalent bulks - one of each poliovirus (Sabin) types 1, 2 and 3. The passage level of the virus in the bulks was: SO+3 for type 1, SO+3 for type 2 and RSO+3 for type 3. The assigned potency was set at: 7.51, 6.51, 6.87 and 7.66 log10 TCID50/ml for type 1, 2, 3 and total virus content, respectively.
- The same bulk materials were used to prepare candidate preparations for bOPV, mOPV1, mOPV2 and mOPV3 in a similar manner, which were established as International Standards by WHO ECBS in 2017. The 1st International Standard for bOPV 1+3 (16/164) was assigned potencies 7.19, 6.36 and 7.32 log10 TCID50/ml for type 1, 3 and total poliovirus content, respectively. The 1st International Standards for mOPV1 (16/196), mOPV2 (15/296) and mOPV3 (16/202) were assigned potencies 7.19, 6.36 and 7.32 log10 TCID50/ml for type 1, 2 and 3 poliovirus, respectively.

Monoclonal Antibody Reagents for OPV potency assays

- Poliovirus type 1 monoclonal antibody serum 02/256 (NIBSC batch number 425)
- Poliovirus type 2 monoclonal antibody serum 02/258 (NIBSC batch number 267)
- Poliovirus type 3 monoclonal antibody serum 02/260 (NIBSC batch number 495)
- NIBSC MAbs are routinely used globally by a number of manufacturers and NRA's for the assay of trivalent OPV.

Cell sensitivity standards

- Low titer monovalent Sabin type 1, 2 and 3 poliovirus reference reagents were prepared using the same bulk materials used to produce the current International Standards for monovalent, bivalent and trivalent OPV with assigned potencies as follows:
  - Type 1 (10/164): 5.5 log 10 CCID50/0.1ml in RD cells and 5.3 log 10 CCID50/0.1 ml in L20B cells;
  - Type 2 (10/166): 5.1 log 10 CCID50/0.1ml in RD cells and 4.8 log 10 CCID50/0.1ml in L20B cells;
  - Type 3 (10/168): 5.3 log 10 CCID50/0.1ml in RD cells and 4.8 log 10 CCID50/0.1ml in L20B cells.

International Standard for anti-poliovirus sera

- The 1st International Standards for anti-poliovirus sera types 1, 2 and 3 were established by the WHO ECBS in 1963 from serotype specific polyclonal antisera produced by the hyper-immunisation of rhesus monkeys with live virus suspensions. Each of the standards was specific to one serotype only. They were established in 1963 and assigned a unitage of 10 IU/vial, for each of the polio serotypes.
- The 2nd IS (66/202) was established by the WHO ECBS in 1991 to replace the 1st International Standards. In contrast to the 1st IS the 2nd IS was a single serum that contained activity against each of the three poliovirus serotypes. The following unitage was assigned to the 2nd IS: 25 IU of anti-poliovirus serum (type 1) human; 50 IU of anti-poliovirus serum (type 2) human; and 5 IU of anti-poliovirus serum (type 3) human.
- The 3rd International Standard for anti-poliovirus sera (Human) types 1, 2 and 3 (82/585) was established by the WHO ECBS in 2006 with assigned unitage of 11, 32 and 3 IU per vial of neutralising antibody to type 1, 2 and 3 poliovirus respectively (22).

International Reference Reagents for neurovirulence assays

- Reference preparations at the SO+2 passage level, designated WHO/I for type 1 virus, WHO/II for type 2 virus and WHO/III for type 3 virus (05/416 now), are available upon request through WHO. These reference preparations are for use in in vivo neurovirulence tests with vaccines. The relevant reference materials should be included in each test of vaccine.
- Virus panels for validation and implementation of the transgenic mouse neurovirulence test, as specified in the WHO SOP, are also available. These include a variety of vaccines with known pass/fail neurovirulence results.

International Standards and Reference Reagents for MAPREC

- International Standards and Reference Reagents were prepared from commercial vaccines and viruses generated by cell culture infection. The list of MAPREC references currently available is as follows:

  - 00/410: MAPREC assay of poliovirus type 1 (Sabin).100% 480-A, 525-C DNA (1st International Reference Preparation).
  - 00/416: MAPREC assay of poliovirus type 1 (Sabin) Low Mutant Virus Reference (1st International Reference Preparation).
  - 00/418: MAPREC assay of poliovirus type 1 (Sabin). Synthetic DNA 2% 480-A, 525-C. WHO (1st International Standard).
  - 00/422: MAPREC assay of poliovirus type 1 (Sabin) High Mutant Virus Reference(1st International Reference Preparation).
  - 97/758: MAPREC analysis of Poliovirus type 2 (Sabin) Synthetic DNA 0.67%481-G (1st International Standard)
  - 98/524: MAPREC analysis of Poliovirus type 2 (Sabin) Synthetic DNA, 100%481-G (1st International Standard)
  - 98/596: MAPREC analysis of Poliovirus type 2 (Sabin), high virus reference 1.21% 481-G (1st International Reference Preparation)
  - 94/790: MAPREC analysis of Poliovirus type 3 (Sabin). Synthetic DNA 100% 472-C. WHO (1st International Standard)
  - 95/542: MAPREC analysis of Poliovirus type 3 (Sabin) Synthetic DNA 0.9% 472-C. WHO (1st International Standard)
  - 96/572: MAPREC analysis of Poliovirus type 3 (Sabin) Low virus reference 0.7%472-C (1st International Reference Preparation)
  - 96/578: MAPREC analysis of Poliovirus type 3 (Sabin) High virus reference 1.1%472-C (1st International Reference Preparation)
  - 97/756: MAPREC analysis of Poliovirus type 2 (Sabin), low virus reference 0.65%481-G (1st International Reference Preparation)

International References for IPV

		Potency (D-Ag/ml)		
Reference	**Year**	**Type 1**	**Type 2**	**Type 3**
IRP	1963			
PU78-02 RP	1978	400	40	160
PU91-01	1991	430	95	285
91/572 2nd IS	1991	430	95	285
BRP No. 1	1991	430	95	285
BRP No. 2	2003	320	67	282
12/104 3rd IS	2013	277	65	248
BRP No. 3	2016	320	78	288

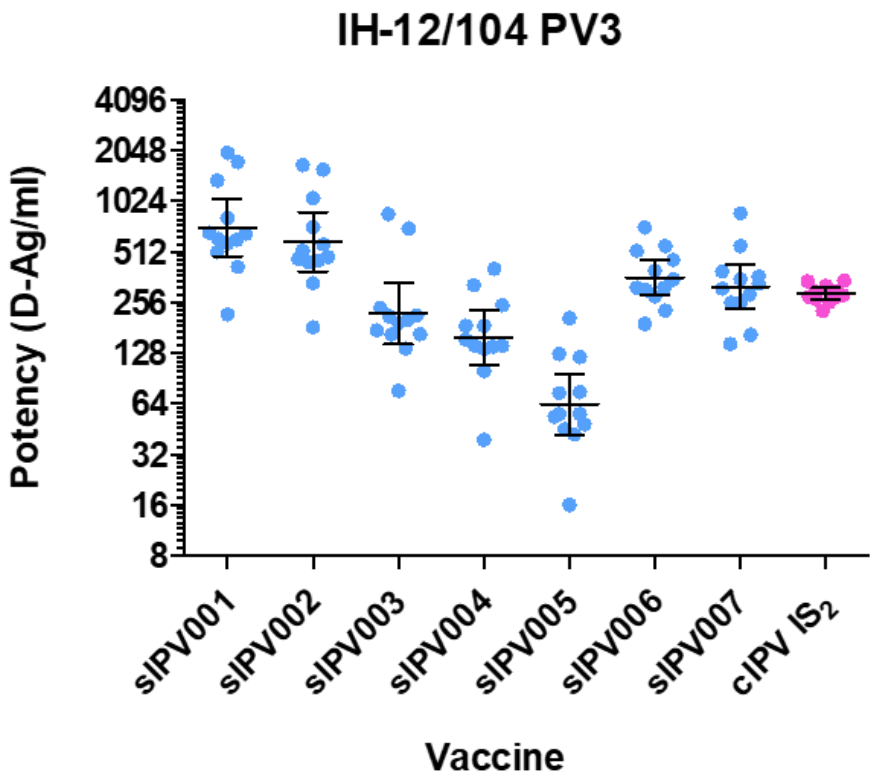
International Standards and Reference Reagents for IPV

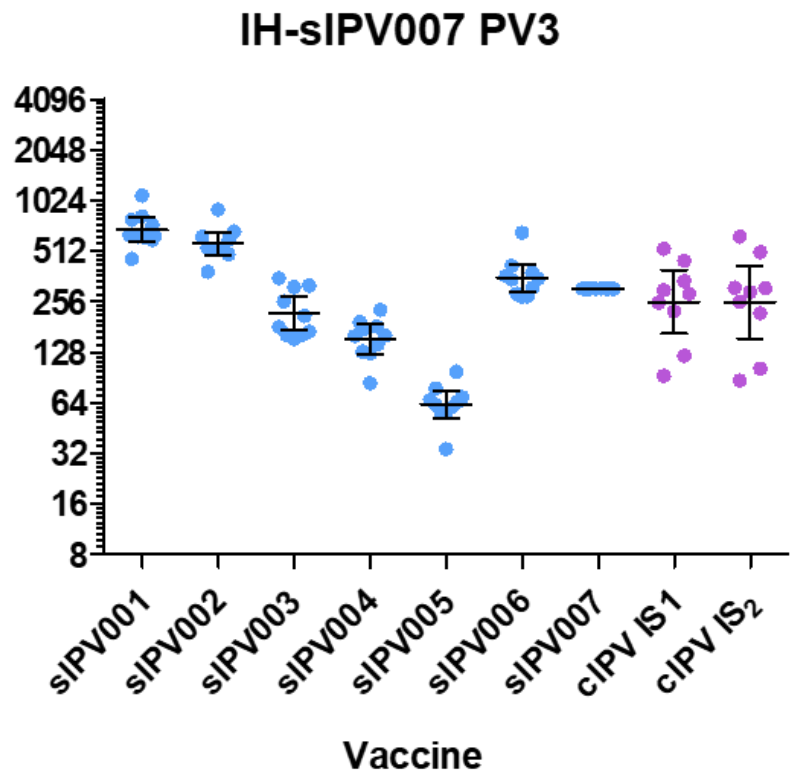
- An International Reference Preparation (IRP) of poliomyelitis vaccine (inactivated) was established by the WHO ECBS in 1962. This preparation was a trivalent blend prepared in 1959 in primary monkey kidney cells from type-1 (Mahoney), type-2 (MEF) and type-3 (Saukett) strains of poliovirus.
- An enhanced potency IPV (PU78-02) from the RIVM was widely used as a reference preparation for control purposes.
- A new reference material (91/574) was established by the WHO ECBS in 1994 as the second WHO International Reference Reagent for in vivo and in vitro assays of IPV. Potencies of 430, 95 and 285 D-antigen units per millilitre were assigned, respectively, to poliovirus types 1, 2 and 3 of this preparation.
- Following inconsistency in the performance of some vials of 91/574, the use of this reference was discontinued in 2010.
- In 2013, the Third WHO International Standard for inactivated poliomyelitis vaccine (12/104) was established by the WHO ECBS using BRP batch 2 as the reference in the study. Potencies of 277, 65 and 248 D-antigen units per millilitre were assigned to poliovirus types 1, 2 and 3, respectively.

International Standards and Reference Reagents for sIPV

- A collaborative study conducted in 2015/2016 found the International Standard for conventional IPV (12/104) unsuitable for measuring the antigen potency of sIPV as a high proportion of invalid assays and large differences between laboratory potency results were found when using 12/104 as a reference to measure the potency of sIPV products.
- Assay validity and between-laboratory variability improved when a sIPV sample was used as a reference to determine the potency of sIPV study samples. The decision was to establish a new International Standard specific for sIPV products
- The First International Standard for sIPV (17/160) was established by the WHO ECBS in 2018 and a new sIPV potency unit, Sabin D-Antigen Unit (SDU) was defined. Potencies of 100 Sabin D-Ag Units (SDU) per millilitre for each of the three poliovirus serotypes were assigned to poliovirus types 1, 2 and 3 (18).
- The new unit is unrelated to the D-antigen unit used to express potency of wIPV.

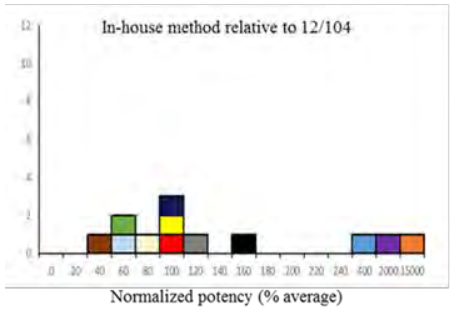
Potency results cIPV vs sIPV reference – PV3

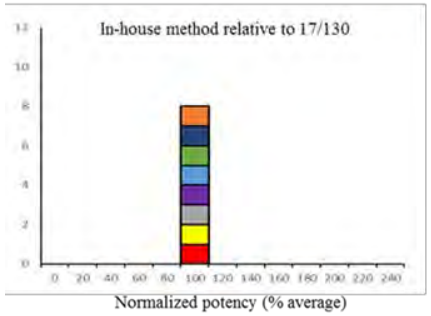


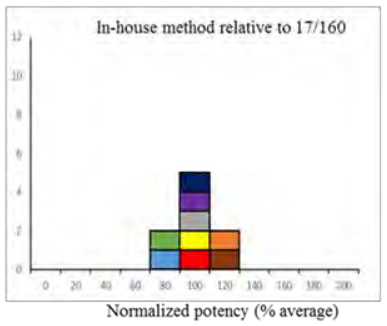


Benefits of biological standardization – Potency of Sabin-IPV product

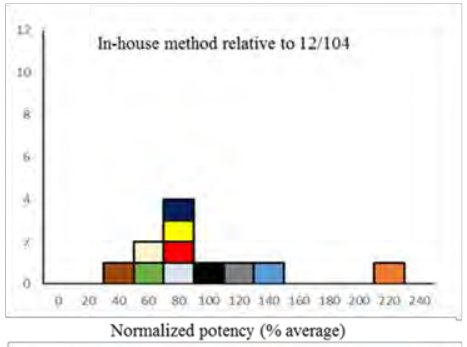
PV1

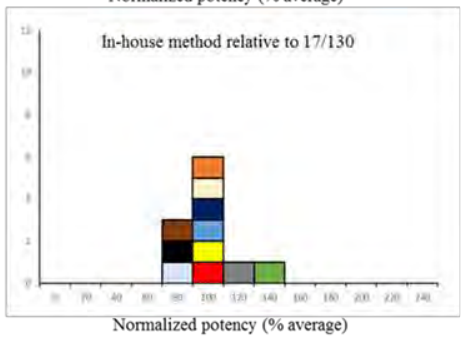


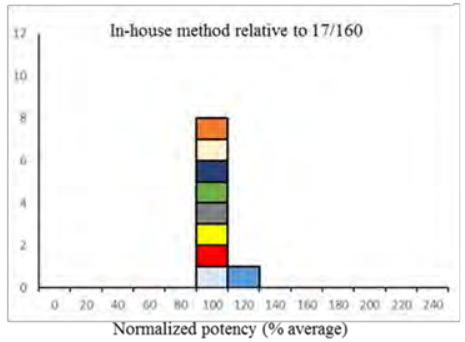




PV3

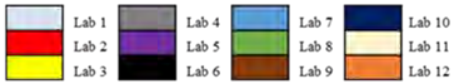






Heterologous reference

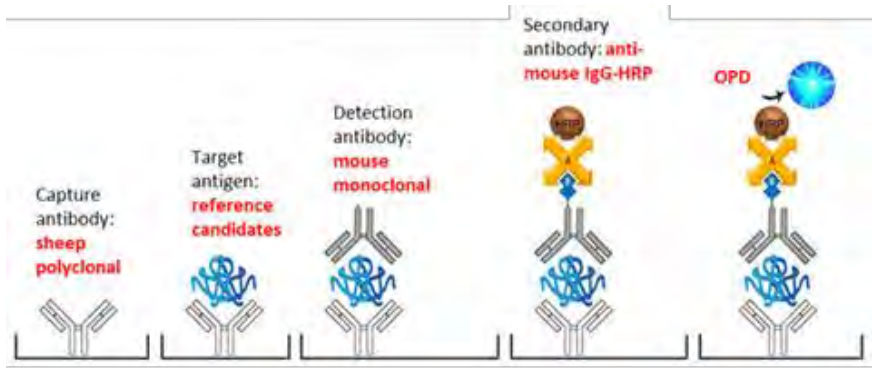
Homologous references



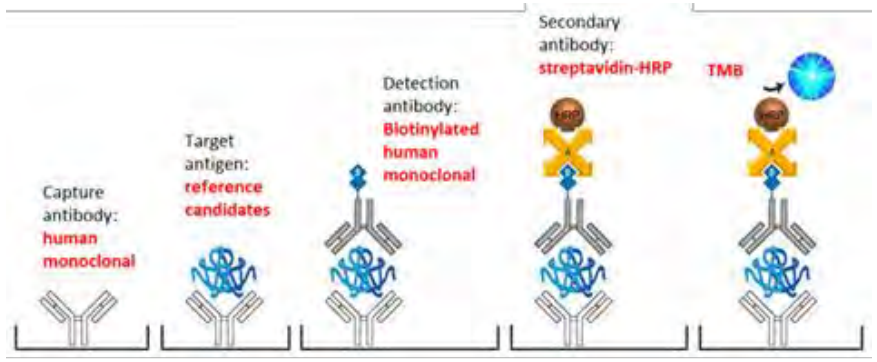
International Standards and Reference Reagents for IPV

- Some differences have been noted in the antigenic profile for different IPV products, highlighting the importance of product-specific assessment of future IPV products, particularly sIPV vs cIPV products.
- For this reason, a new International Standard for sIPV (17/160) was established by the WHO ECBS in 2018 and a new sIPV potency unit, Sabin D-Antigen Unit (SDU) was defined.
- In addition, WHO Universal Reagents for the D-Antigen potency testing of Inactivated Polio Vaccines were established by ECBS in 2022.
- A standardised protocol was developed using human monoclonal type specific capture antibodies, 20/250, 20/252, 20/254 and the human monoclonal cross reactive detection antibody, 20/256.
- International standards and reference reagents for the control of *in vivo* potency assays in rats are under investigation.

Standardization of Sabin-IPV *in vitro* potency assays



Mouse MAbs



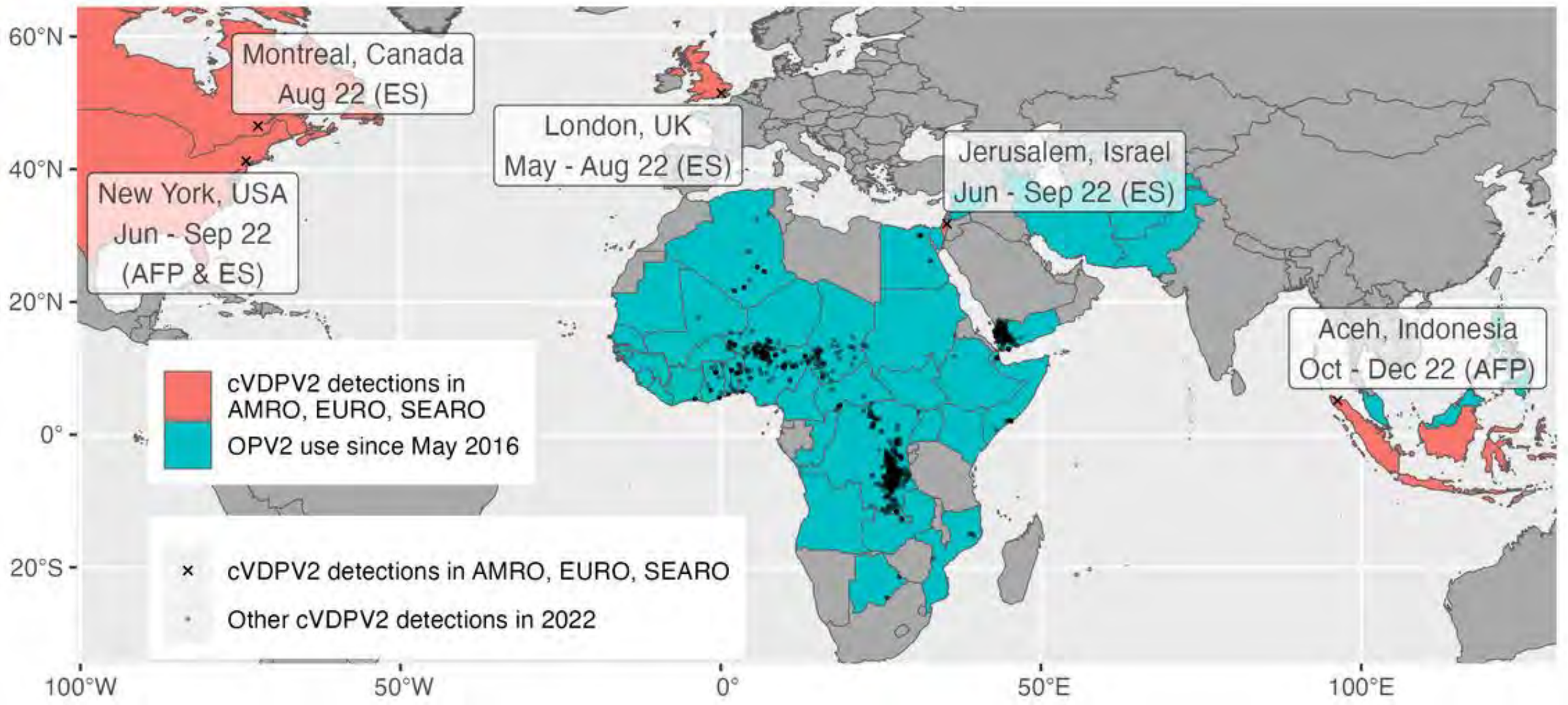
Human MAbs

New WHO International Standards for nOPV type 1, 2, and 3

Background / Rationale

- Sabin poliovirus strains in oral polio vaccine (OPV) are genetically unstable quickly losing their attenuating mutations and reverting to a neurovirulent phenotype causing vaccine-derived poliovirus (VDPV) outbreaks in areas of low immunity.
- For this reason, genetically-modified novel OPVs (nOPVs) with improved genetic stability, less likely to revert to a neurovirulent phenotype, have been developed for each of the three PV serotypes, which will be essential to support the global polio eradication endgame.
- nOPV2 is being used under EUL to respond to cVDPV2 outbreaks and is being assessed for WHO prequalification, while nOPV1 and nOPV3 are being tested in clinical trials.
- We intend to produce WHO International Standards for monovalent nOPV1, nOPV2 and nOPV3 as well as trivalent nOPV (batch size: 4,000 vials each IS).
- Intended use is for potency assays of nOPV products for the quality control of vaccine bulks and finished products.
- Anticipated users are vaccine manufacturers, National Control Laboratories and research and development consortiums developing new safer poliovirus vaccine strains.

Spread of cVDPV remains a key concern



Source: why-is-polio-making-a-comeback-and-what-can-we-do-about-it. https://speakingofmedicine

nOPV2: A New Tool for cVDPV2 Outbreak Response

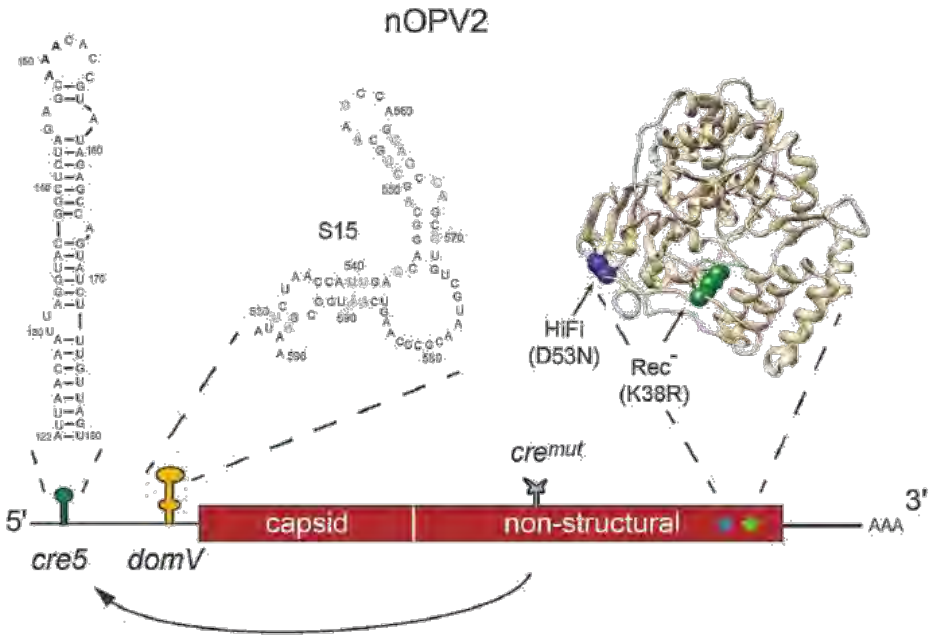
- **The novel oral polio vaccine type 2 (nOPV2)** is a next-generation version of the existing cVDPV2 outbreak response vaccine, mOPV2

- Clinical trials have shown that nOPV2 provides comparable protection against type 2 poliovirus while being more genetically stable and therefore less likely to revert to a form that can cause paralysis in under-immunized communities. **This means that nOPV2 could help stop the spread of cVDPV2 outbreaks**.

- nOPV2 has been in development for over 10 years and it is being used under WHO Emergency Use Listing (EUL).

- nOPV1 and nOPV3 are been assessed in clinical trials.

nOPV2 Genome with modifications



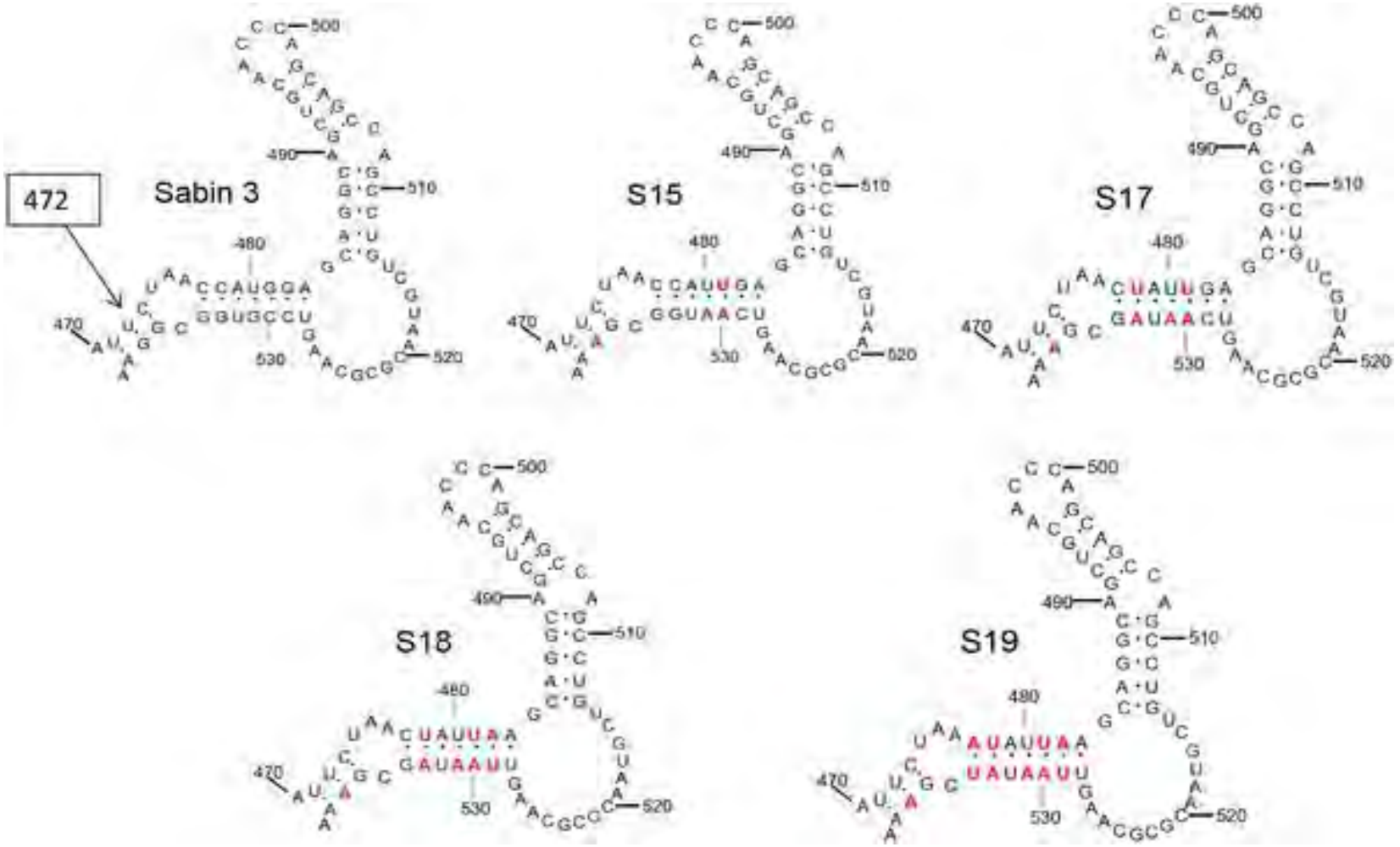
Relevant publication on the nOPV2 Modifications and Source of this photo: Ming TY, Bujaki E, Dolan PT, Smith M, Wahid R, Konz J et al. Engineering the Live-Attenuated Polio Vaccine to Prevent Reversion to Virulence. Cell Host and Microbe 2020; 27(5):736-751.E8

Indicative Timeline

Milestone	Date
Sourcing material	December 2023
Process development	March 2023
Definitive fill	April 2024
Collaborative Study	November 2024
Submission to ECBS	February 2025 (June 2025)

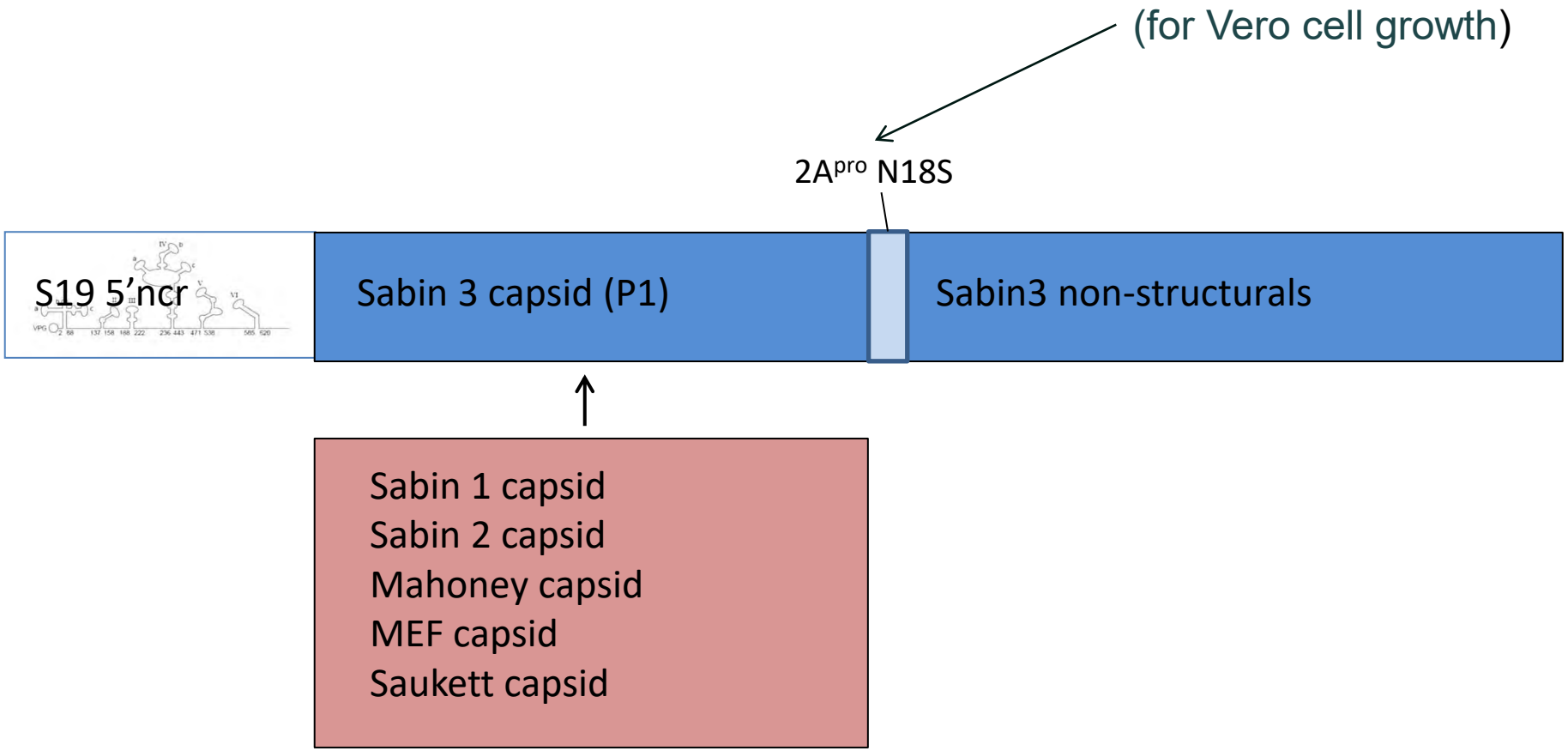
New WHO S19 hyper-attenuated poliovirus type 1, 2 and 3 Reference Reagents

Genetic manipulation of domain V of 5’NTR – S19 PV strains



Base paired stem loop structures in domain V of the 5’ noncoding region of type 3 poliovirus.

S19-poliovirus strains



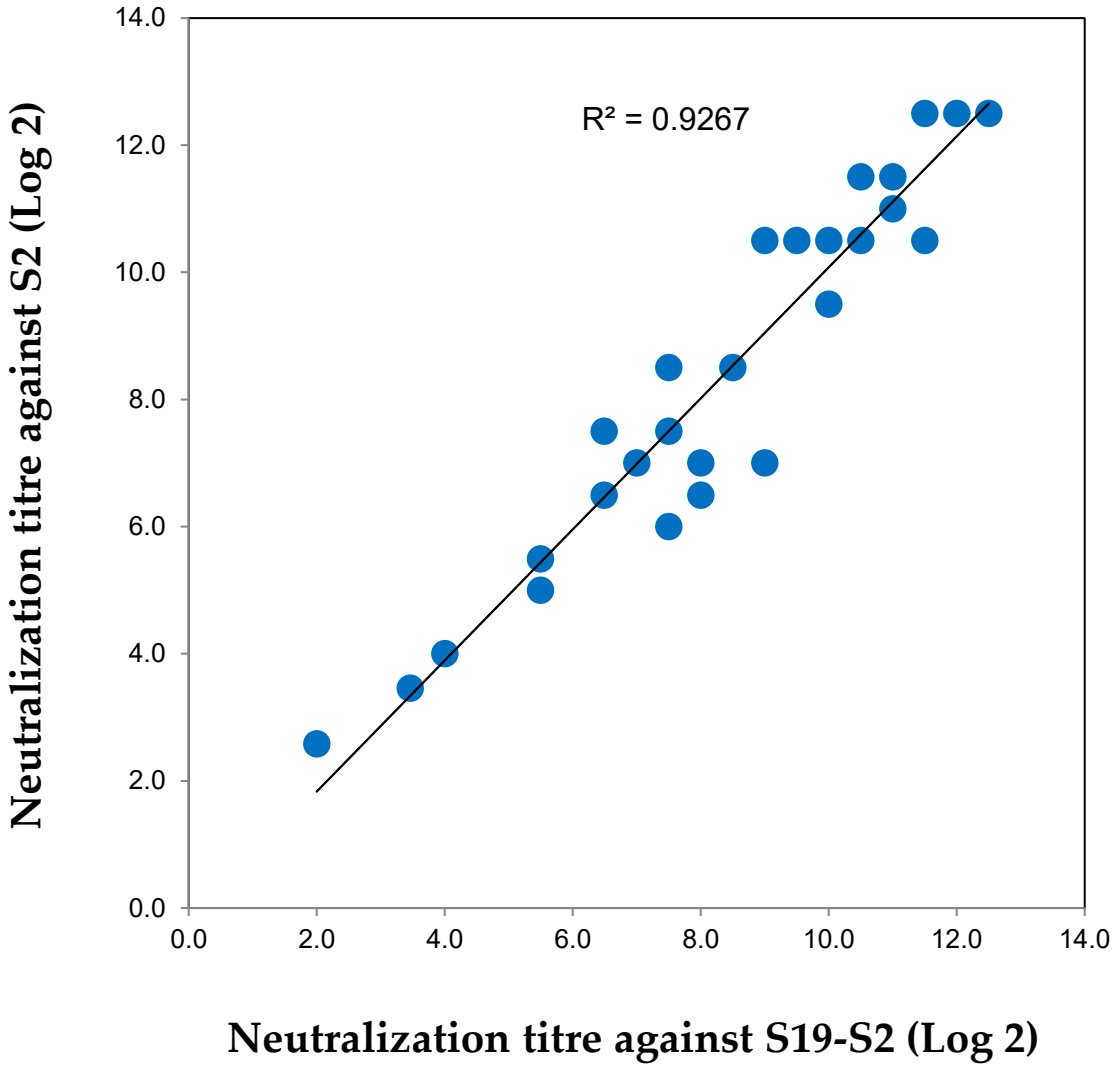
S19-poliovirus strains

- Very low infectivity
  - > 1 million-fold increase in PD_{50} in transgenic mouse spinal cord
  - Oral ID_{50} $>10^{12}$ $CCID_{50}$ in non-human primates
- High yields in cell culture
- Genetically stable
  - no reversion after >20 passages under selective conditions
- Wild-type and VDPV capsid sequences can be used
- Unaltered antigenicity and immunogenicity
- Can be used as seeds for IPV production – assessment in progress
- Can be used as challenge strains for serology assays requiring lower containment – validation in progress (human sera + rat IPV sera)

Use of S19-poliovirus strains

Virus Stocks	IPV Production	IPV QC	IgG QC	Serology Assays	Cell Sensitivity
S19S1_N18S	√				
S19S2_N18S	√				
S19S3_N18S	√				
S19S1		√		√	?
S19S2		√		√	?
S19S3		√		√	?
S19Mah_N18S	√				
S19MEF1_N18S	√				
S19Skt_N18S	√				
S19Mah		√	√		
S19MEF1		√			
S19Skt		√			

Immune response in humans – S2 vs S19-S2



Differences not statistically significant

	p-value
Log Data + Paired t-test	0.813
Wilcoxon Signed Rank Test	0.136

Acknowledgments

Alison Tedcastle	FDA, USA
Elaine Pegg	Lankenau Institute, USA
Gill Cooper	WHO, Switzerland
Laura Crawt	Vaccine manufacturers
Peter Rigsby	NRAs
Helen Fox	BMGF, USA
Andrew Macadam	PATH, USA
	Collaborating laboratories

WHO biological standardization:

An update

Dr Ivana Knezevic, WHO/MHP/HPS/TSS/NSB

31st October 2023

Jakarta, Indonesia

Outline of presentation

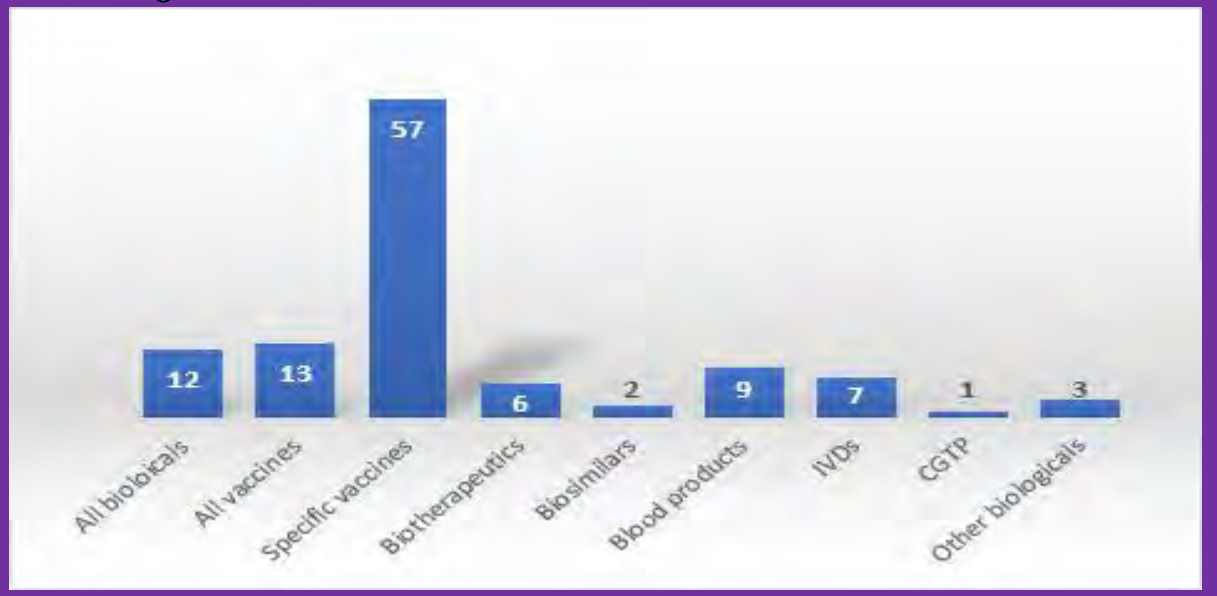
World Health Organization

- Update on WHO standards for Vaccines, BTP and CGTP
  - written standards
  - measurement standards
- WHO Collaborating Centers and Custodian Laboratories
- ECBS reports – TRS 1045 and TRS 1048
- Consultations and workshops: 2023 – 2024
- ECBS meetings in 2023 and 2024

WHO norms and standards for biologicals

Global written standards (111)

https://www.who.int/groups/expert-committee-on-biological-standardization



Global measurement standards (more than 400)

Measurement standards: essential elements for development, licensing and lot release

Scientific evidence

1) Standardization of assays
2) Further development and refinement of QC tests
3) Scientific basis for setting specifications

WHO written standards for regulatory evaluation of vaccines

1. WHO Guidelines on NC and C evaluation that apply to vaccines:

1.1. Nonclinical evaluation of vaccines (TRS 927, ECBS 2003)
1.2. Nonclinical safety evaluation of DNA vaccines (TRS 941, ECBS 2005) - discontinued
1.3. Nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (TRS 987, 2013)
1.4. **Clinical** evaluation of vaccines (TRS 1004, ECBS 2016)
1.5. Guidelines for assuring the quality, safety and efficacy of plasmid DNA vaccines (TRS 1028, ECBS 2020)
1.6. Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations (TRS 1039, ECBS 2021)

2. WHO Good Practices: GMP, GLP, GCP

3. Cell substrates for vaccine production, stability evaluation of vaccines, vaccine lot release, post-approval changes etc

4. Guidelines/ Recommendations for specific types of vaccines: polio, rabies, influenza, pneumo, DTP and combined vaccines, rota, malaria, typhoid, HPV, RSV etc

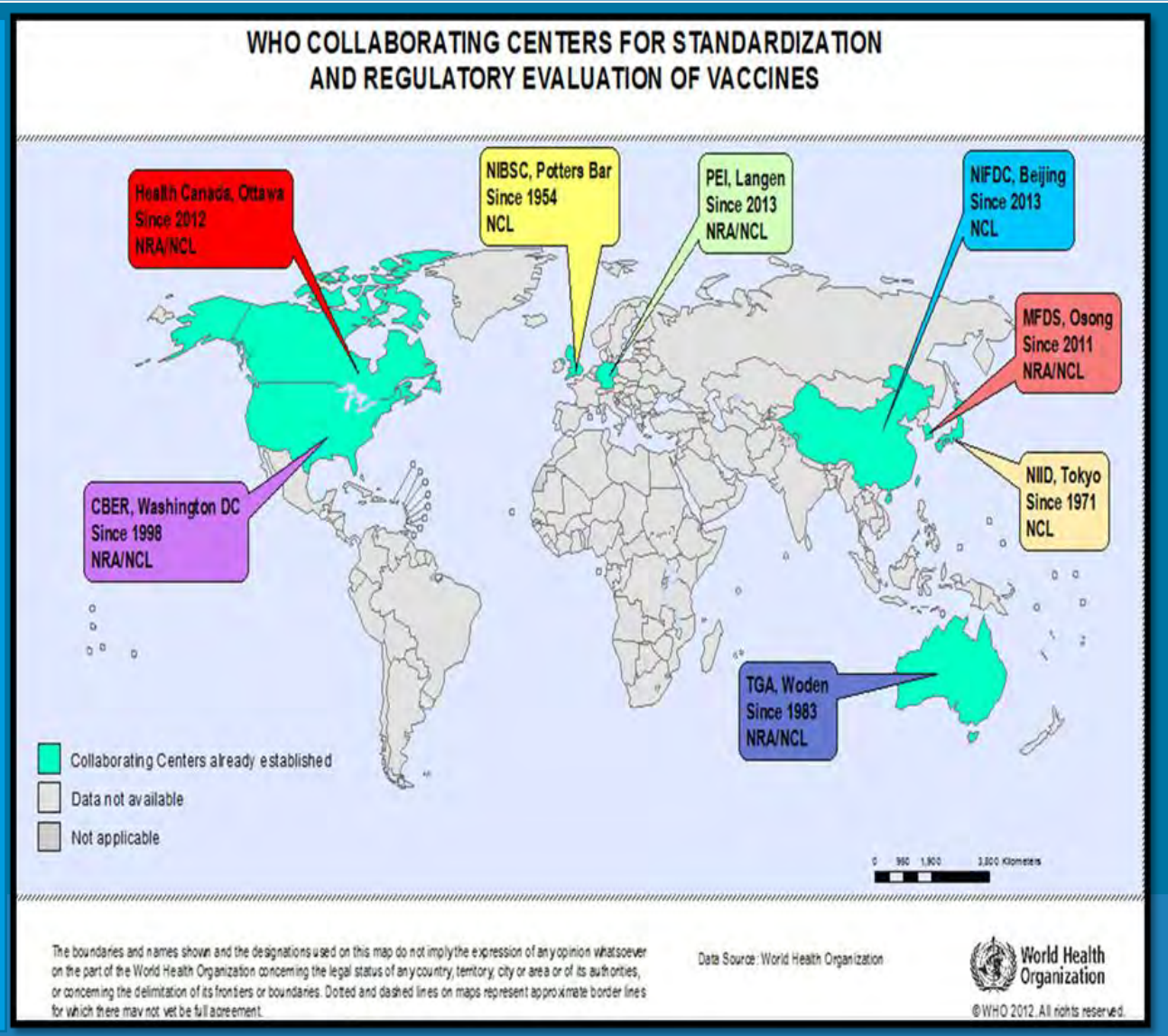
WHO CCs and Custodian Laboratories for Biological Standardization

- **Input provided to the following:**
  - Measurement standards: MHRA (NIBSC) with the input from CCs and other laboratories
    - Vaccines and related substances
    - Biotherapeutics
    - Blood products and related substances
    - In vitro diagnostics
    - Cell and gene therapies
    - PHE (eg, SARS-CoV-2)
    - high-throughput sequencing standards
  - Written standards
  - Implementation workshops

- **CBER re-designation by February 2024**

- WHO CC network for standardization of vaccines – meeting postponed to 2024

- **WHO custodian laboratories for measurement standards: 1) MHRA (NIBSC), 2) PEI, 3) CBER/US FDA and 4) EDQM (antibiotics)**

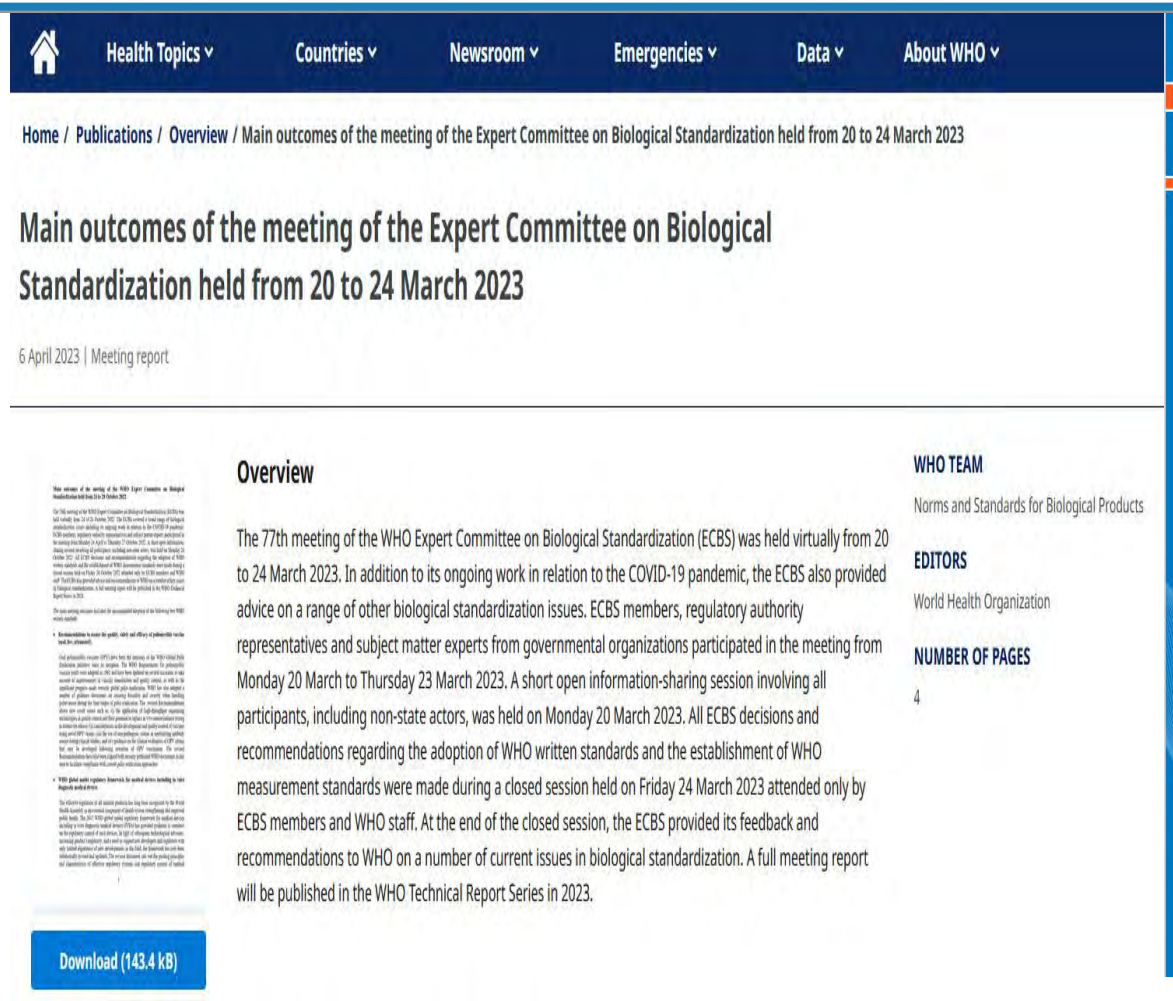


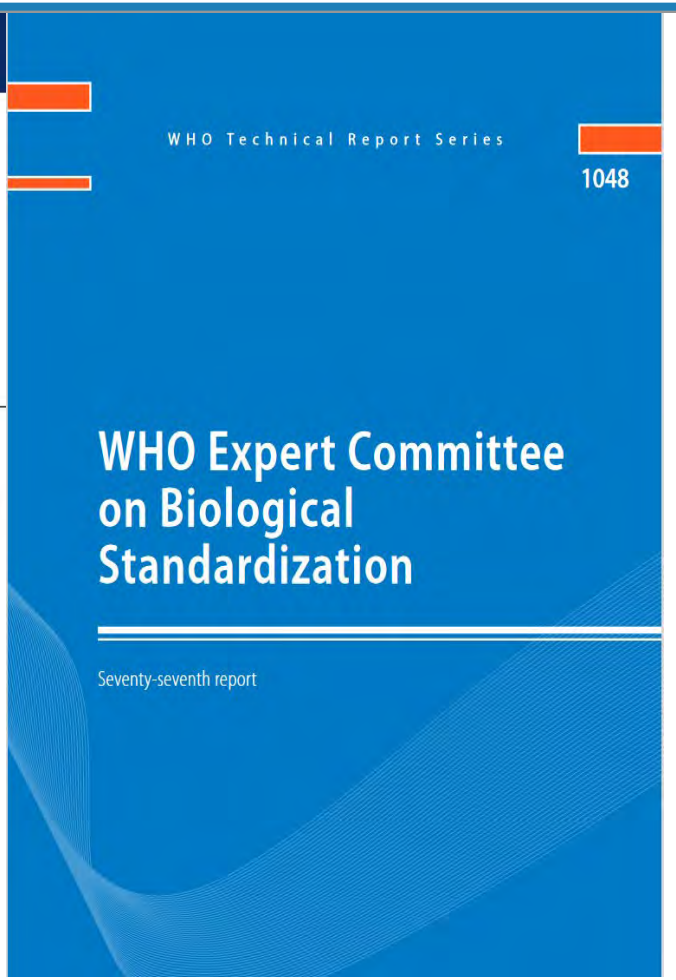
Report of 77th ECBS meeting held on 20-24 March 2023

WHO Technical Report Series 1048

1. Executive Summary published on WHO web site on 6 April 2023: https://www.who.int/publications/m/item/main-outcomes-of-the-meeting-of-ecbs-20-to-24-mar-2023

2. ECBS report (TRS 1048) published on 4 Oct 2023





Main outcomes of 77th ECBS meeting (20-24 March 2023)

World Health Organization

- **2 written standards adopted:**
- **Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases**
- **Considerations in developing a regulatory framework for human cells and tissues and for advanced therapy medicinal products**
- **8 new and 3 replacement WHO International reference preparations established**
- **12 proposals for new standards endorsed**
- In addition, it was recommended to add two further antibody preparations to the First WHO International Reference Panel of antibodies to SARS-CoV-2 variants of concern established at its previous meeting.
- A pilot study to explore the potential utility of a prospective reference reagent for lipid-nanoparticle-encapsulated messenger RNA (mRNA) products was endorsed.

78th ECBS meeting held on 16 - 19 Oct 2023

1. Executive Summary published on WHO web site on 26 October 2023: https://www.who.int/publications/m/item/78th-ecbs-meeting-october-2023

2. ECBS report (TRS) will be published in 2024

Main outcomes of 78th ECBS meeting (16-19 Oct 2023) - 1

World Health Organization

- **1 written standard for vaccines adopted:**
- Guidelines on preparedness for regulatory oversight of vaccines used in pandemics in importing countries
- **6 new and 5 replacement WHO International reference preparations established**
- **7 proposals for new standards endorsed**

- **Additional topics discussed:**
  - Issues arising from the ongoing COVID-19 pandemic:
    - 1) Mab for COVID-19 and 2) convalescent plasma
  - Animal testing in WHO documents
  - A need for discontinuation of written and measurement standards in the near future

Main outcomes of 78th ECBS meeting (16-19 Oct 2023) - 2

World Health Organization

Table 1
WHO international reference materials established by the ECBS in October 2023

Material	Unitage	Status
Biotherapeutics other than blood products		
Alpha-fetoprotein (human)	7800 IU/ampoule	Second WHO International Standard
Follicle-stimulating hormone and luteinizing hormone for bioassay (human, urinary)	177 IU/ampoule FSH 170 IU/ampoule LH	Sixth WHO International Standard
Thyroid-stimulating hormone (human, pituitary)	11.7 mIU/ampoule	Fourth WHO International Standard
Blood products and related substances		
Thrombin activatable fibrinolysis inhibitor (plasma)	Activity: 0.87 IU/ampoule Antigen: 0.92 IU/ampoule Antigen: 7.43 µg/ampoule (expanded uncertainty limits = 7.05–7.82 with k=2 taken to correspond to a 95% level of confidence)	First WHO International Standard
In vitro diagnostics		
Protein S (plasma)	0.71 IU/ampoule activity 0.83 IU/ampoule free antigen 0.88 IU/ampoule total antigen	Third WHO International Standard
Q fever (*Coxiella burnetii*) antibodies (human plasma)	100 U/ampoule for Phase I antigens 16 U/ampoule for Phase II antigens	WHO International Reference Reagent
Standards for use in high-throughput sequencing technologies		
Gut microbiome DNA extraction (whole cell)	No unitage assigned	WHO International Reference Reagent
Standards for use in public health emergencies		
SARS-CoV-2 RNA for NAT-based assays	7.50 $\log_{10}$ IU/ampoule	Second WHO International Standard
Vaccines and related substances		
Nipah virus antibodies for use in binding assays (human serum)	250 IU/ampoule anti-glycoprotein IgG	First WHO International Standard
Nipah virus antibodies for use in neutralization assays (human serum)	250 IU/ampoule	First WHO International Standard
Ross River virus antibodies for use in neutralization assays (human plasma)	500 U/vial	WHO International Reference Reagent

WHO written standards for biologicals: recently adopted and new/ revision under consideration

1. COVID-19 related documents (recent and upcoming):

1.1. Guidelines for assuring the quality, safety and efficacy of plasmid DNA vaccines (TRS 1028, ECBS, Aug 2020)

1.2. Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations (ECBS, Oct 2021)

1.3. Guidelines for the production and QC of mAbs for use in humans – replacement of Annex 3 of WHO TRS 822 (TRS 1043, ECBS, Apr 2022)

1.4. Guideline for the preclinical and clinical evaluation of mAbs and related products for the prevention and treatment of infectious diseases (ECBS, Apr 2023). Disease specific supplements for COVID-19, RSV, rabies, malaria, HIV to be developed in 2023-2025

1.5. Manual for the establishment of national and other secondary standards for antibodies against infectious agents focusing on SARS-CoV-2 (ECBS, Apr 2022) and preparation of the case studies for the implementation workshop in 2023

2. Revision of PIP guidelines (TRS 1004, Annex 7) to expand the scope and to provide guidance for vaccines for pandemic and emergency use **(ECBS, Oct 2023)**

3. Revision of Guidelines for PAC for vaccines (TRS 993, Annex 4) to review reporting categorization of some PACs and to include risk-based approach and reinforce reliance mechanism **(ECBS, Oct 2025)**

4. Revision of Guidelines for rota vaccines (TRS 941, annex 3) – revision due to the experience gained since 2006, new generation rotavirus vaccines **(ECBS, Oct 2024)**

5. Animal use in testing vaccines and biotherapeutic products – Review of the Report on 3Rs in WHO guidelines presented to the ECBS in October 2023 and follow up actions

WHO written standards: revision/new/ implementation under consideration from 2023/ 2024 onwards

I Revision of the following:

1.1. Recommendations for YFV vaccines (TRS 978, annex 5)
1.2. Guidelines for dengue vaccines (TRS 979, annex 2)
1.3. Recommendations for MMR vaccines (TRS 840, annex 3)
1.4. Guidelines for Vaccine Lot Release (TRS 978, annex 2)
1.5. Recommendations for the preparation, characterization and establishment of international and other biological reference standards (TRS 932, annex 2)
1.6. Recommendations for BCG vaccines (TRS 771, annex 12)
1.7. Guidelines for malaria vaccines (TRS 980, annex 3)

II Standardization of enteric vaccines – new documents in coming years

III Implementation workshops:

3.1. Polio vaccines: 31 October – 2 Nov 2023
3.2. Manual for secondary standards: 14 – 16 November 2023
3.3. Cell and gene therapy products: May 2024
3.4. Biosimilars: July 2024

Main outcomes of the WHO PAC meeting, 20-21 Sep 2023, hosted by PEI, Germany (1)

- Agreed to revise the current guideline to meet the need of industry and regulators
  - The potential merge of the PAC guidelines for vaccines and biotherapeutics.
  - It was agreed to revise the paragraph on reliance and recognition.
  - It might be useful to envisage the following cases during the revision:
    - NRAs accept the assessment report and decision of another NRA
    - NRAs accept notification of the decision only (or with data package from industry).
    - NRAs rely on the NRA of the country of origin
  - A defined review period is essential for companies to implement the change in an efficient manner.
  - To have a validation step before the evaluation procedure.

Main outcomes of the WHO PAC meeting, 20-21 Sep 2023, hosted by PEI, Germany (2)

- **Annual strain changes** of flu and COVID-19 vaccines, an expedited review should be taken into account. It was also agreed that there is a need to review the specific sections pertaining to regular strain/variant changes in the PAC guidelines.
- To look at the different appendixes for further refinement and to amend the text where appropriate
  - emphasize on multiple related changes
  - replace the current timelines with a timespan (e.g. for moderate changes 60 days to 3 months)
  - review categorization of some changes.
- To review the categorization of some changes linked to GMP.
- Definition of the implementation date:
  - the implementation date is the date when the change is implemented by the company when a sufficient number of agencies have approved the change.
- The topic of post-approval change management protocol is already mentioned in the guideline but the paragraph could be reviewed and amended if necessary.

Revision of Guidelines to assure the quality, safety and efficacy of live attenuated rotavirus vaccines, TRS 941, Annex 3

- WHO set up drafting group and convened discussions in 2022
- WHO organized broad informal consultation meeting to discuss the revision during 15-17 Nov 2022 – meeting report available on WHO web site
- 1st draft revision was prepared, and circulated among the consultation meeting participants for review during April- May 2023: 16 sets of comments were received
- 2nd draft revision was then prepared and posted on WHO website for **1st round Public Consultation, 21 Jul - 22 Sep 2023**: 13 sets of comments received and analyzed
- Subsequently further draft will be prepared. Target submission to ECBS in June 2024, for review at ECBS in Oct 2024.

Workshop to implement Manual for establishment of secondary standards – case studies

- In Indonesia from 14-16 November 2023
- Participants (33) including regulators, industry from WP, SEA and EM regions
- Facilitators: experienced scientists from WHO CCs and Institutes that are preparing secondary standards
- Case studies on calibration of SARS CoV-2, HPV, RSV antibody standards

Workshop to implement Manual for establishment of secondary standards – participating countries

- Vietnam
- Korea R.
- China
- Malaysia
- Thailand
- India (TBC)
- Indonesia
- Bangladesh
- Saudi Arabia
- Tunisia
- Egypt
- Pakistan
- Iran
- IFPMA
  - GSK
- DCVMN
  - Bharat Biotech International Ltd and Bio-Manguinhos

Biosimilars

Completed: To clarify the data requirements for biosimilars

- **Revision of WHO guidelines, April 2022 (TRS No. 1032)**


Annex 3

Guidelines on evaluation of biosimilars

Replacement of Annex 2 of WHO Technical Report Series, No. 977

- **Article publication, Ann NY Acad Sci 1521, 2023**


DOI: 10.1111/nyas.14965

ORIGINAL ARTICLE

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

WHO guidelines on biosimilars: Toward improved access to safe and effective products

Hye-Na Kang[1] | Meenu Wadhwa[2] | Ivana Knezevic[1] | Clive Ondari[1] | Mariangela Simao[1]

Ongoing & Planning: Implementation of GLs

- **2 topics identified and case studies under development (Dec 2023)**
  - Small molecules (Insulins)
    - CMC and non-clinical aspects (also cover device issue)
    - Clamp trial – the pivotal clinical trial (also cover immunogenicity issue)
  - Large molecules (mAbs)
    - Streamlined evaluation
- **Implementation workshop (July 2024)**
  - In EM including some countries from AF region
  - Review country situation
  - Hand on training for regulators by using the developed case studies
- **Article to be submitted:** The role of WHO international reference standards throughout the product life-cycle of biosimilars **(Oct 2023)**

World Health Organization

3Rs in WHO guidelines – review from 2020 to 2023

Review of animal testing requirements in WHO Guidelines for vaccines and biological therapeutics: a proposal to implement 3Rs principles

UK NC3Rs conducted a review to determine the extent of animal testing recommended in WHO guidelines for lot release purposes

- Determine where in the guidelines that animal methods are recommended for lot release testing, whether in vitro alternative methods may exist and/or if better language surrounding 3Rs should be incorporated into these guidelines
    - WHO Guidelines were reviewed by international working groups (regulators, manufacturers, academics)
- Surveys are also being conducted of manufacturers and NRA/NCLs to understand where there may be hesitancy or reluctance in adopting 3Rs methods for lot release testing
- Regional workshops were organized
- Final report is to provide proposals to WHO ECBS on how to proceed with implementing 3Rs into guidance documents for vaccines and biological therapeutics

Timelines:

- October 2019: Presented to ECBS
- June 2020: Project officially started
- April 2022: Mid-project report to ECBS
- October 2023: Final report to ECBS

ECBS meetings in 2023 and 2024

World Health Organization

- 16-19 October 2023 (hybrid meeting)
  - Preparatory meetings with the ECBS: 14 June, 10 July and 12 Sep 2023
  - Agenda and timetable provided in May, revised and updated
  - Submission of CS reports to WHO by 1 July 2023 and publication of BS documents on WHO web site for PC from mid July to mid Sep and the beg of Oct 2023
- 11-15 March 2024 (virtual meeting)
  - Preparatory meetings with the ECBS: Jan and Feb 2024
  - Submission of CS reports to WHO by 15 Dec 2023 with the aim to publish BS documents on WHO web site for PC from mid Jan to mid Feb 2024

Many thanks to:

World Health Organization

…team (NSB/TSS/HPS/MHP/WHO)

…colleagues in BTT/TSS/HPS/MHP/WHO and RSS/PQT/MHP/WHO

…members of WHO drafting and Working Groups

…colleagues from Collaborating Centers and Custodian Laboratories

…many individual experts

…stakeholders

Further information and contact

Dr Ivana Knezevic (email: knezevici@who.int) on behalf of NSB/TSS team

Biological standardization website:

Expert Committee on Biological Standardization (who.int)

Session III. Use of molecular tests in the QC of OPV and IPV

Overview of molecular methods and their utility in the QC of polio vaccines

Kostya Chumakov

George Washington University

Presentation plan

- History of Oral Polio Vaccine
- Rationale for genetic stability testing
- Monkey test
- MAPREC
- Next Generation (Deep, High-throughput) sequencing
  - Its use as a replacement of MAPREC
  - Potential replacement of animal neurovirulence testing
- Consistency monitoring of sIPV
  - Consensus sequence can be generated by HTS or Sanger methods

Dr Albert Sabin
1904 - 1993

Oral Polio Vaccine (OPV)

- Weakened “attenuated” virus
- Selected from the pre-exiting attenuated variants within wild-type stocks
- Natural route of administration
- Comprehensive immunity
- “Herd” effect through transmission to contacts

Starting from the early 1960s
used throughout the world

(except in Finland, Sweden, and Netherlands)

PROPERTIES OF ATTENUATED POLIOVIRUSES AND THEIR BEHAVIOR IN HUMAN BEINGS*

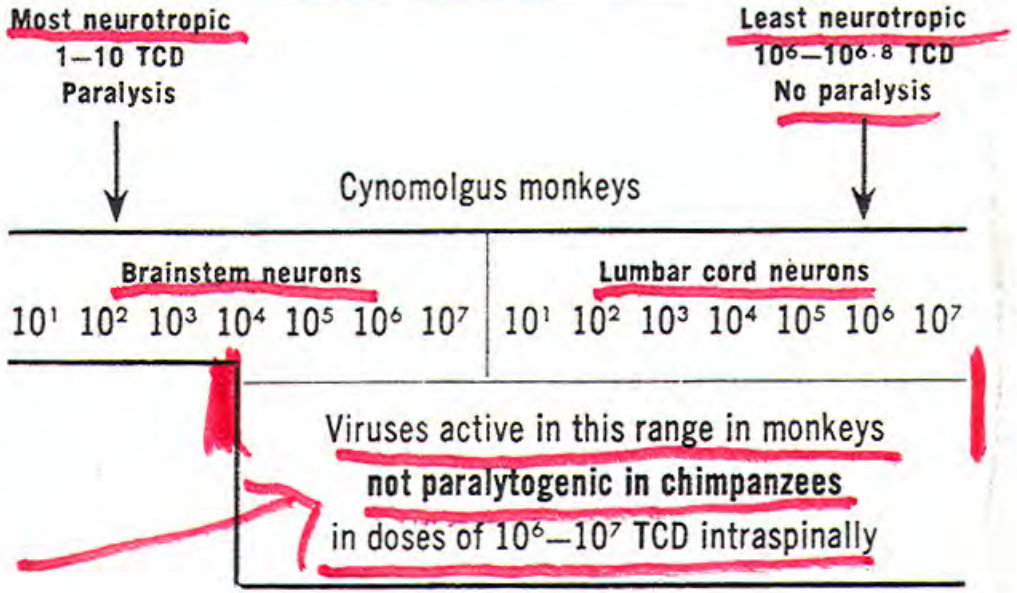
BY

ALBERT B. SABIN

Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio

TABLE 1

NEUROTROPIC SPECTRUM OF KNOWN POLIOVIRUSES IN RELATION TO DIFFERENT PRIMATE NEURONS



- Virus stocks are a mixture of particles with different level of neurotropism.
- Virulent particles replicate to a higher titer but take longer to reach maximum
- Attenuated particles replicate to a lower titer but do it slower

Kostya

– please note

heterogeneous population in Leon KP34 as demonstrated by marked differences in spinal neurovirulence of individual plaque progeny in highly diluted cultures

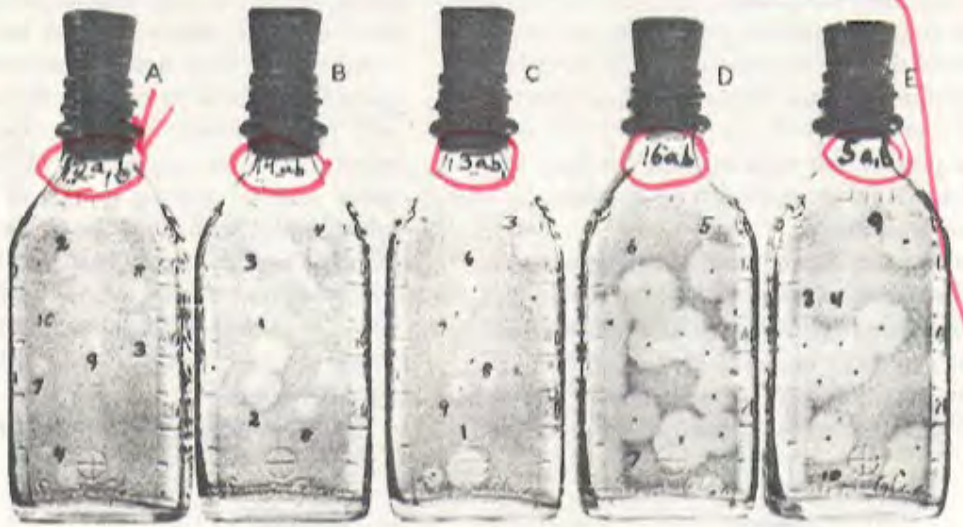
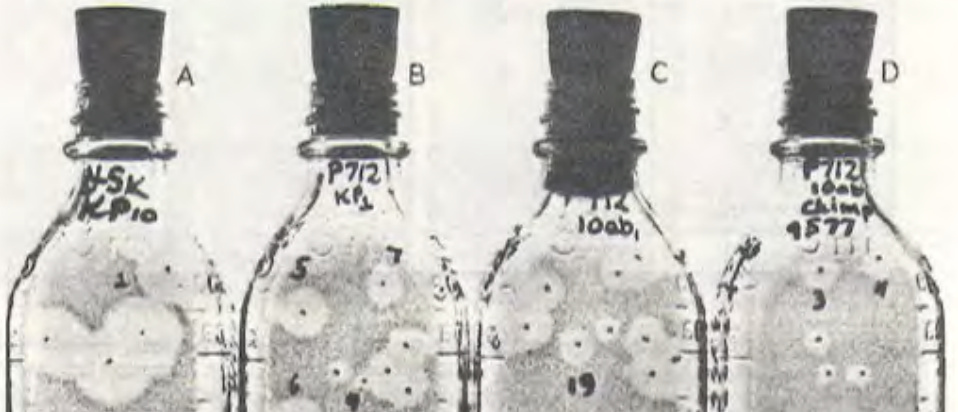


FIGURE 2. The progeny of five different triply purified plaques derived from attenuated Type 3, Leon KP 34 virus previously purified by three terminal dilution passages. All cultures were grown on the same lot of monkey kidney cells and were photographed six days after inoculation. The results of spinal tests in monkeys were as follows:

Dilution of culture fluid	Incidence of paralysis with indicated stock				
	A	B	C	D	E
Undiluted	0/10	0/10	3/5	3/5	5/5
10^{-1}	0/10	0/10	2/5	3/5	4/5

!!

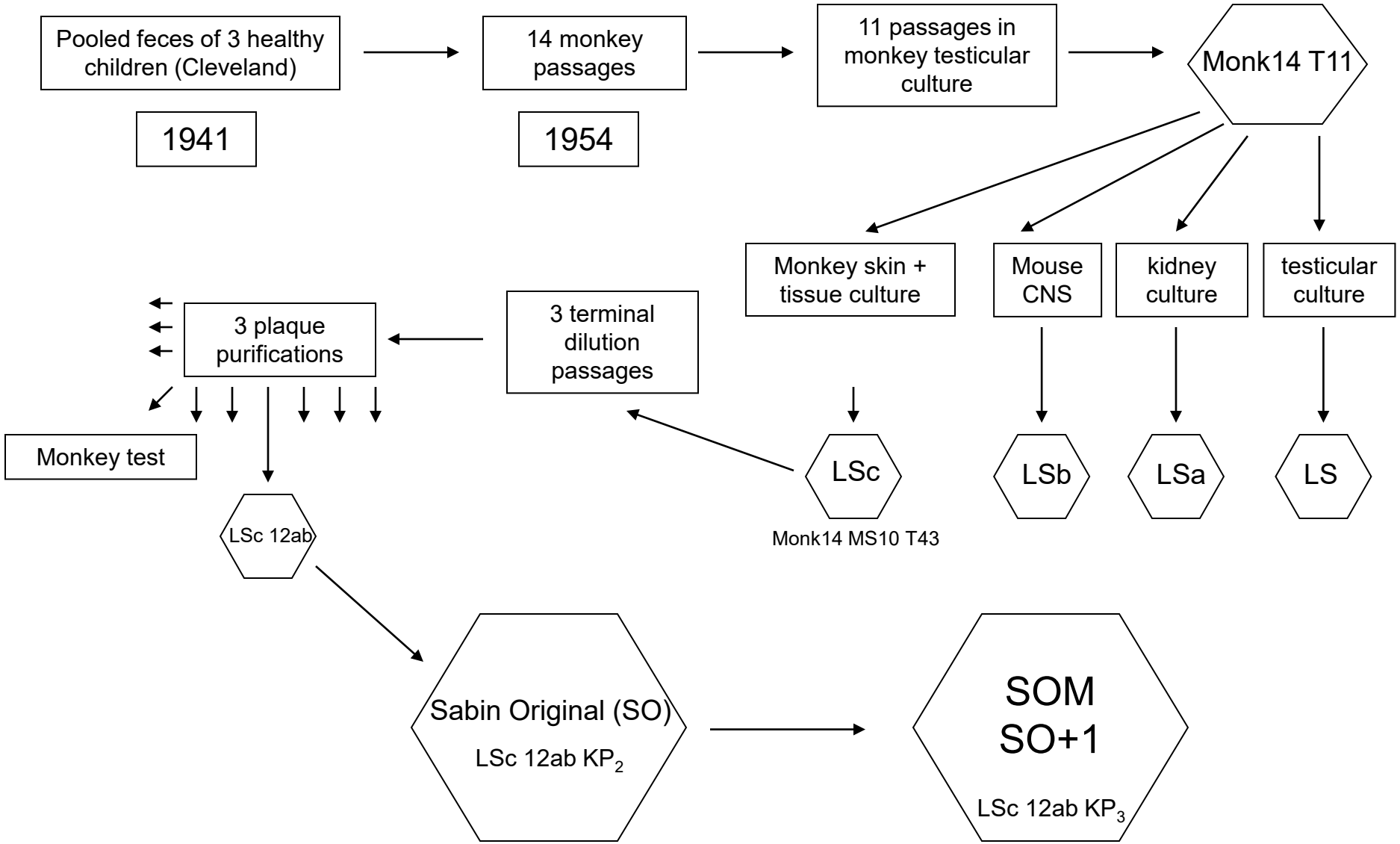


- Virulent particles generate larger plaques than attenuated particles

TABLE 3

THE INFLUENCE OF CONCENTRATION OF $NaHCO_3$ AND pH ON THE YIELD OF INFECTIVE VIRUS PARTICLES FROM MONKEY KIDNEY EPITHELIAL CELLS DESTROYED IN VITRO BY HIGHLY NEUROTROPIC AND LEAST NEUROTROPIC VARIANTS OF TYPE 3 LEON VIRUS*

Concentration of $NaHCO_3$ in medium (per cent)	pH during propagation of virus	PFU/ml. of culture fluid at time all cells were destroyed				Ratio
		Highly neurotropic Leon KP_3		Least neurotropic Leon 12 a_1b		
0.22	8.1 - 8.0 - 8.0 - 7.8	7×10^7	(48)†	2.2×10^7	(61)	3
	7.5 - 7.5 - 7.4 - 7.3	6.8×10^7	(48)	3.3×10^7	(61)	2
0.055	7.4 - 7.0 - 7.5 - 7.3 - 6.8	7.4×10^7	(48)	8×10^6	(91)	9
0.028	7.3 - 6.8 - 7.3 - 6.8 - 7.0	6.6×10^7	(54)	3.2×10^6	(106)	20
0.22	6.4 or less	1.8×10^7‡	(100)	4.2×10^6	(115)	4
0.055	6.4 or less	7.8×10^6	(100)	1.2×10^6	(115) to (161)	6.5
0.028	6.4 or less	3.2×10^6	(120)	1×10^5	(100) to (115)	32



October 10, 1956

Sabin, A. B., and R. L. Boulger. 1973. History of Sabin attenuated poliovirus oral live vaccine strains. J. Biol. Stand. **1:**115–118.

Passage in cell culture and in the gut of vaccine recipients leads to the loss of attenuation

Dr. Sabin required vaccine manufacturers to test every batch of vaccine for neurovirulence in monkeys

WHO Monkey neurovirulence test

To measure residual virulence of Sabin strains

- 24*2=48 monkeys inoculated intraspinally
  - 24 with new vaccine lot and 24 with reference
- Observed for 17 days for signs of paralysis
- All monkeys sacrificed for histological examination
- Lesions in CNS are scored and compared
- Vaccine lot “passes” if lesions are not greater than in reference vaccine
- ~200 monkeys were killed to QC one lot of trivalent vaccine

Monkey neurovirulence test is a product consistency test

- There is no evidence that failure of MNVT leads to unsafe vaccine
- However, it indicates a breach in manufacturing consistency and drift in the direction of higher neurotropism of vaccine virus

- MNVT often yields variable results, is very expensive, takes a lot of time, requires specialized expertise, and is inhumane
- Therefore, there was a strong need to find a surrogate test that could replace MNVT
  - Currently there is an alternative neurovirulence test based on transgenic mice

Increased neurovirulence associated with a single nucleotide change in a noncoding region of the Sabin type 3 poliovaccine genome

D. M. A. Evans*, G. Dunn*, P. D. Minor*, G. C. Schild*, A. J. Cann†, G. Stanway†, J. W. Almond†, K. Currey‡ & J. V. Maizel Jr‡

* National Institute for Biological Standards and Control, Holly Hill, Hampstead, London NW3 6RB, UK
† University of Leicester, University Road, Leicester LE1 7RH, UK
‡ Laboratory of Molecular Genetics, National Institutes of Health, Bethesda, Maryland 20205, USA

Most of the small number of cases of poliomyelitis which occur in countries where Sabin's attenuated poliovirus vaccines are used are temporally associated with administration of vaccine and involve polioviruses of types 2 and 3 (ref. 1). Recent studies have provided convincing evidence that the Sabin type 2 and 3 viruses themselves may revert to a neurovirulent phenotype on passage in man[2–6]. We report here that a point mutation in the 5′ noncoding region of the genome of the poliovirus type 3 vaccine consistently reverts to wild type in strains isolated from cases of vaccine-associated poliomyelitis. Virus with this change is rapidly selected on passage through the human gastrointestinal tract. The change is associated with a demonstrable increase in the neurovirulence of the virus.

Table 1 Base at position 472, time of isolation, neurovirulence and temperature sensitivity of Sabin type 3 vaccine-derived strains of poliovirus

Virus	Base at position 472	Time of isolation after vaccination	Mean histological lesion score	rct marker test*
Sabin vaccine	U		0.36	>5.5(rct⁻)
DM1	U	24 h	ND	ND
DM2	U	31 h	1.58	6.13 (rct⁻)
DM3	U/C	35 h	ND	ND
DM4†	C	47 h	2.48	5.71 (rct⁻)
DM38	C	18 days	ND	ND
DM119	C	3–4 weeks	3.34	0.25 (rct⁺)

Mean histological lesion scores were determined using the standard WHO neurovirulence test[14]. The range of mean histological lesion scores of a type III attenuated refer… strain in eight tests carried out over the past 2 yr is 0.43–0.79.
ND, not determ…
* The rct ma… logarithmic diff… showing reduct… sensitive (rct⁻).
† The sequen… DM4 were dete… was sequenced … RNA, annealin… by the dideoxy… polymerase I[13].

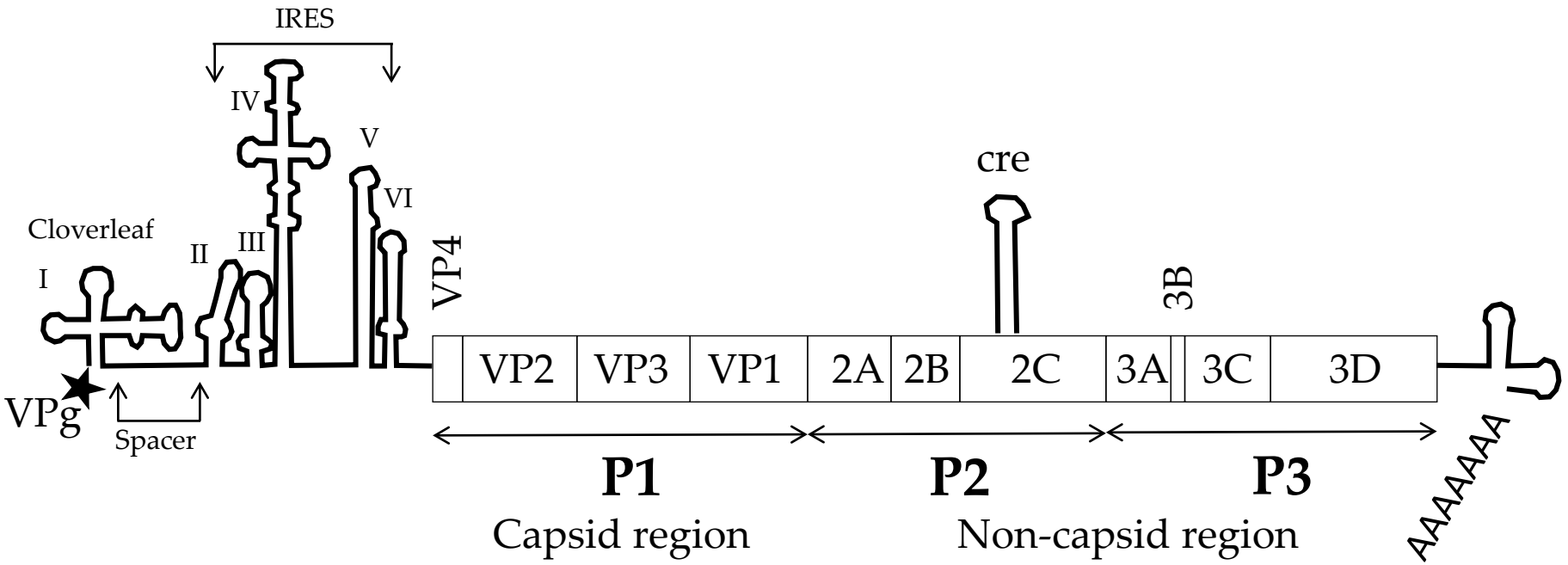
We therefor… to cytidine a… ated cases … phenotype o…
Twelve str…

Table 2 Base at position 472 of the poliovirus genome in primary vaccinees

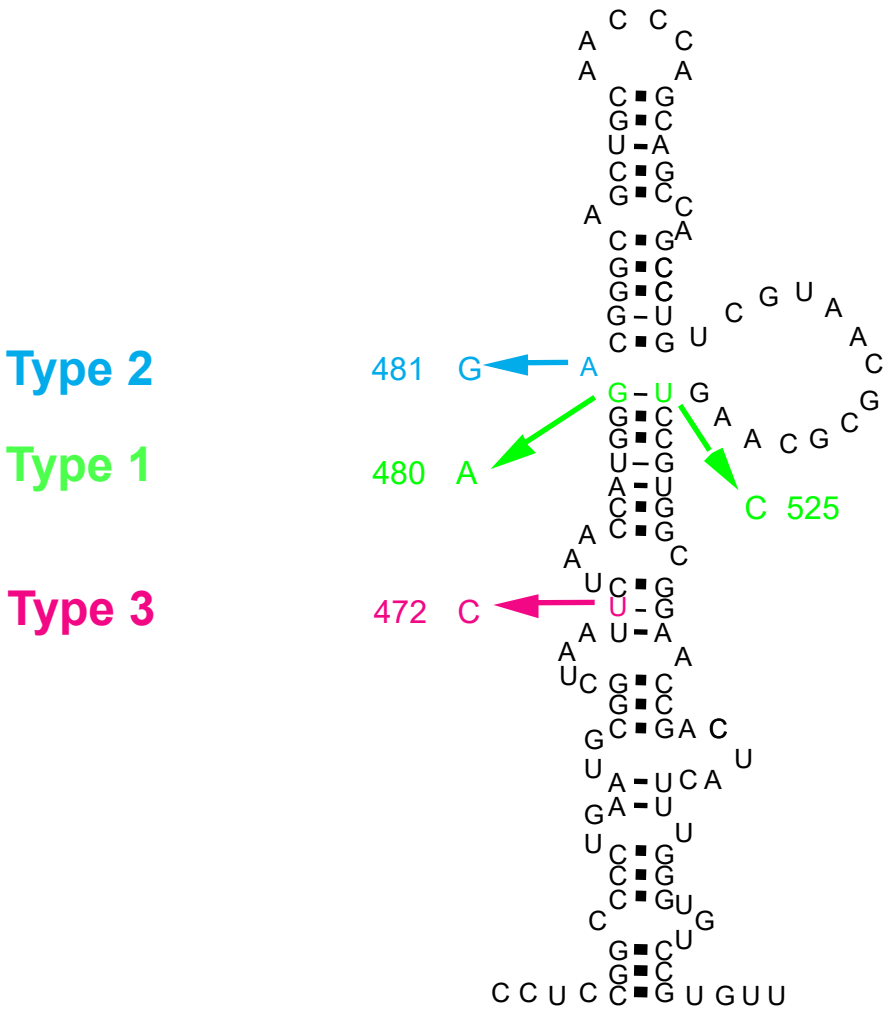
Day post-vaccination	Vaccinee KT1	KT2	KT3
1	U	*	U
2	U/C	*	U
3	C	*	C
4	C	C	C

Faecal samples were taken daily from three vaccinated infants less than 1 yr old, who received vaccine of the same origin as DM (Table 1).
* Isolates of poliovirus type 3 not available.

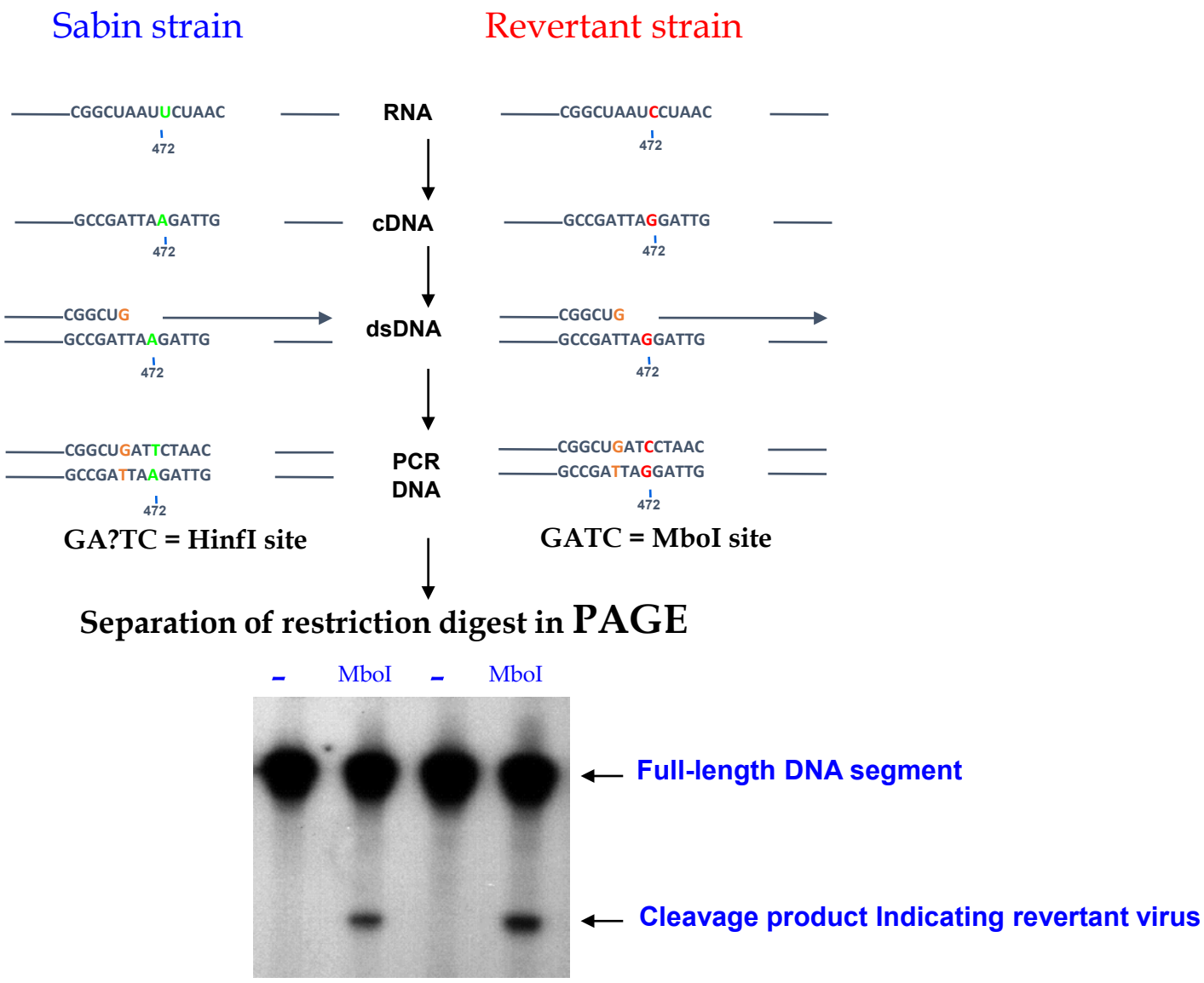
Genome Structure of Enteroviruses



Neurovirulent mutations in domain V of the IRES element



MAPREC assay for neurovirulent revertants in type 3 OPV



Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 199–203, January 1991
Medical Sciences

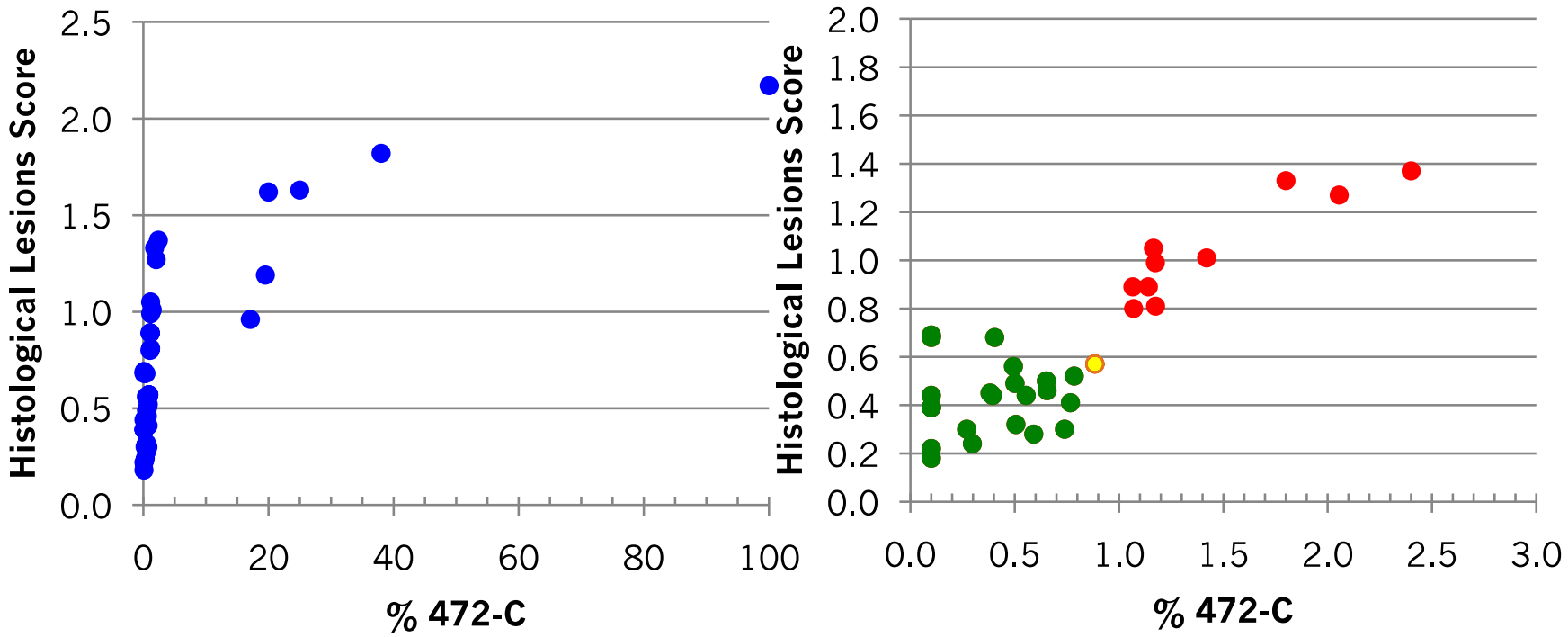
Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine

(attenuation/type 3 poliovirus/polymerase chain reaction/restriction enzyme analysis)

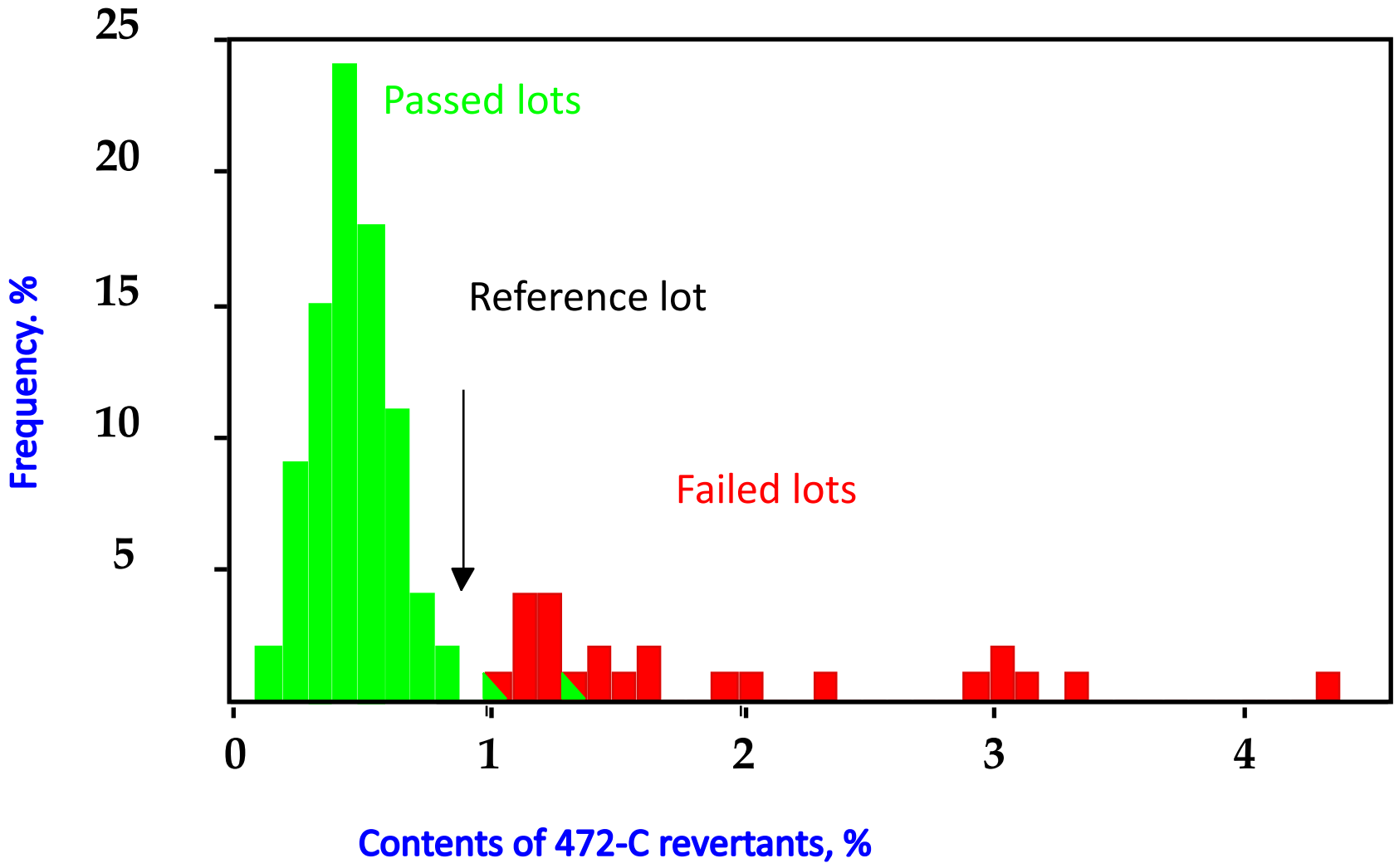
KONSTANTIN M. CHUMAKOV*, LAURIE B. POWERS*, KEVIN E. NOONAN†, IGOR B. RONINSON†, AND INESSA S. LEVENBOOK*

*Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892; and †Department of Genetics, University of Illinois at Chicago, Chicago, IL 60612

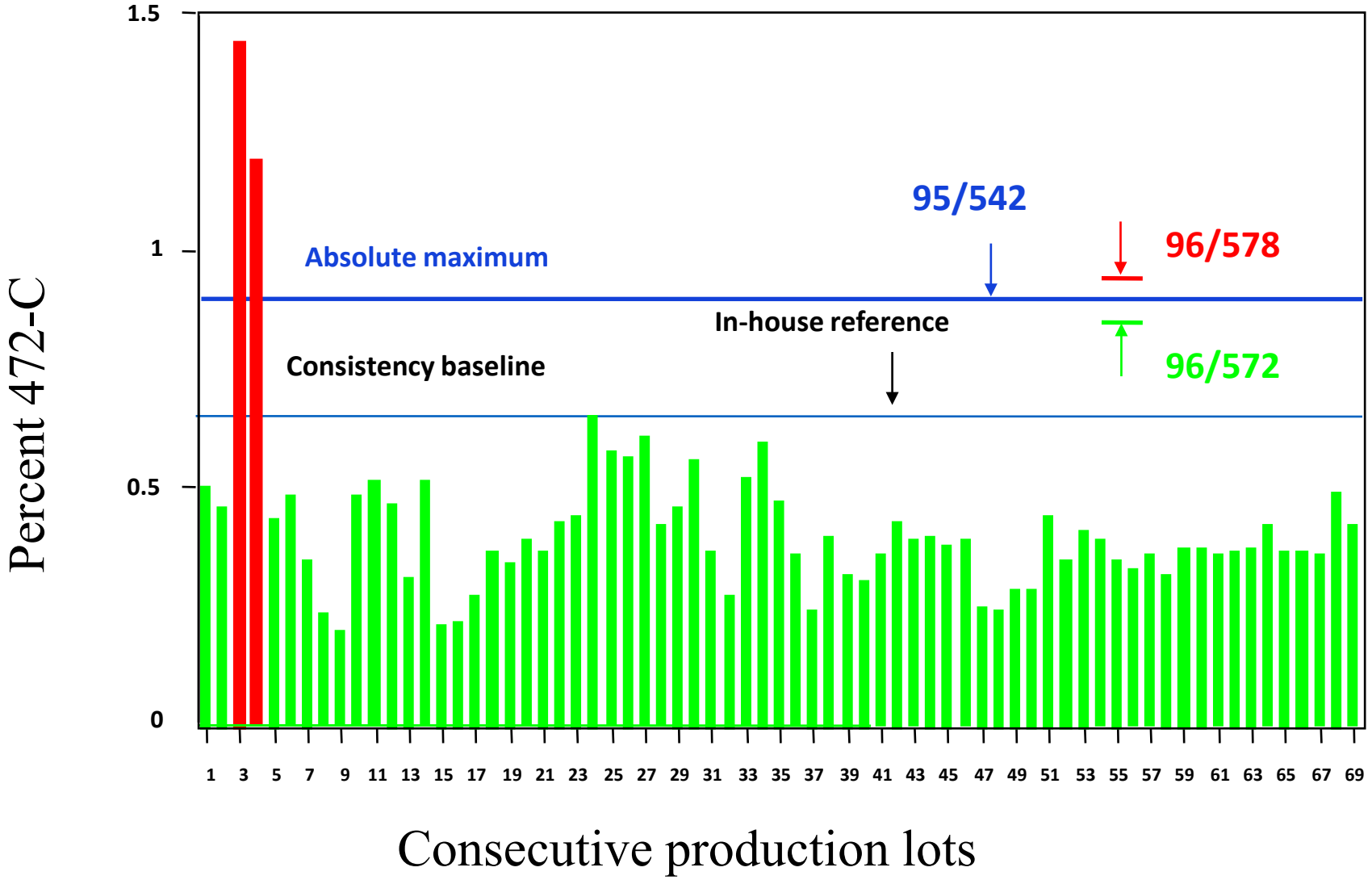
Communicated by Albert B. Sabin, October 10, 1990 (received for review August 16, 1990)



Contents of 472-C revertants in lots of type 3 OPV



Pass-Fail Decision



Regulatory role of MAPREC

- An International Collaborative Studies on MAPREC tests for all three serotypes of OPV were conducted in the 1990s
- WHO Expert Committee on Biological Standardization (ECBS) approved MAPREC as an *in vitro* test of preference for lot release of OPV
- WHO recommendation for manufacture and control of OPV recommend MAPREC in combination with monkey or Tg-mouse neurovirulence test
- If MAPREC is performed rct_{40} marker test can be omitted

Why do we need an alternative to MAPREC?

- MAPREC tests only one genomic position
  - A method testing for all potential mutations would be preferable
- MAPREC test requires a highly skilled personnel and specialized equipment
- MAPREC requires the use of radioactive isotopes
  - An alternative protocol based on fluorescent dyes is available but has a lower dynamic range
- Some labs experience over time an unexplained baseline drift defined by reference materials

www.pnas.org/cgi/doi/10.1073/pnas.1012537107 PNAS | November 16, 2010 | vol. 107 | no. 46 | 20063–20068

Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines

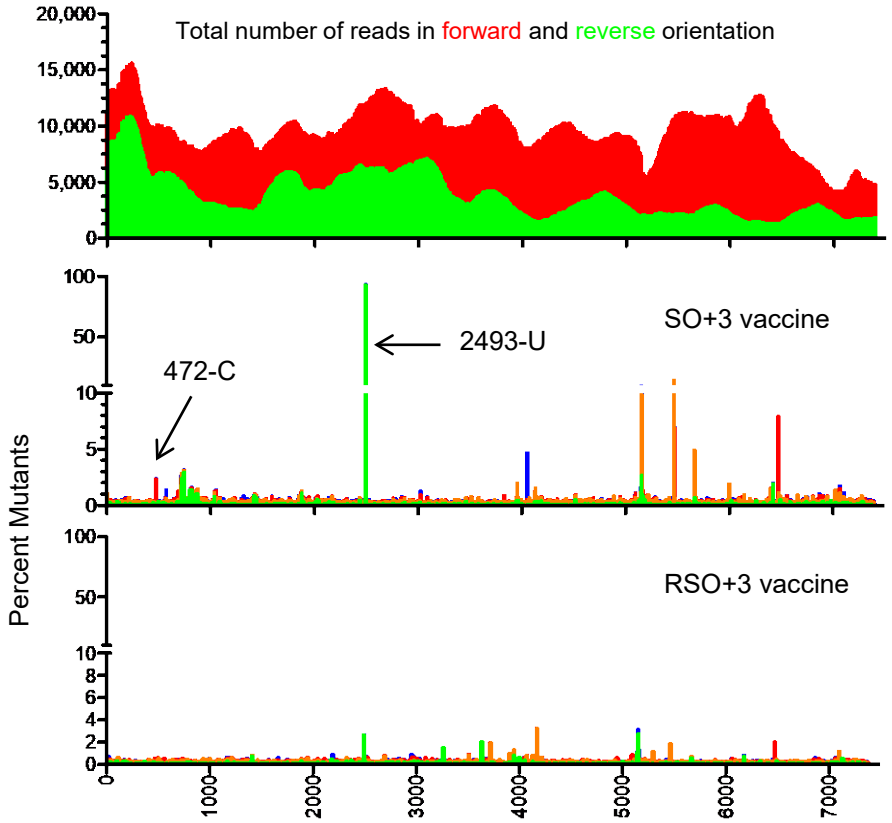
Alexander Neverov and Konstantin Chumakov[1]

Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD 20852

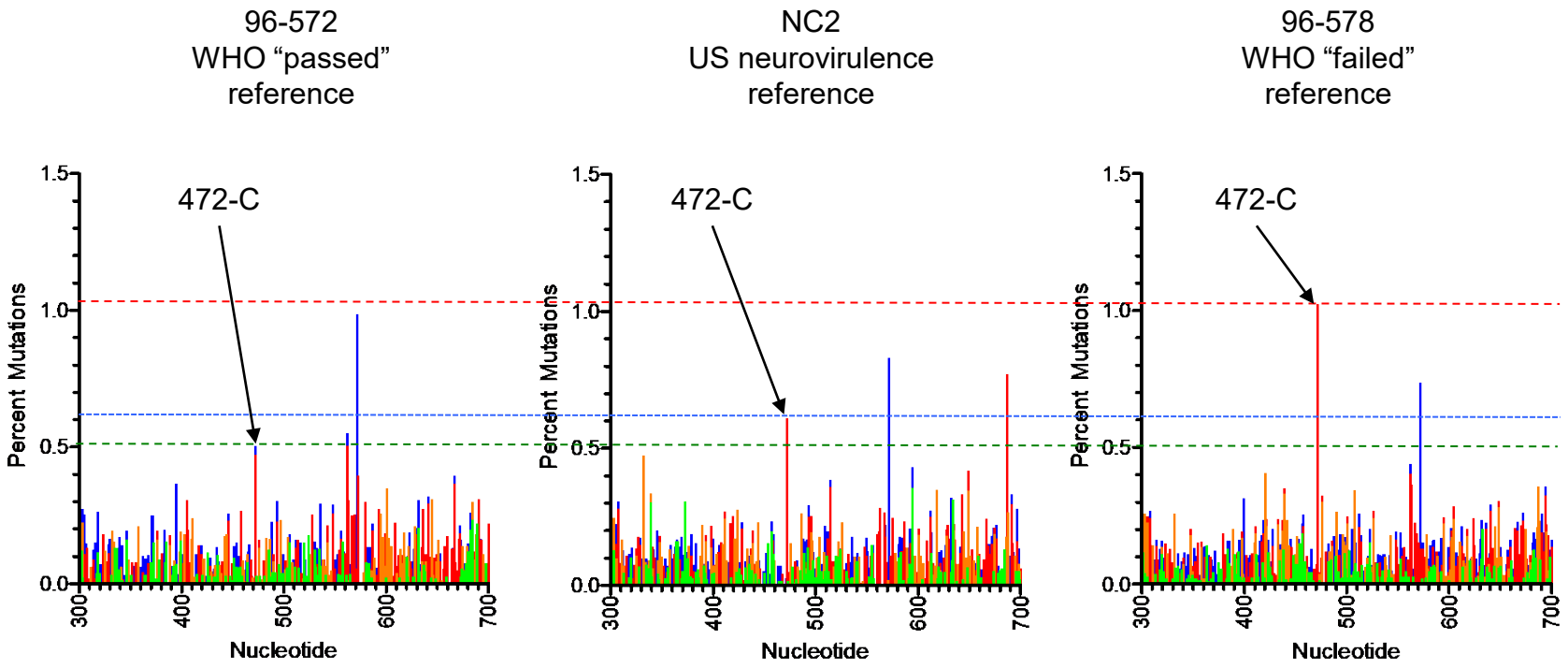
Edited* by Robert H. Purcell, National Institutes of Health, Bethesda, MD, and approved October 6, 2010 (received for review August 24, 2010)

Intrinsic genetic instability of RNA viruses may lead to the accumulation of revertants during manufacture of live viral vaccines, requiring rigorous quality control to ensure vaccine safety. Each

uation. MAPREC is currently recommended by the World Health Organization (WHO) for screening of batches of OPV before they can be released for use in humans (11, 18).



HTS data for type 3 OPV references

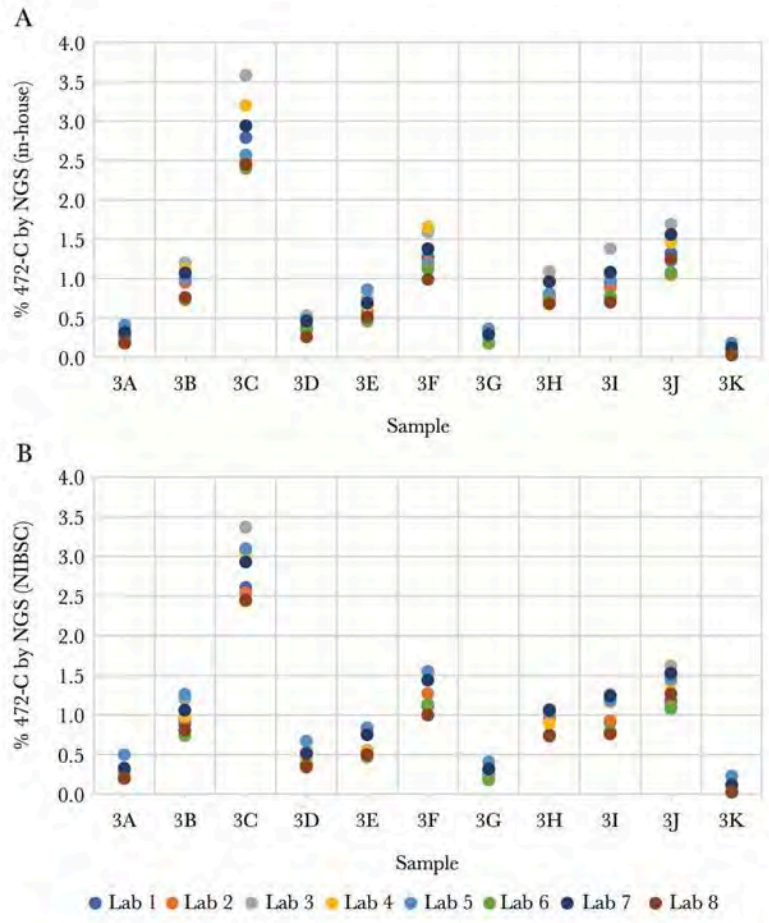


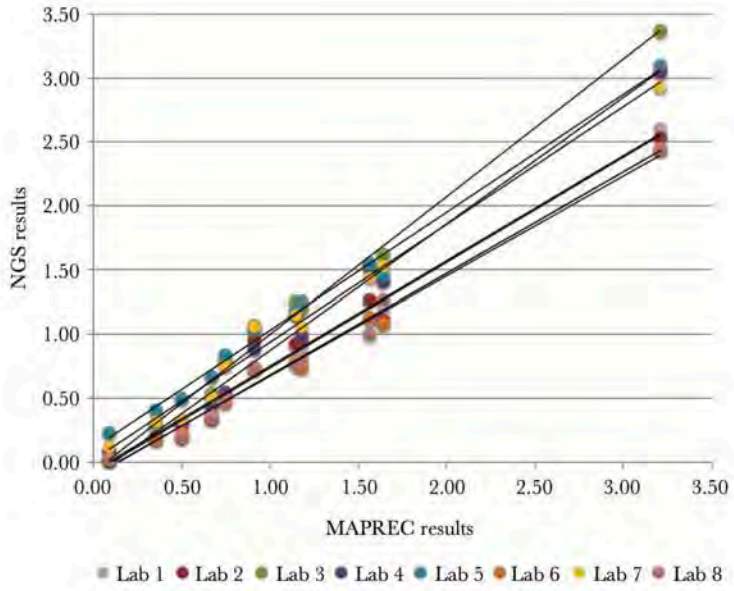
The Journal of Infectious Diseases

ACADEMICSUBJECTS/MED00860

The Use of Next-Generation Sequencing for the Quality Control of Live-Attenuated Polio Vaccines

Bethany Charlton,[1] Jason Hockley,[2] Majid Laassri,[3] Thomas Wilton,[1] Laura Crawt,[1] Mark Preston,[4] NGS Study Group, Peter Rigsby,[2] Konstantin Chumakov,[3] and Javier Martin[1]



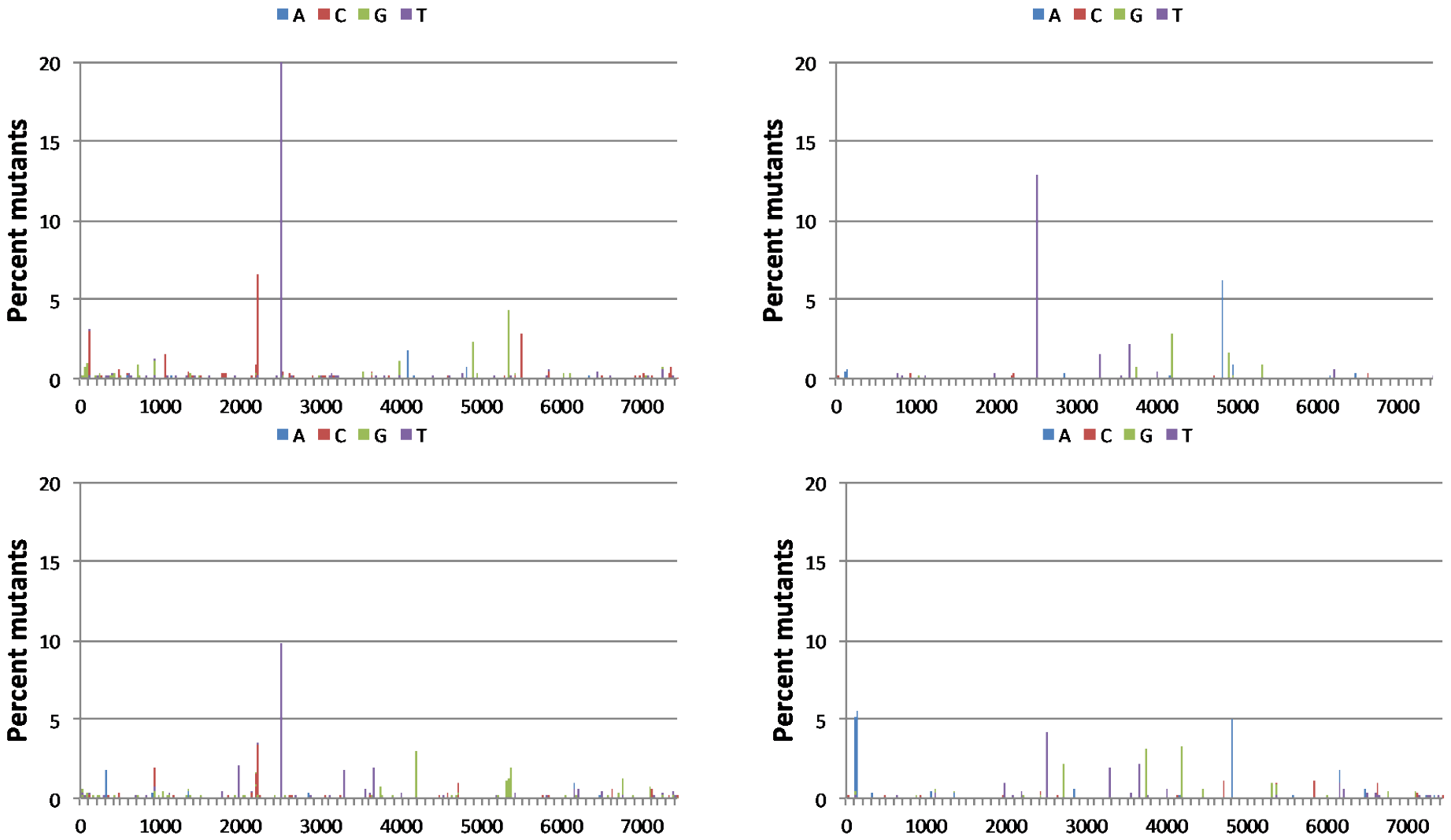


Current status of HTS as a replacement of MAPREC

- Collaborative studies on all three serotypes of OPV demonstrated excellent correlation between the results of MAPREC and HTS
- WHO Expert Committee on Biological Standardization (ECBS) recommended HTS test for 5’-UTR mutations as an alternative to MAPREC
- HTS can be used in conjunction with existing MAPREC reference materials, or new references could be developed for HTS
- Currently PATH in collaboration with NIBSC and CBER/FDA is working on the development and validation of new reference materials
  - A Collaborative Study is scheduled to begin later this or early next year

Can HTS replace animal testing?

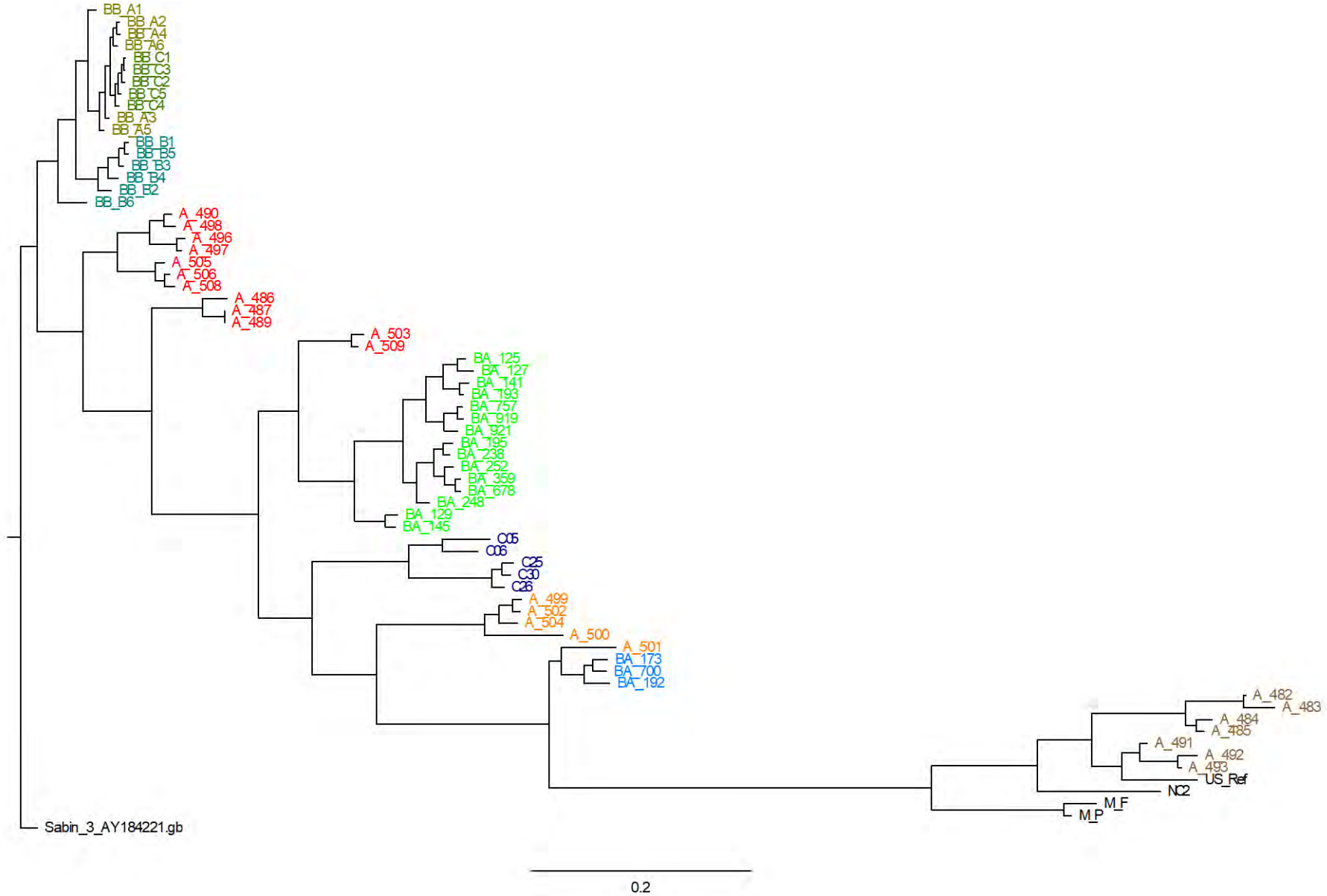
SNP profiles of OPV3 made form different seed viruses



Unique pattern of mutations in vaccines made by difference manufacturers

Nucleotide, Protein, Amino Acid						A	B	C
2189	A->C	VP3	143	Thr->Pro		4.7 ± 1.16	0.4 ± 0.09	0.1 ± 0.10
2493	C->T	VP1	6	Thr->Ile		5.2 ± 2.96	13.0 ± 2.84	3.5 ± 1.48
2696	A->G	VP1	74	Thr->Ala		0.6 ± 0.22	0.1 ± 0.01	6.0 ± 3.43
3723	A->G	2A	116	Gln->Arg		1.4 ± 0.26	1.2 ± 0.15	6.2 ± 2.67
4696	G->C	2C	194	Val		0.7 ± 0.50	0.4 ± 0.14	2.9 ± 0.99
4791	G->A	2C	226	Arg->His		0.5 ± 0.51	5.6 ± 0.69	1.0 ± 2.24
5323	T->G	3A	74	Gly		2.4 ± 1.68	0.2 ± 0.02	0.0 ± 0.02

Comparison of SNP profiles of several OPV products



Alternative bioinformatic methods developed at the University of Haifa will be presented by Dr. Julia Panov
They are used to:

- Identify vaccine lots with SNP profiles that significantly differ from historically established baseline
- Identify nucleotide changes associated with this inconsistency
- Suggest potential phenotypic manifestations of the changes, facilitating interpretation of their significance to make regulatory decisions

What is the purpose of animal testing?

- Monkey and Tg-mouse tests are NOT safety tests, but rather consistency tests
- Contents of 5’-UTR mutations predicts the results of MNVT
- In the last decades no OPV lot failed animal neurovirulence testing
  - All rare cases of the failure were associated with increased levels of 5’-UTR mutations
- If a lot passes MAPREC or HTS test for these mutations, then the only reason for animal testing is to ensure consistency of manufacture
- Mutational profiles of vaccine virus are more informative than MNVT
  - MNVT measures neurovirulence only, which is easier to test by HTS
  - HTS can detect any breach of manufacturing consistency, including potential changes in antigenic characteristics
  - HTS can also serve as an identity test

ECBS agreed that after appropriate validation whole-genome HTS could be considered as a replacement of animal testing

How HTS can be used for lot release?

- During the establishment of OPV production first several batches of vaccine should be tested in animals as well as by generating whole-genome single-nucleotide polymorphism (SNP) profiles by HTS
  - new manufacturer or major change in production conditions, new seed virus, etc.
- After consistency of manufacture is established, only HTS can be used
- If a breach in consistency occurs:
  - Careful review of the specific sequencing data should be conducted
  - Based on the results, animal testing should be performed
  - If the result of animal testing is acceptable, the SNP database is updated

Can HTS be used to monitor consistency of sIPV production?

- Sabin virus is inactivated and therefore the presence of small amounts of neurovirulent mutants is not critical
- Complete reversion of Sabin strains is undesirable because of the biosafety concerns
- Mutations can also affect antigenic sites, so molecular consistency is important
- Consensus sequence of vaccine virus is sufficient to prove consistency
- Both Sanger and HTS methods could be used to generate whole-genome consensus sequences
  - HTS is preferable as it is easier and faster than Sanger method

Acknowledgements

• **Alex Neverov**, *CBER/FDA*	First use of HTS for OPV QC
• **Majid Laassri**, *CBER/FDA*	HTS data generation
• **Tatiana Zagorodnyaya**, *CBER/FDA*	HTS data generation
• **Leonid Brodsky**, Haifa University	Bioinformatics
• **Julia Panov**, Haifa University	Bioinformatics
• **Javier Martin**, *NIBSC*	Collaborative studies
• **Manasi Majumdar**, *NIBSC*	Collaborative studies
• **Kutub Mahmood**, *PATH*	Support and coordination

Thank you

Health Canada Santé Canada

Canada

Recommended use of HTS in current WHO TRS for the control of polio vaccines

Tong Wu, Ph.D.
Vaccine Quality Division 3, Health Canada
Oct 31 – Nov 2, 2023, Jakarta, Indonesia

List of current WHO TRS concerning the quality, safety and efficacy of poliomyelitis vaccines

1. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated), Annex 3, TRS No 993 (https://www.who.int/publications/m/item/poliomyelitis-vaccines-inactivated-annex-3-trs-no-993)

2. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated), Annex 3, TRS No 1024 (Amendment to Annex 3 of WHO Technical Report Series, No. 993) (https://www.who.int/publications/m/item/poliomyelitis-vaccines-annex-3-trs-no-1024)

3. Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated), Annex 2 TRS No 1045 (https://www.who.int/publications/m/item/recommendations-to-assure-the-quality--safety-and-efficacy-of-poliomyelitis-vaccines-(oral--live--attenuated)--annex-2)

Definition of High-throughput sequencing (HTS)

- Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)

  A next generation sequencing (NGS) technology based on sequencing of individual nucleic acid molecules that allows each nucleotide to be sequenced multiple times (massively parallel or deep sequencing), thereby enabling the detection and quantitation of sequence heterogeneities including single nucleotide polymorphisms (SNPs).

- HTS is also known as deep sequencing or next generation sequencing

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

- Tests for adventitious viruses in cell cultures
  - New molecular methods with broad detection capabilities are being developed for the detection of adventitious agents. These methods include …c) **high-throughput sequencing**. These methods might be used in the future to supplement existing methods or as alternative methods to both in vivo and in vitro tests after appropriate validation and with the approval of the NRA (A.3.1.3.2).
- Additional tests on seeds from attenuated strains
  - Suitable in vitro tests should be performed on the master seed from attenuated strains derived by recombinant DNA technology. The tests may include full genome characterization by nucleotide sequencing or **deep sequencing** techniques and demonstration of genetic and phenotypic stability on passage under production conditions. (A.3.1.3.3)

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

- Identity test for virus seeds and single harvest
  - The strain identity of each of the three serotypes may be determined by standard or **deep nucleotide sequence** analysis or by a suitable molecular technique (A.4.3.3).
- Additional tests for purified monovalent pools produced from attenuated poliovirus
  - Suitable in vitro tests should be performed on purified monovalent pools derived from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or **deep sequencing** techniques (A.4.4.2.7).

Amendment to Annex 3 of WHO Technical Report Series, No. 993 (Annex 3, TRS No 1024)

- Additional tests on seeds from attenuated strains
  - Suitable in vitro tests should be performed on the master seed produced from attenuated strains derived by recombinant DNA technology. Such tests may include full genome characterization by determining consensus nucleotide sequences, or **deep sequencing** techniques and demonstration of genetic or phenotypic stability on passage under production conditions (Revised section A.3.1.3.3).
- Additional tests for purified monovalent pools produced from attenuated seeds
  - Suitable tests should be performed on purified monovalent pools produced from attenuated strains derived by recombinant DNA technology. Tests may include full genome characterization by nucleotide sequencing or **deep sequencing** techniques (Revised section A.4.4.2.7).

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)

- Tests for virus strains
  - In addition, it is recommended that the presence of sequence heterogeneities across the entire genome of OPV is determined by **HTS** and documented as a reference for future characterization of the virus seed lots (A.3.2.1).
- Tests on virus master, sub-master and working seed lots
  - New virus seed lots used for OPV production should be evaluated for molecular consistency using a suitable test, **such as HTS**, and should meet the acceptance criteria approved by the NRA (A.3.2.3).
  - Tests for adventitious viruses in cell cultures (A.3.2.3.1)
- in vitro test for the control of monovalent bulk
  - The MAPREC assay may be replaced by alternative molecular biology methods (**such as HTS**) that demonstrate an equivalent or better level of sensitivity following validation, and with the approval of the NRA. The current MAPREC reference materials might also be useful for HTS assays for Sabin OPV upon suitable validation. Alternatively, new reference materials might be needed for this purpose (A.4.4.7.1.2).

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)

- Neurovirulence tests for Sabin OPV
  - It is possible that the in vivo neurovirulence test could be omitted in the future when manufacturing consistency has been established based on the results of both in vivo and whole genome **HTS**. However, additional experience and data are required to establish suitable acceptance criteria for whole genome HTS performed for the control of Sabin OPV (A.4.4.7.2).
- Neurovirulence tests for nOPV
  - Where the results of manufacturing, preclinical and clinical studies have demonstrated the genetic stability of the attenuation to the satisfaction of the NRA, the in vivo MNVT may be omitted for routine manufacturing control of nOPV with the agreement of NRA.
  - Only monovalent bulks that meet the acceptance criteria using a validated **HTS** are used to formulate the final product.
    (A.4.4.7.3)

HTS method – Challenges

- **Setting acceptance criteria**
  - The acceptance criteria for percentage of mutations at positions found to be variable under the conditions used by the manufacture should be based on the molecular characteristics of vaccine batches shown to be safe and immunogenic in clinical studies, or vaccine batches that have met the acceptance criteria of an in vivo NVT. When mutations arise at additional positions, a risk assessment should be performed to assess the potential impact on neurovirulence based on current understanding of the genetic basis for the attenuation (A.4.4.7.3).
- **Method validation and control**
  - Where HTS method is used it should be validated using appropriate standards and materials, and acceptance criteria approved by the NRA. At this point the use of HTS remains a work in progress and is a subject of international collaborative study that may result in the establishment and availability of appropriate reference materials with defined acceptance criteria (A.3.2.3.2).

Summary

- HST can be used:
  - to supplement existing methods or as an alternative for the detection of adventitious agents.
  - to confirm the identity of attenuated virus seeds.
  - to demonstrate genetic and phenotypic stability on passage of virus seeds under production conditions.
  - to monitor molecular consistency of virus seeds and monovalent bulk derived from attenuated poliovirus.
  - potentially, to replace the in vivo neurovirulence tests for the control of poliomyelitis vaccines (oral, live, attenuated) for routine manufacturing control *(ongoing study to validate).*

Alternatives to Animal Testing for Vaccine Release

– *Use of HTS as alternate for animal neurovirulence testing release testing and genetic stability for polio vaccines*

Kutub Mahmood, PhD
Scientific Director, CVIA, PATH

WHO workshop on “Implementation of international standards for the quality control of polio vaccines including OPV and IPV

31 October- 2 November 2023
Jakarta, Indonesia

PATH

Background on Animal NVT

- Animal Safety Testing for neurovirulence performed routinely in the manufacturing process for live attenuated OPV (including novel OPVs)
- Large number of animals used in NVT for routine vaccine safety testing. Very expensive
- NVT is performed in monkeys (M-NVT) or polio-virus receptor transgenic mice (Tgm-NVT).
  - ~30 monkeys or 80 Tg mice are sacrificed for QC release of one lot of OPV
- Monkeys use restricted in some countries, and only one supplier of Tgm mice.
- Need highly trained expertise in intra-spinal inoculations. Very few labs can perform NVT
- Replacement of animal testing with *in vitro* high throughput sequencing (HTS) also referred to as Next Generation Sequencing (NGS) assays is highly desirable and recommended.

PATH

1. Background on the HTS Project for OPV and IPV for Vaccine Lots Release Testing

- Grant from BMGF (INV-017623) in Nov 2020 for HTS use in replacing animal NVT
- Virtual Workshop conducted over three days Apr 12th, 14th and 20th 2021 (~120 representatives from 32 organizations (manufacturers, national authorities, and other testing laboratories)
- Conducted a global survey with vaccine manufacturers and NCLs to gauge interest in participating in this project (overwhelming a 'yes' response) May 2021
- Submission of concept note to WHO ECBS on use of HTS for OPV/IPV based on the previously completed HTS collaborative study with Sabin type 3 - submission in June 2021
- WHO-ECBS meeting in Oct 2021 feedback of the effort from meeting summary as below:

…….. a collaborative study will be required, specifically on reference reagent generation for NGS testing. ……

- Under a PATH MTA agreement, vaccine material bulks received from some (n=4) polio vaccine manufacturers for this efforts and send to collaborators (MHRA and CBER-FDA).

PATH

2. Background on HTS Project for OPV and IPV for Vaccine Lots Release Testing

- Establishment of agreements with NIBSC(MHRA) and CBER-FDA in March 2022
- A second concept note to WHO ECBS on use of high-throughput sequencing (HTS) technologies in the quality control testing and release of vaccine materials - submission in June 2022
- WHO-ECBS meeting in Oct 2022 and endorsement of the effort as below:

During the current meeting, the utility of high-throughput sequencing (HTS) technologies in the quality control of vaccines was extensively discussed……. The ECBS recognized that such approaches will have far-reaching implications for ensuring the quality, safety and efficacy of all live attenuated vaccines…… (ECBS meeting summary Nov2022)

- Bioinformatics Tools development work initiated with University of Haifa, Tauber Center, under a contract with PATH. Subcontract agreement in Q1-2022
- Planning and execution of collaborative study led by MHRA.
- Bioinformatics Training workshop (planned following study completion)

PATH

Animal NVT safety testing for OPVs

Intermediate	Animal Neurovirulence			
	Until 2002	**2002-2013**	**2013 onwards**	**202x**
MVS	MNVT	MNVT	MNVT	HTS
WVS	MNVT	MNVT	TgmNVT	HTS
Monovalent bulk	MNVT	TgmNVT / MAPREC	TgmNVT, MAPREC	HTS

WHO TRS RECOMMENDATION:- All OPV producers should generate HTS data to support with the replacement of NVT with HTS, with acceptance criteria, reference controls established

PATH

World Animals Day- Oct 4th

St. Francis of Assisi
Died 3rd Oct 1228

A patron with great love of animals


PATH

Key takeaways from the Virtual Workshop – Apr 2021

Key takeaways from this workshop meeting included the following:

- With an NGS proof of concept now in place, the next step is to build a consensus for how to operationalize and refine the interpretive approach, methods, sequencing of files, etc., Specifically, the workshop attendees felt that the highest priority was to complete the NGS replacement of the restriction enzyme cleavage (MAPREC) assay by establishing clear analytical process with reference reagents development and standard to reach pass-fail decisions.
- Attendees requested a training workshop on how to use the NGS platform once development is complete.
- For the longer-term application of using NGS to replace routine animal neurovirulence tests, further development will be needed, as whole genome NGS evaluates complete genomic positions as opposed to MAPREC and may show more variants. Therefore, it will be important to lean on historic OPV data.
- Manufacturers with NGS experience indicated that there is a need for a revision of the OPV product-based WHO Technical Report Series (TRS# 980) to include language for use of NGS. In addition, they indicated monkey neurovirulence test (MNVT) and NGS data do not always align, but both fell well within the reference limit.
- Defining the limits of what is allowable versus what constitutes a problematic breach of consistency is a challenge. Some potential ways to define these limits were identified but will require continued manufacturer engagement.

PATH

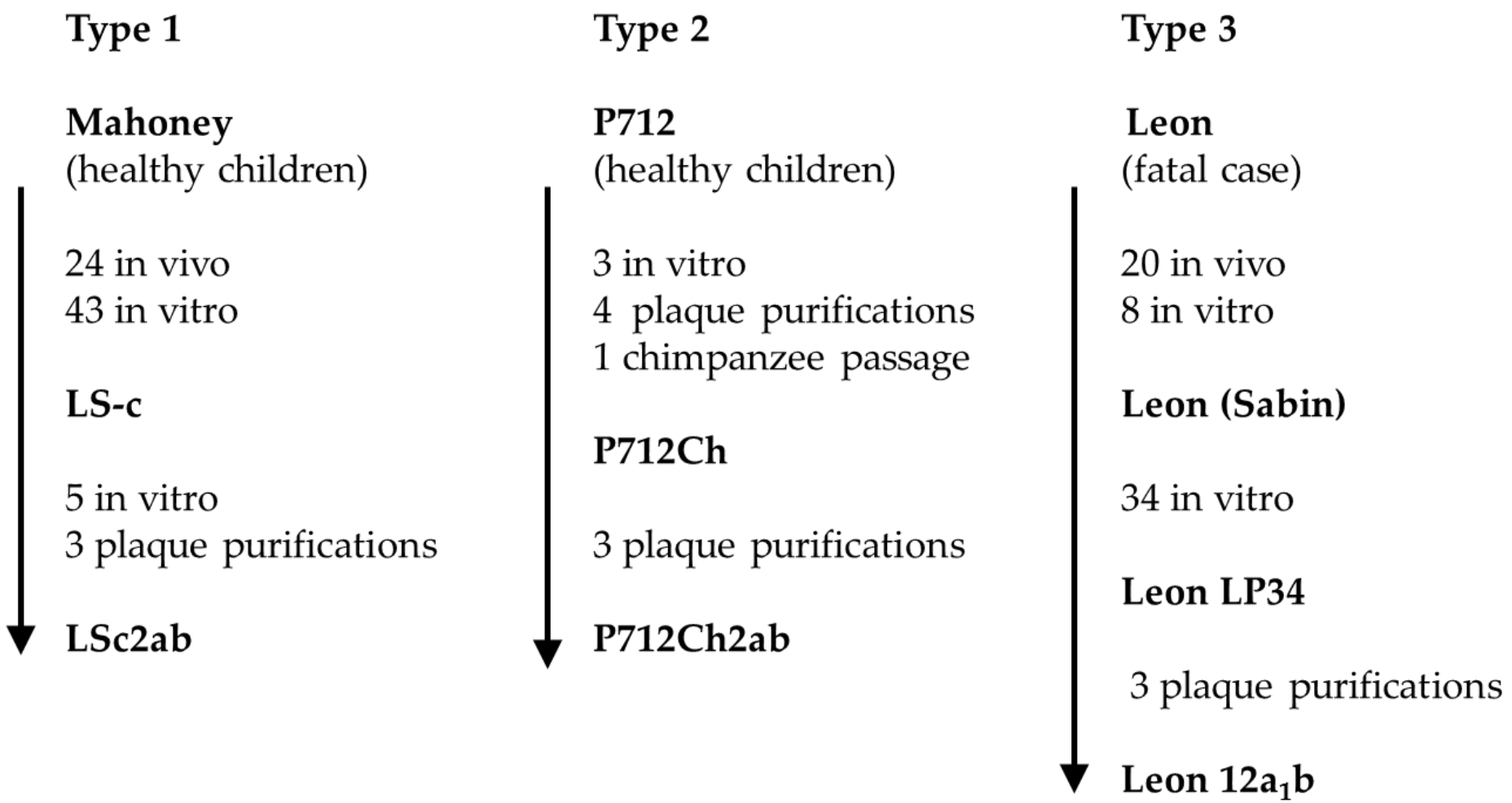
Medicines & Healthcare products Regulatory Agency

Ongoing effort towards HTS standardization for QC of polio vaccines

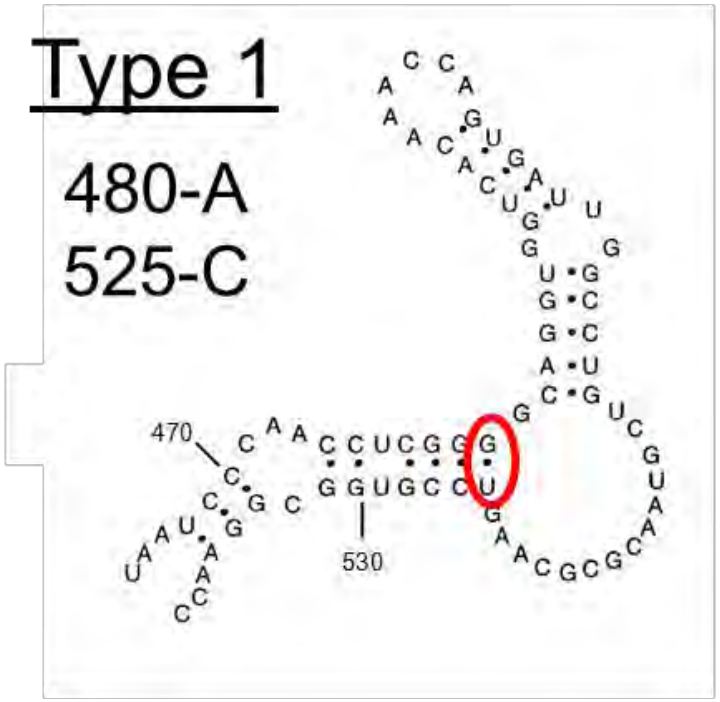
WHO workshop on implementation of international standards for
The quality control of polio vaccines including OPV and IPV
31 October- 2 November 2023
Jakarta, Indonesia

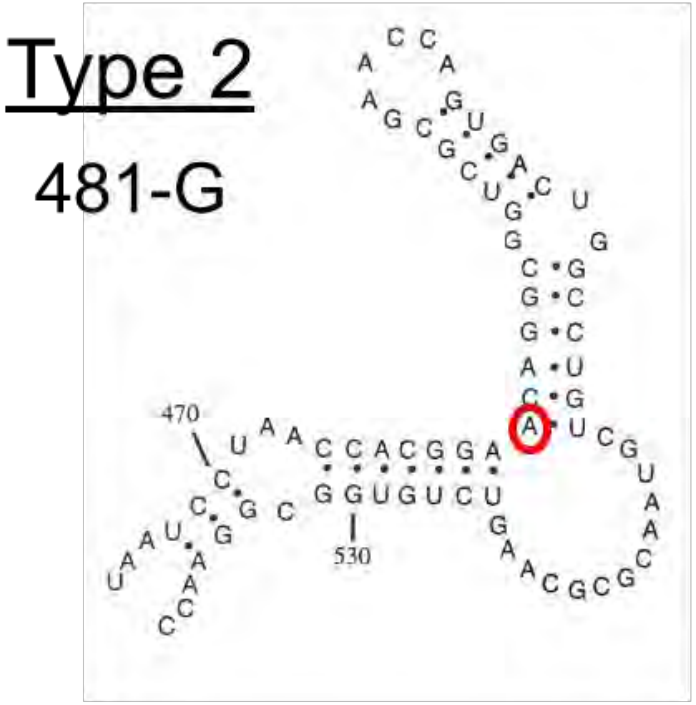
Javier Martin

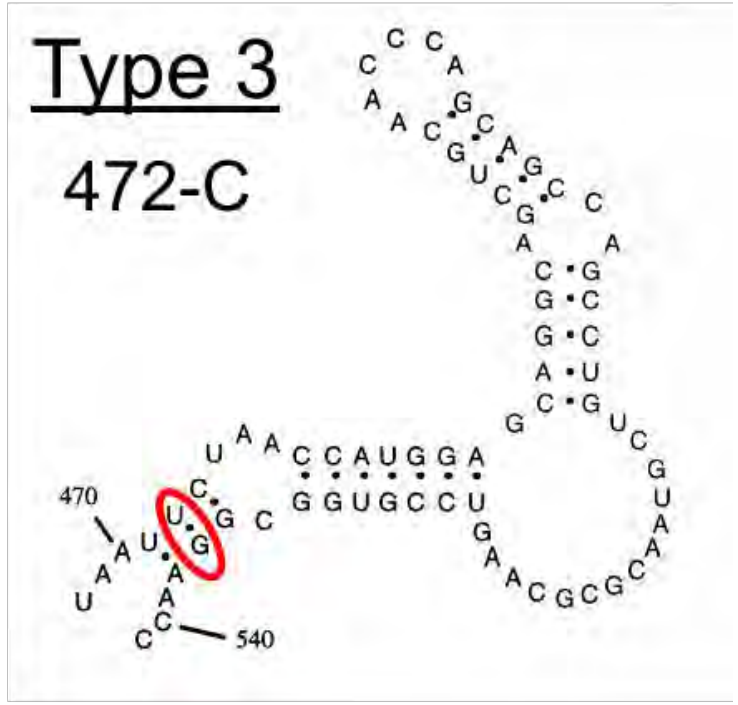
Development of Sabin live-attenuated OPV strains



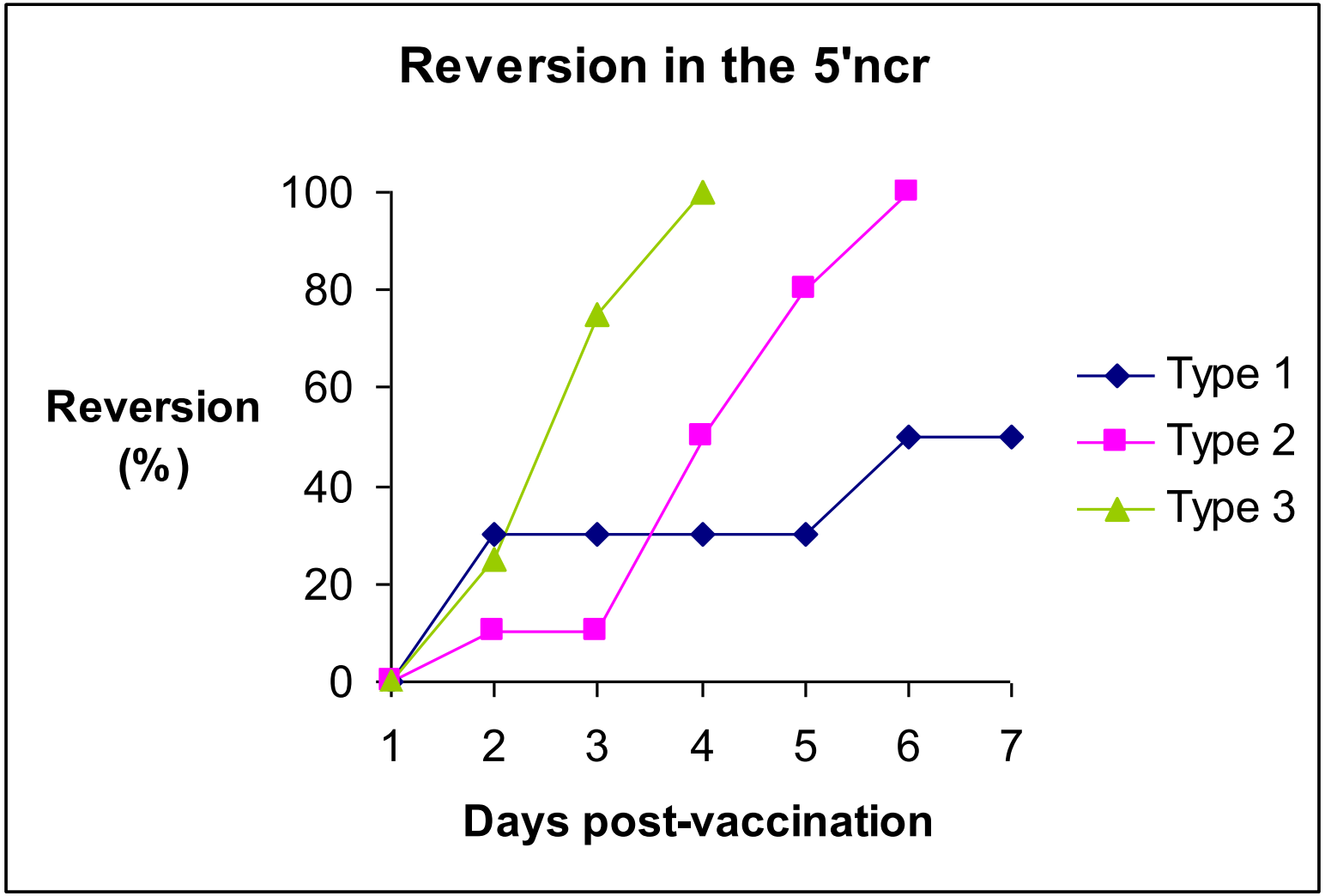
Poliovirus 5’NTR Domain V Mutations







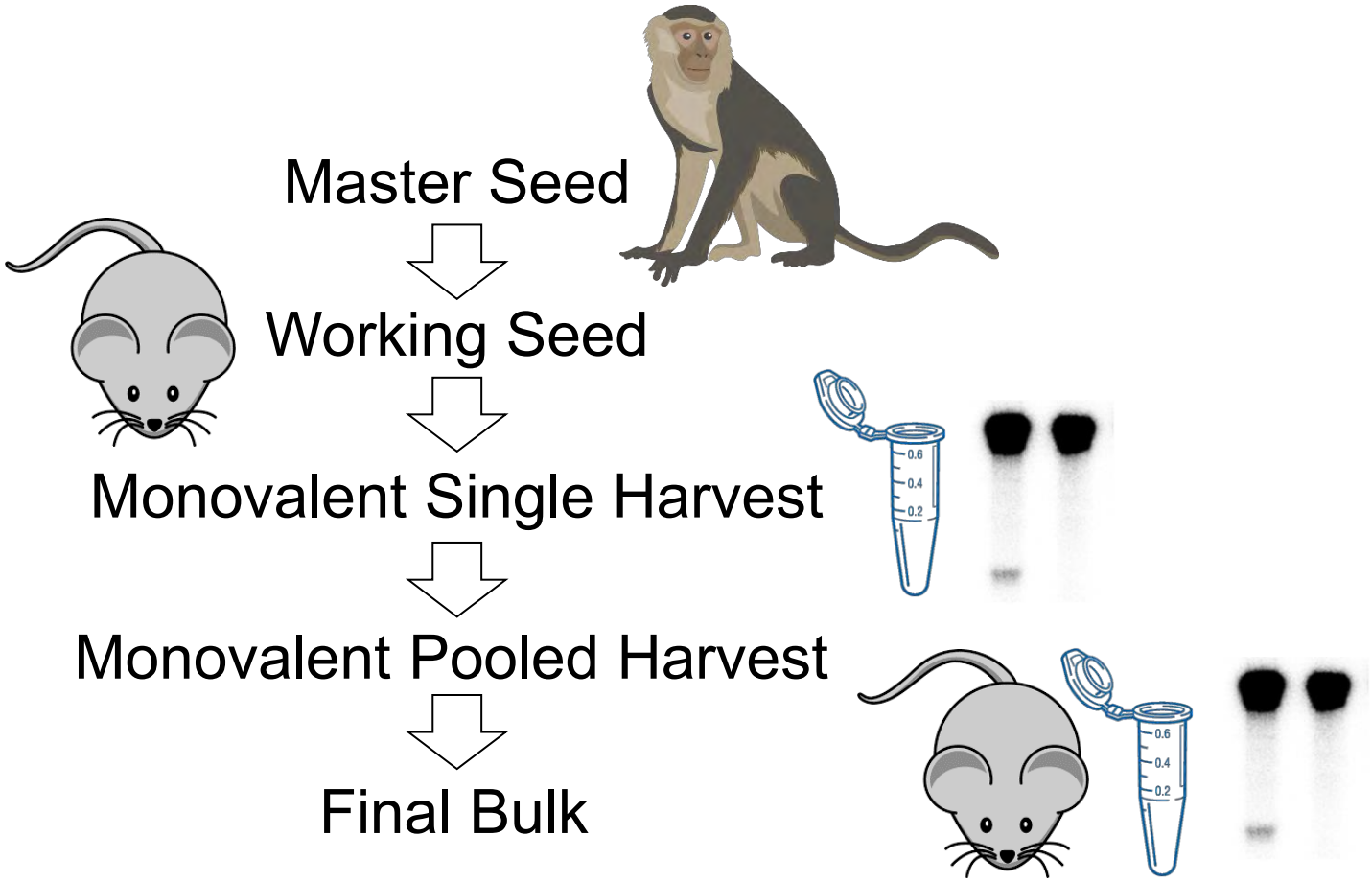
Genetic stability of OPV strains



Quality control of OPV

- OPV prepared by serial passage of wild poliovirus
- Virus weakened by incorporation of mutations
- Low risk of disease 1:10^6 doses, VAPP
- Potency: titration in cell culture
- Safety: reversion to virulence

OPV safety testing before NGS



OPV safety testing through time

OPV Production	**Testing**			
	Until 2002	*2002-2013*	*2013 onwards*	*202X onwards*
Master Seed	MNVT	MNVT	MNVT	MNVT
↓	↓	↓	↓	↓
Working Seed	MNVT	MNVT	TgmNVT	TgmNVT
↓	↓	↓	↓	↓
Monovalent Single Harvest	-	MAPREC	MAPREC	MAPREC
↓	↓	↓	↓	↓
Monovalent Pooled Harvest	MNVT	TgmNVT MAPREC	TgmNVT MAPREC	TgmNVT MAPREC
↓				
Final Bulk Vaccine (mOPV, bOPV, tOPV)				

N

G

S

High Throughput (Next Generation, or Deep) Sequencing

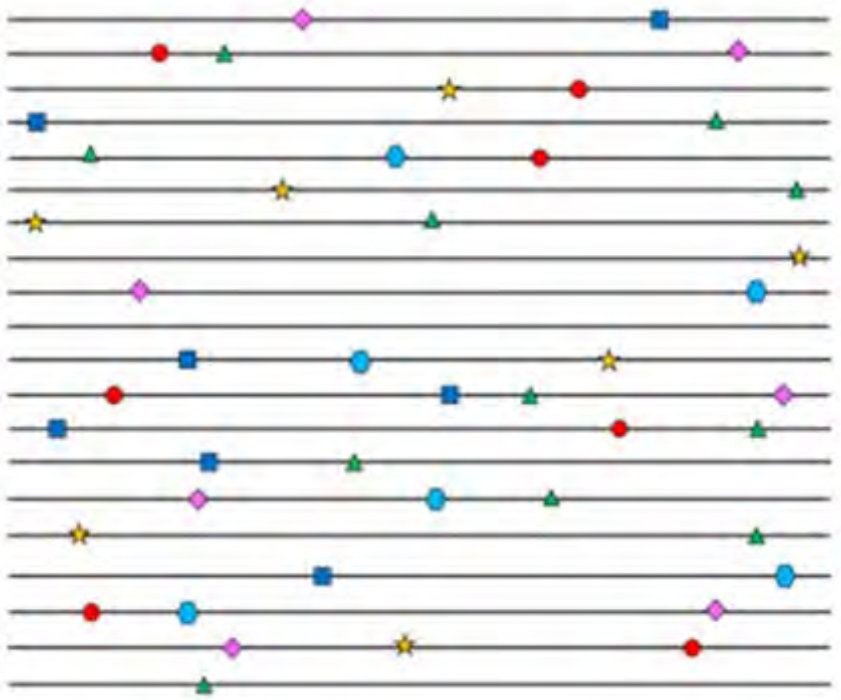
- High throughput allowing multiplexing
- Allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP)
- Decreasing cost per sample for large amounts of data
- High efficiency
- Technically simpler and more robust than MAREC

Early research evidence of potential of NGS for OPV

Neverov A and Chumakov K. **Massively parallel sequencing for monitoring genetic consistency and quality control of live viral vaccines**. PNAS, 2010

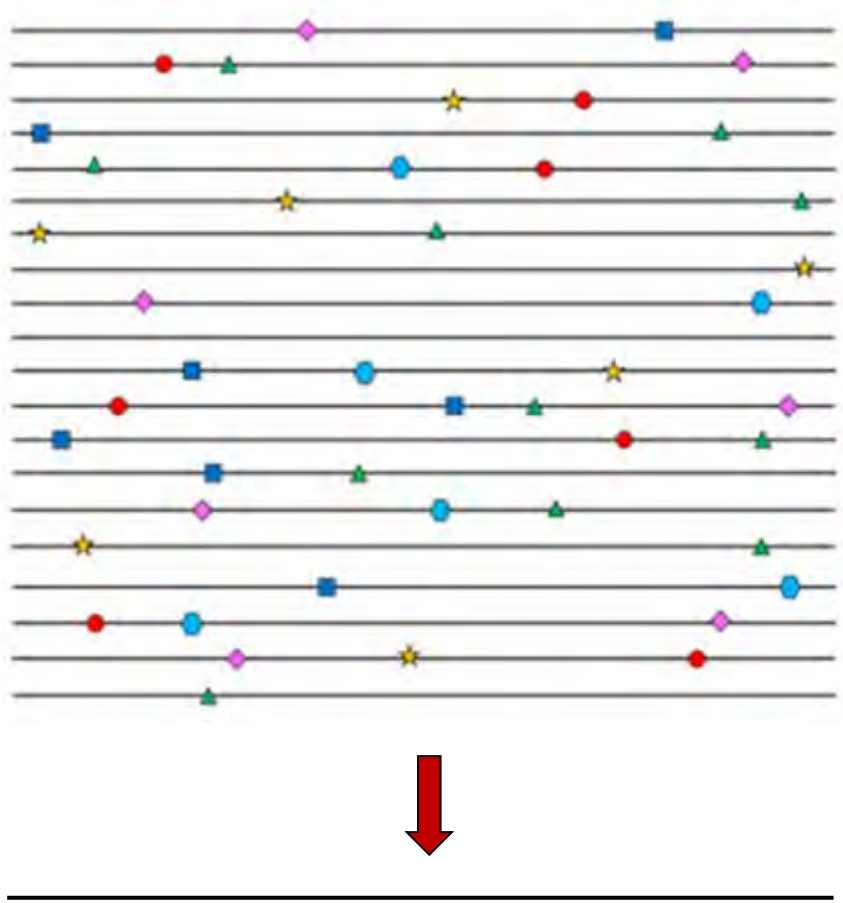
Sarcey E *et al*., **Quantifying low-frequency revertants in oral poliovirus vaccine using next generation sequencing.** J Virol Methods, 2017.

Viral quasispecies



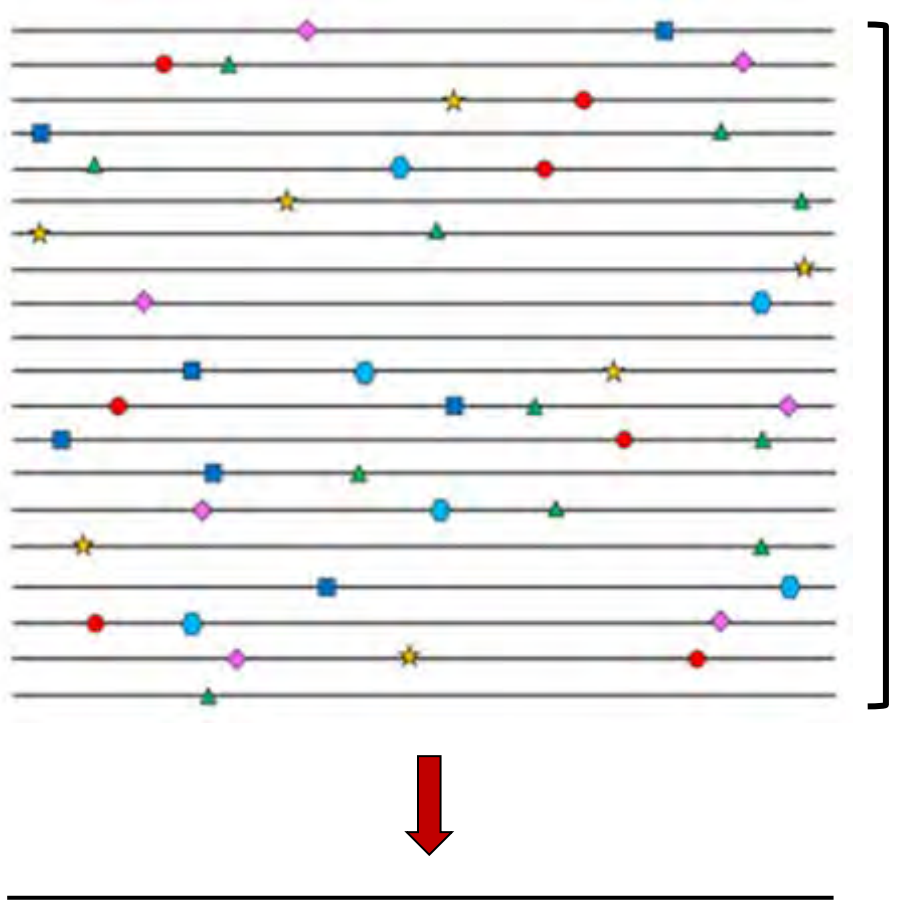
RNA viruses exist as **viral quasispecies**, which refers to a population structure that consists of extremely large numbers of variant genomes, termed mutant spectra, mutant swarms or mutant clouds. Fuelled by high mutation rates, mutants arise continually, and they change in relative frequency as viral replication and selection proceeds.

Nucleotide sequence analysis of viral genomes



Consensus sequence by **Sanger sequence analysis**

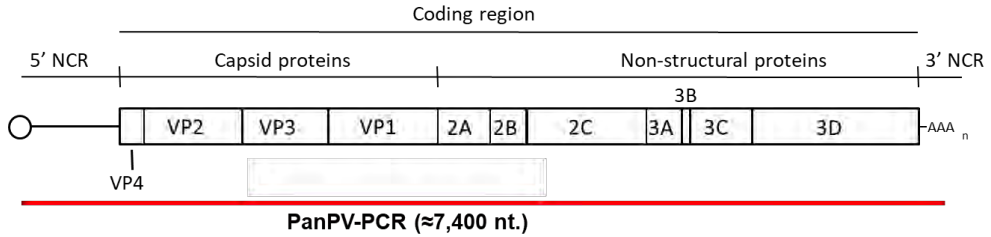
Nucleotide sequence analysis of viral genomes



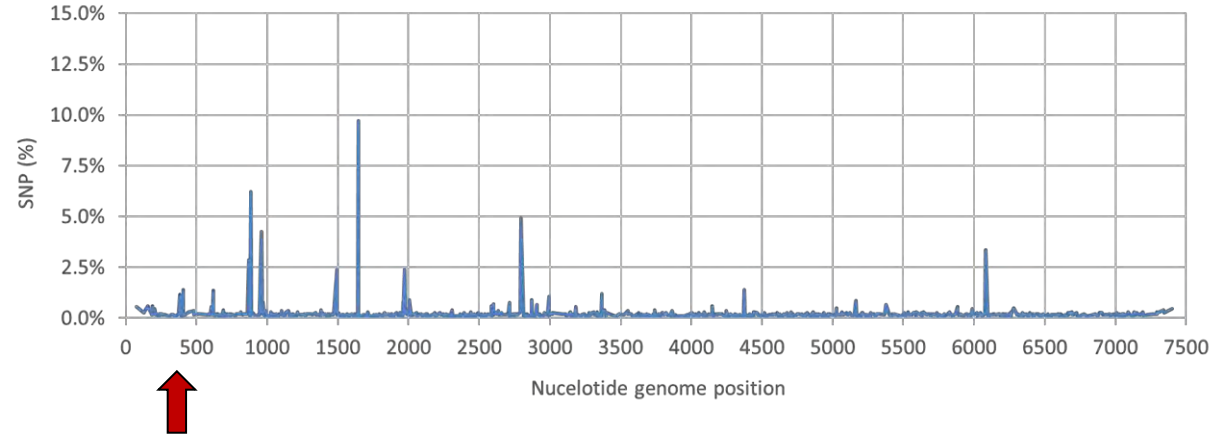
High Throughput (Next Generation, or Deep) Sequencing allows sequencing of individual molecules, accurately measuring variant mutations – Single Nucleotide Polymorphism (SNP) at each genome nucleotide position, creating a unique SNP profile (*molecular fingerprint*) for any given virus preparation that can be compared to that of other related (e.g. master seed vs working seed vs bulk produced from the same seed virus) or unrelated virus preparations (e.g. vaccine bulks from different seeds/manufacturers) .

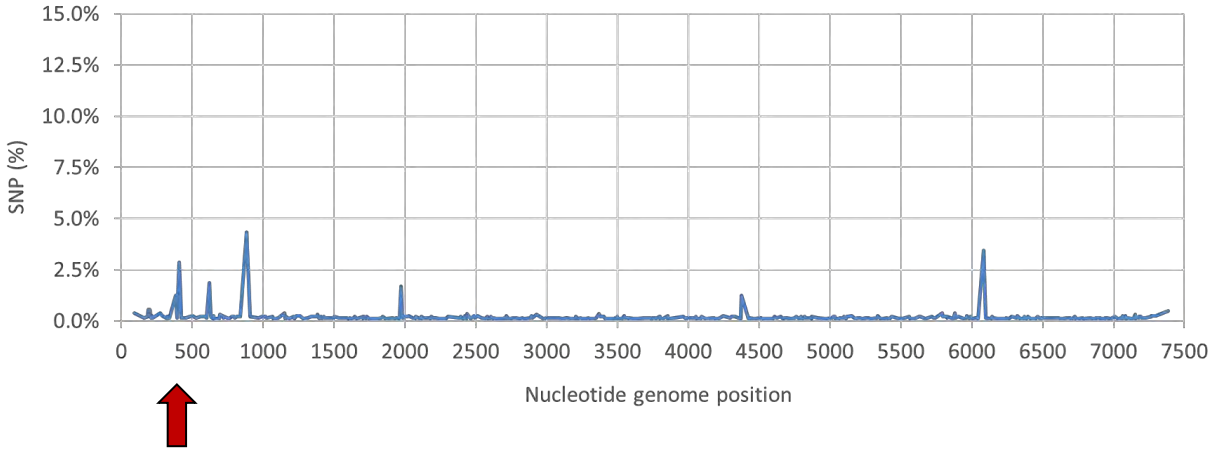
Consensus sequence by **Sanger sequence analysis**

NGS analysis of OPV strains – SNP analysis



Samples analyzed	OPV sample
RNA extraction	High Pure Viral RNA kit (Roche)
cDNA-PCR synthesis	Whole-genome Pan-PV PCR
Library preparation	Nextera XT reagents
NGS	MiSeq Nextera™ XT kit v2
Data analysis	**Geneious R10** *Mapping to Sabin reference* *SNP calling*



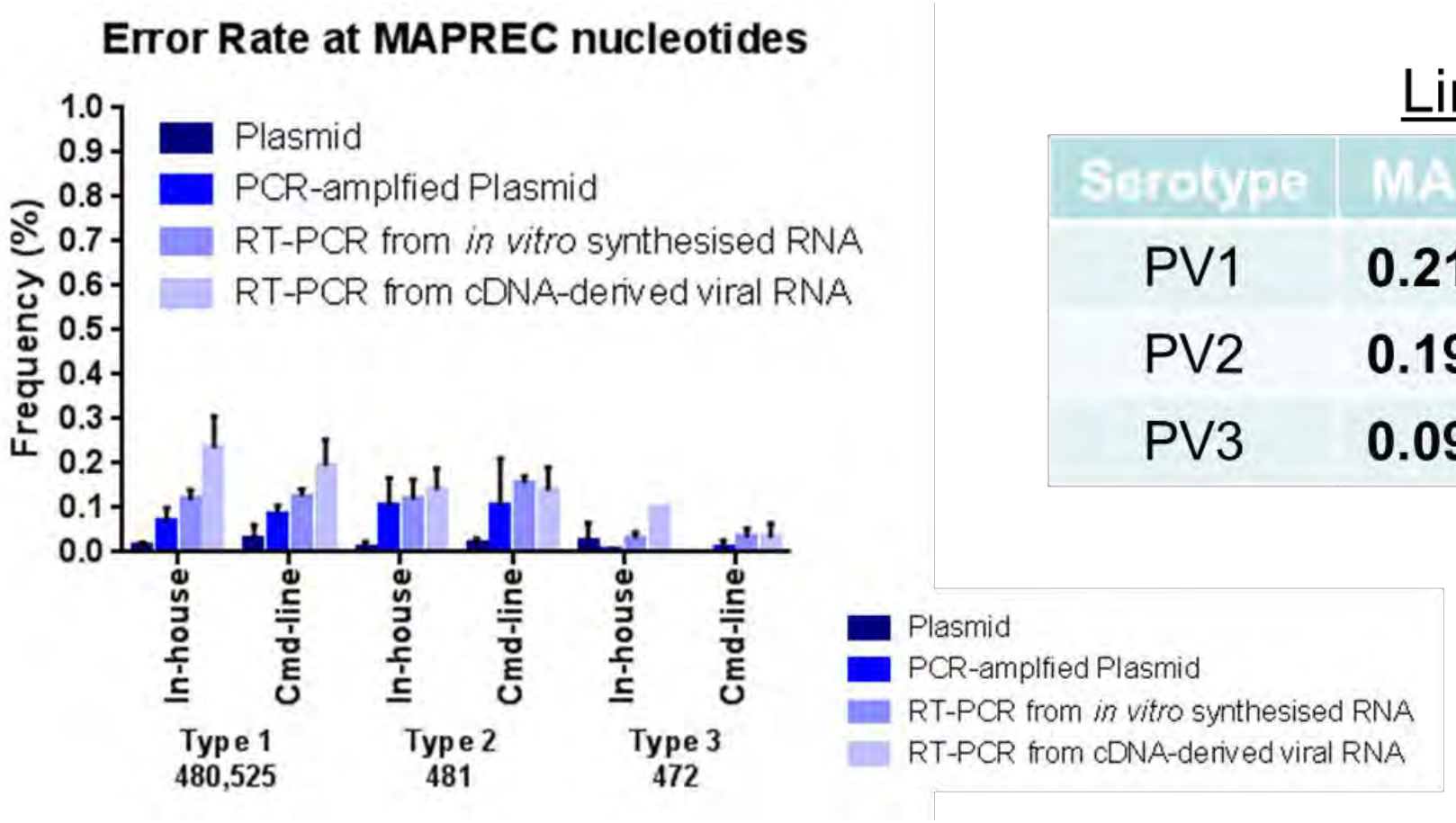


MAPREC mutation

NGS of OPV for the assessment of molecular consistency

- Phase 1:
  - Can NGS be used as a replacement for MAPREC to quantify 5'-NTR mutations?
- Phase 2:
  - Can whole-genome sequence SNP profiles be used as a replacement for NVT eventually removing the need for animal testing?
  - This would be useful for both OPV and OPV seeds used for Sabin-IPV production, to monitor not only the preservation of attenuation mutations but of any other mutations that might affect antigenicity/immunogenicity
- A collaborative study is planned to answer the above questions.
- Objectives of the collaborative study include:
  - A WHO SOP for NGS analysis of whole-genome poliovirus genomes to be developed
  - There is also a need for developing suitable reference standards and analytical methods for NGS assays of all three OPV types
  - An appropriate test format and analytical process to establish assay validity and pass/fail decisions should be developed

NGS is sensitive to detect low frequency variants in the linear range of raw MAPREC data



Linearity

Serotype	MAPREC Linear Range
PV1	**0.21** ± 0.07 – **2.75** ± 0.24
PV2	**0.19** ± 0.06 – **4.28** ± 0.24
PV3	**0.09** ± 0.09 – **3.21** ± 0.21

NGS for OPV3 - International Collaborative Study

The Journal of Infectious Diseases

MAJOR ARTICLE

The Use of Next-Generation Sequencing for the Quality Control of Live-Attenuated Polio Vaccines

Bethany Charlton,[1] Jason Hockley,[2] Majid Laassri,[3] Thomas Wilton,[1] Laura Crawt,[1] Mark Preston,[4] NGS Study Group, Peter Rigsby,[2] Konstantin Chumakov,[3] and Javier Martin[1]

[1]Division of Virology, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [2]Division of Biostatistics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [3]US Food and Drug Administration, Silver Spring, Maryland, USA, and [4]Division of Bioinformatics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom

World Health Organization

WHO/BS/2019.2359
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 21 to 25 October 2019

Report on the WHO collaborative study to investigate the utility of next generation sequencing (NGS) as a molecular test of virus stocks used in the manufacture of Poliovirus vaccine (Oral)

Javier Martin[1, 5], Kostya Chumakov[4, 5], Jason Hockley[2], Thomas Wilton[1], Laura Crawt[1], NGS Study Group (see Appendix 2), Mark Preston[3], Peter Rigsby[2] and Bethany Charlton[1]

Division of Virology[1], Biostatistics[2] and Bioinformatics[3]
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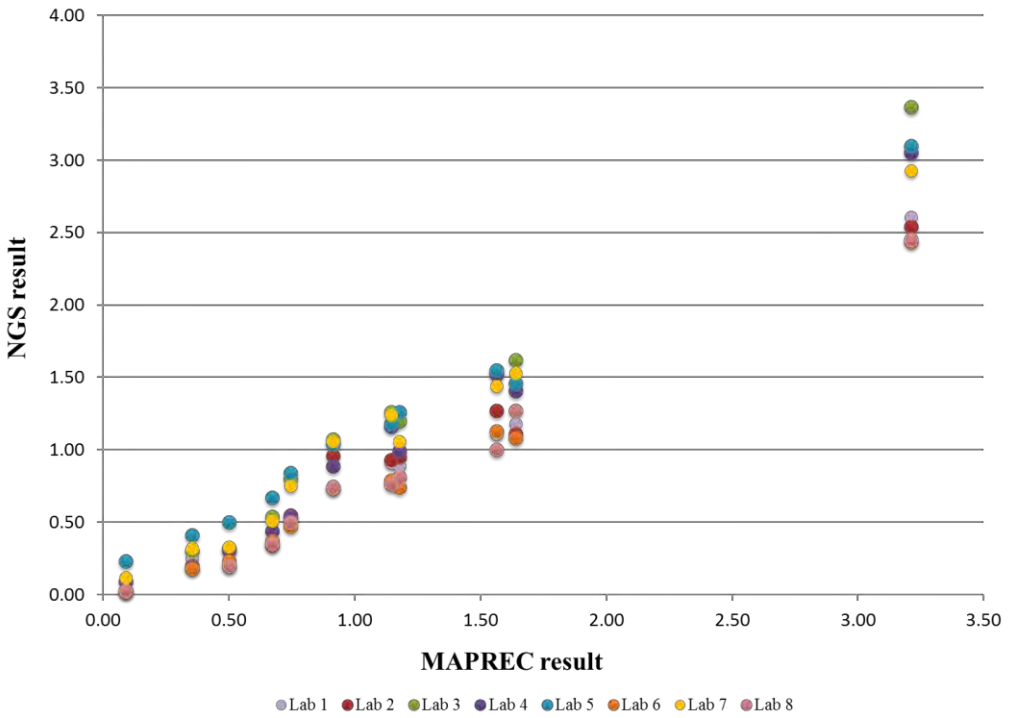
South Mimms, Potters Bar, Herts, EN6 3QG, UK
US Food and Drug Administration[4], Silver Spring, MD 20993, USA.
[5]Study Coordinators
E-mail: Javier.Martin@nibsc.org; konstantin.chumakov@fda.hhs.gov



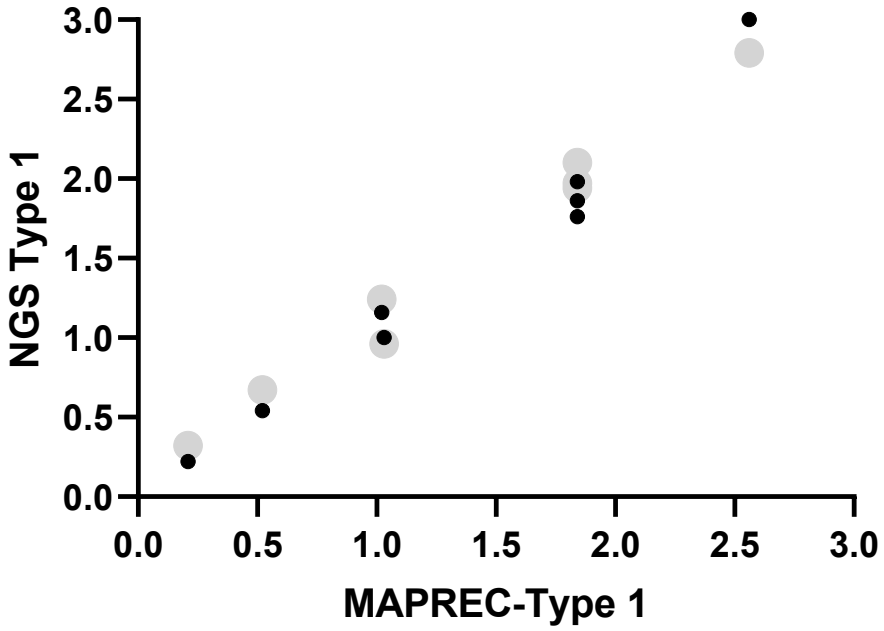
Pearson correlation coefficient

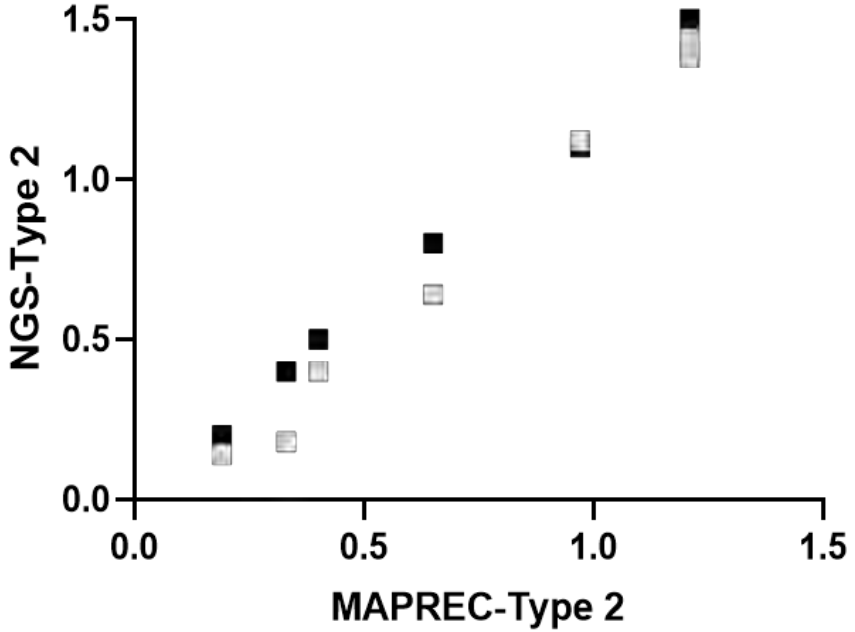
Analysis	Lab								
	1	2	3	4	5	6	7	8	Overall
In-House	0.997	0.979	0.994	0.994	0.990	0.995	0.995	0.992	0.996
NIBSC	0.996	0.984	0.995	0.993	0.996	0.996	0.991	0.993	0.996

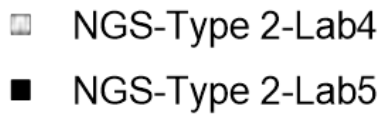
Summary of Phase 1 - Presented to and endorsed by ECBS in 2019

- There was no difference in mean estimates or levels of variability between in-house and NIBSC calculations
- Intra-lab and between-lab variability in 472C NGS measurements was lower than that observed for MAPREC
- There was excellent agreement between laboratories in 472C NGS measurements for all samples, with values showing very low background noise and covering a range of 472C content suitable to make pass-fail decisions
- There was high correlation between NGS and MAPREC 472C measurements (Pearson correlation coefficient >0.995 for all labs)
- There were minor differences in slopes of NGS vs MAPREC regression lines between laboratories which means that specific considerations are required to make pass-fail decisions using NGS
- The overall conclusion is that NGS produced equivalent results to MAPREC when study samples were ranked by 472C content values resulting in the same pass-fail outcome – ***NGS can be used as an alternative to MAPREC for type 3 OPV***

NGS for OPV1 and OPV2 - International Collaborative Study







Type	Lab		
	4	5	Overall
PV1	0.988	0.993	0.994
PV2	0.996	0.999	0.999

Pearson correlation coefficient

Recommendation endorsed by WHO ECBS 2022 and now included in the WHO TRS for OPV: **NGS can be adopted as an alternative test to MAPREC** for measuring the combined 480A + 525C and 481G content **for type 1 and 2 OPV** seeds and lots, respectively, for quality control and batch release purposes.

NGS established as an alternative to MAPREC

World Health Organization

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Geneva, 21 to 25 October 2019

Report on the WHO collaborative study to investigate the utility of next generation sequencing (NGS) as a molecular test of virus stocks used in the manufacture of Poliovirus vaccine (Oral)

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Type 3 - 2019

World Health Organization

WHO/BS/2022.2438
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 24 to 28 October 2022

Report on the WHO collaborative study to investigate the utility of next generation sequencing (NGS) as a molecular test of virus stocks used in the manufacture of Type 1 and 2 Poliovirus vaccine (Oral)

Manasi Majumdar[1], Bethany Charlton[1], Thomas Wilton[1], Kutub Mahmood[3], Peter Rigsby[2], Kostya Chumakov[4,5] and Javier Martin[1,5]

Division of Virology[1] and Biostatistics[2]
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PATH[3], Suite 200, 2201 Westlake Avenue, Seattle, WA 98121, USA
US Food and Drug Administration[4], Silver Spring, MD 20993, USA

Type 1 and 2 - 2022

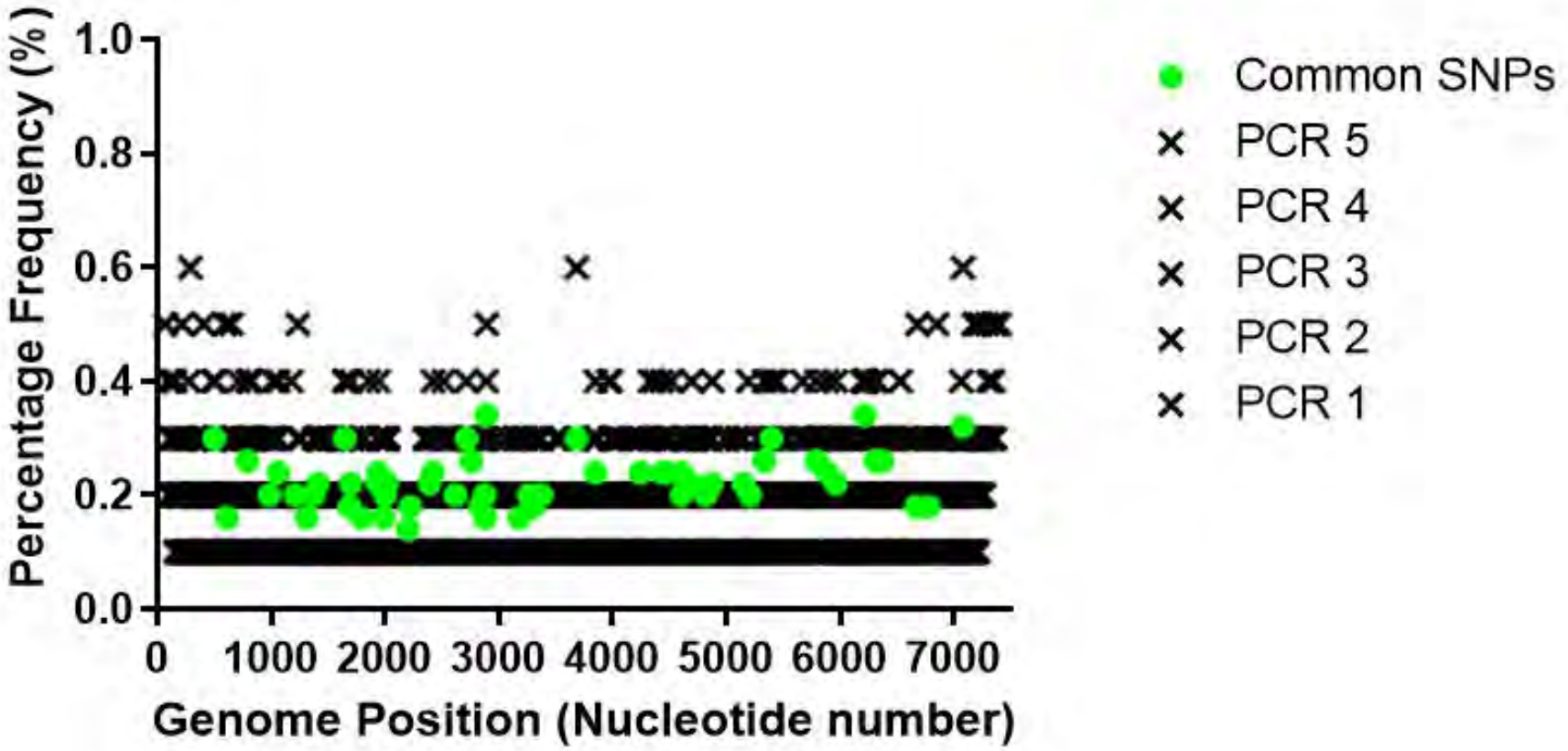
NGS of OPV for the assessment of molecular consistency

- Phase 1:
  - Can NGS be used as a replacement for MAPREC to quantify 5'-NTR mutations?
- Phase 2:
  - Can whole-genome sequence SNP profiles be used as a replacement for NVT eventually removing the need for animal testing?
  - This would be useful for both OPV and OPV seeds used for Sabin-IPV production, to monitor not only the preservation of attenuation mutations but of any other mutations that might affect antigenicity/immunogenicity
- A collaborative study is planned to answer the above questions.
- Objectives of the collaborative study include:
  - A WHO SOP for NGS analysis of whole-genome poliovirus genomes to be developed
  - There is also a need for developing suitable reference standards and analytical methods for NGS assays of all three OPV types
  - An appropriate test format and analytical process to establish assay validity and pass/fail decisions should be developed

Whole-genome NGS analysis of OPV

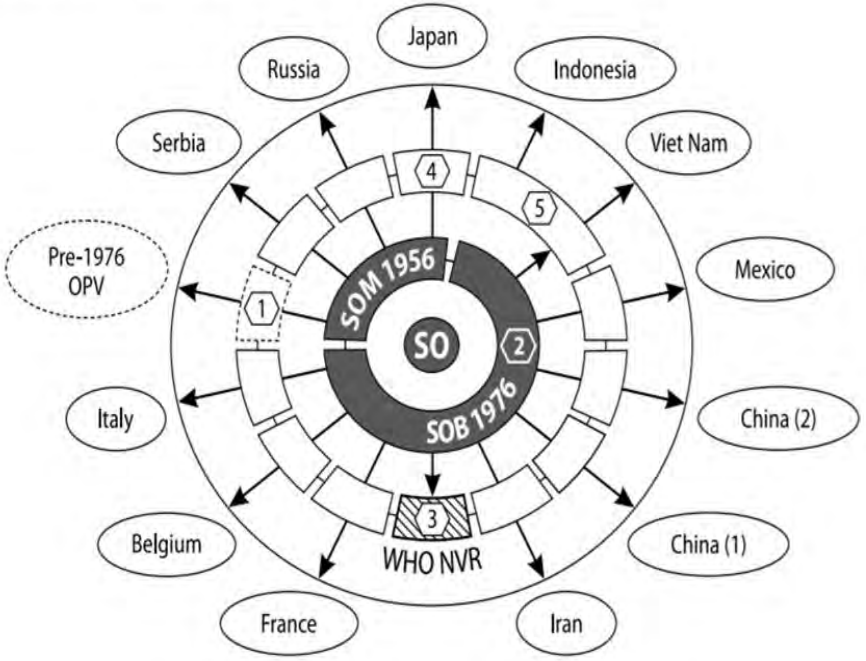
- A large amount of work in this field has been conducted at NIBSC in the last 5 years.
- NGS protocols using the Illumina MiSeq platform have been developed for the analysis of whole-genome poliovirus PCR products.
- The protocol typically involves the analysis of 3-5 replicate whole-genome PCR products from the same RNA preparation extracted from a poliovirus vaccine product.
- Single nucleotide polymorphisms (SNPs) defined as substitutions at single nucleotide positions in the genome that are present in a sufficiently large fraction of the population were quantified to obtain a specific SNP whole-genome profile for each vaccine product.
- Mean SNP replicate values were obtained and SNPs at >1% were used in the analysis.
- Original vaccine seeds, historical reference vaccines and current vaccines with known MNVT, TgmNVT and/or MAPREC results were used for the validation of the NGS protocols.

Whole genome background SNP profile – OPV3 clone



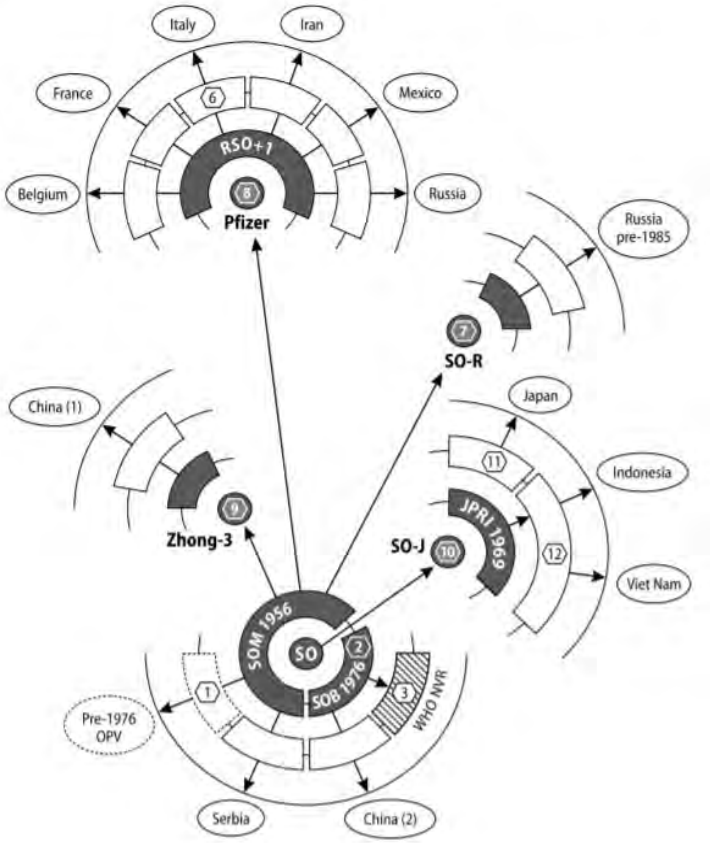
Passage history of OPV seeds

Figure 2.1
History of seed virus and reference materials used to produce type 1 and type 2 OPV from Sabin 1 and Sabin 2

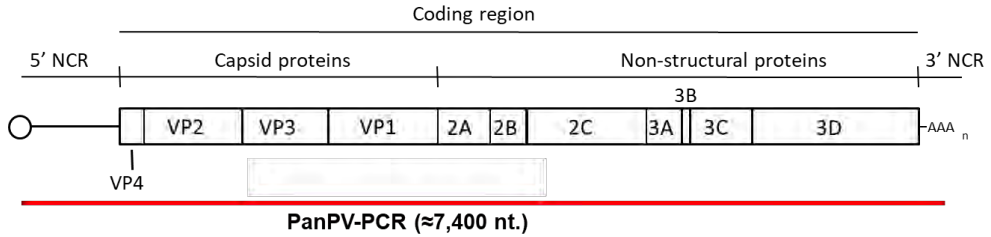


WHO Technical Report Series No. 980, 2014

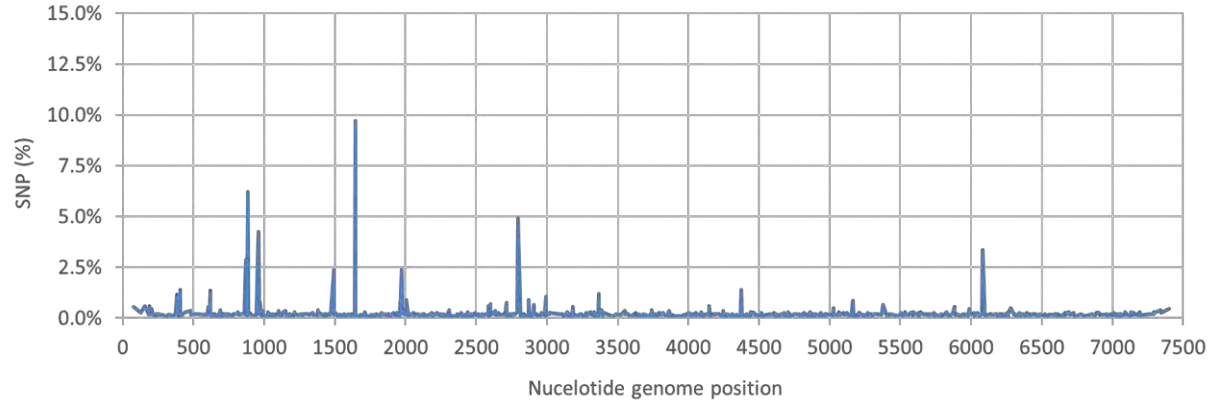
Figure 2.2
History of seed virus and reference materials used to produce type 3 OPV from Sabin 3

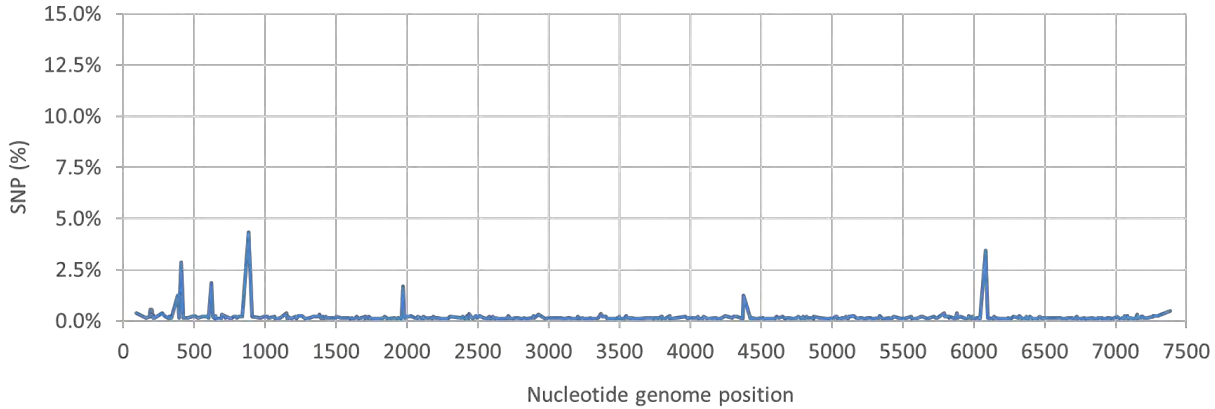


NGS analysis of OPV strains – SNP analysis

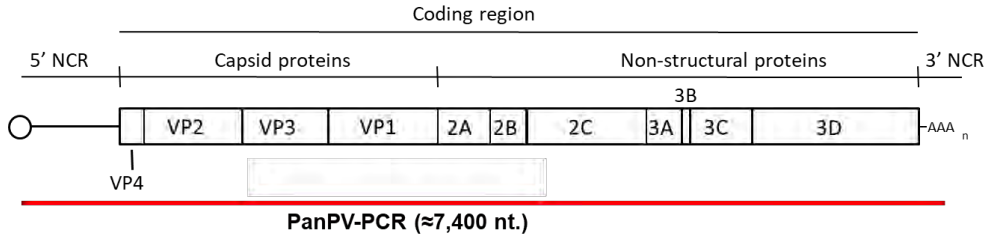


Samples analyzed	OPV sample
RNA extraction	High Pure Viral RNA kit (Roche)
cDNA-PCR synthesis	Whole-genome Pan-PV PCR
Library preparation	Nextera XT reagents
NGS	MiSeq Nextera™ XT kit v2
Data analysis	**Geneious R10** *Mapping to Sabin reference* *SNP calling*





NGS analysis of OPV strains – SNP analysis



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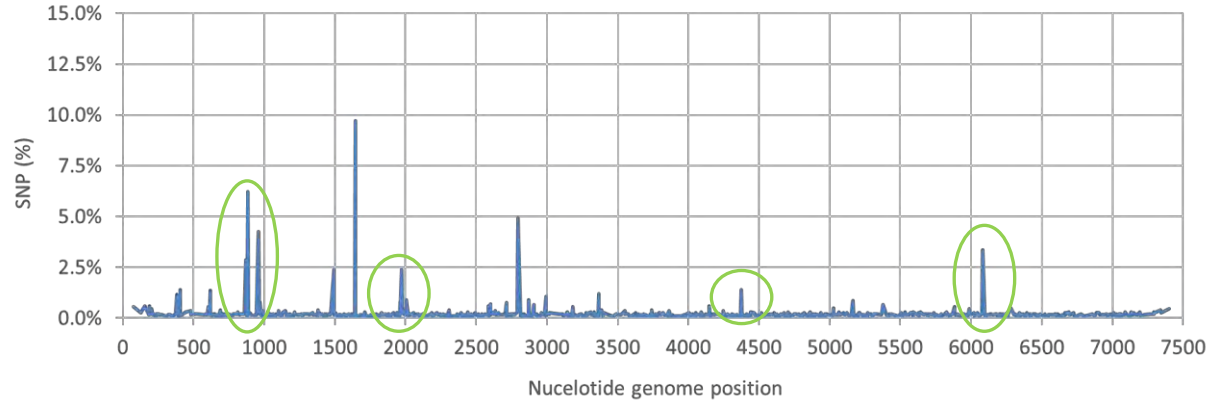
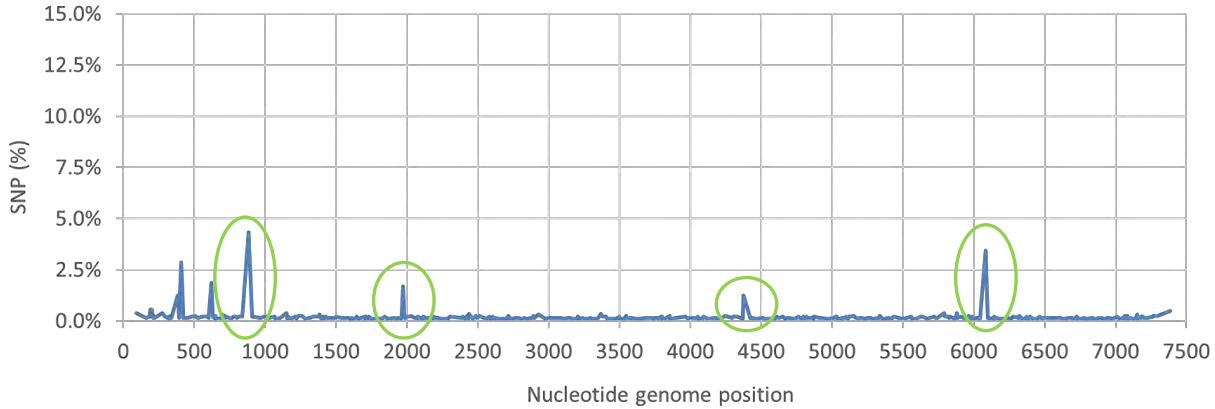
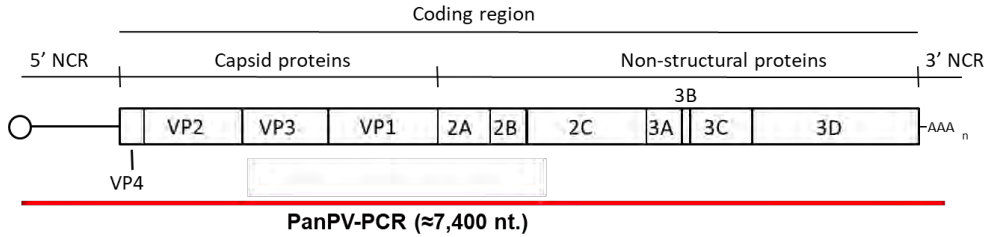




NGS analysis of OPV strains – SNP analysis



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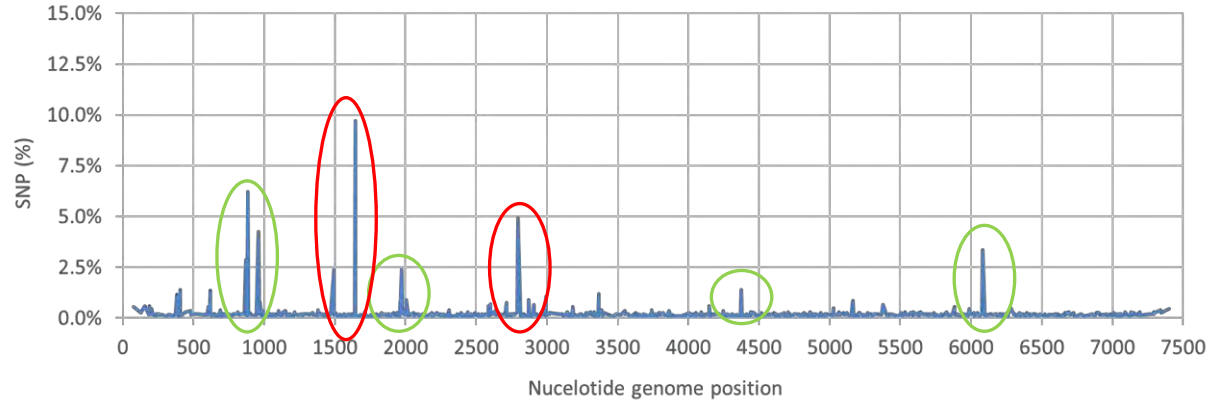

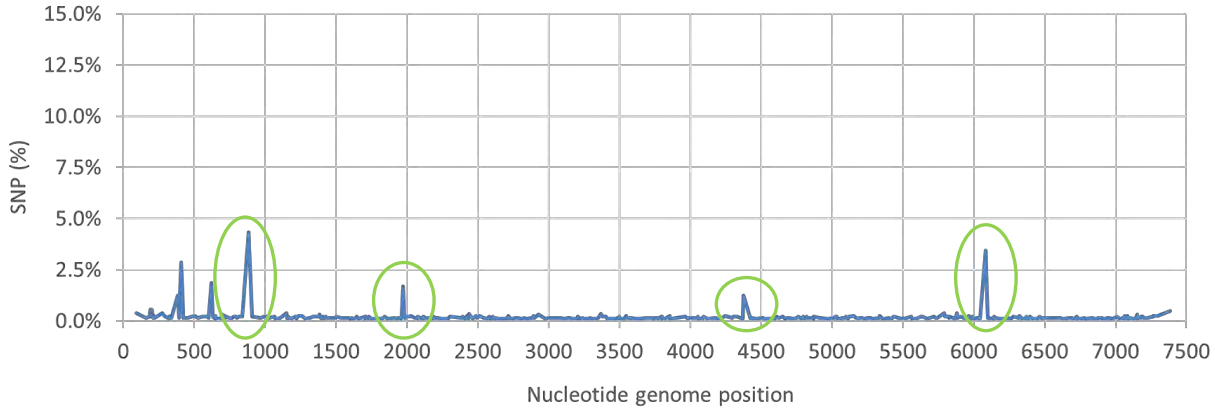

Summary

- Whole-genome NGS analysis demonstrated high consistency in vaccine production of most OPV products by different manufacturers.
- Whole-genome SNP profiles were highly consistent between vaccine products from the same manufacturer.
- Different vaccine seeds and associated products were found to contain unique SNP profiles.
- Vaccine products from manufacturers using the same vaccine seed were found to contain common SNPs unique to that seed but also manufacturer-specific SNPs often associated with the cell substrate used for vaccine production (e.g. monkey kidney vs vero vs human diploid cells).
- These results suggest that whole-genome NGS analysis has a great potential as a QC test for OPV measuring vaccine production consistency and potentially replacing neurovirulence testing using animals

Collaborative study to support Phase 2

- Objectives
  - Identify suitable SOPs for whole-genome NGS analysis of OPV by assessing intra-lab and between-lab variability
  - Establish WHO International/Reference Standards to support whole-genome NGS analysis of OPV
  - Design a process for test format, test validity and pass-fail decision-making
- Samples to be included (for each serotype):
  - 0% mutation cDNA-derived control virus
  - 1 candidate at the pass % MAPREC mutation limit from FDA
  - 1 candidate at the pass % MAPREC mutation limit from MHRA
  - Low Mutant Reference Virus
  - Low Mutant Reference Virus
  - 1 failed vaccine
  - 1 vaccine from manufacturer 1
  - 1 vaccine from manufacturer 2
  - 1 vaccine from manufacturer 3
  - 1 historical reference vaccine suitable for WGS analysis (containing mutations at different % across the genome)
- Participants:
  - MHRA, FDA, Haifa University, manufacturers, NCAs, PATH

Acknowledgements

- Majid Laassri (FDA)
- Tatiana Zagorodnyaya (FDA)
- Kostya Chumakov (FDA)
- Amy Rosenfeld (FDA)
- Leonid Brodsky (Haifa University)
- Julia Panov (Haifa University)
- Bethany Charlton (MHRA)
- Thomas Wilton(MHRA)
- Laura Stephens (MHRA)
- Edward Mee (MHRA)
- Ryan Mate (MHRA)
- Jason Hockley (MHRA)
- Peter Rigsby (MHRA)
- Dimitra Klapsa (MHRA)
- Manasi Majumdar (MHRA)
- Javier Martin (MHRA)
- Vajra Allan (MHRA)
- Kutub Mahmood (PATH)

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Ongoing effort towards HTS standardization for QC of polio vaccines

WHO workshop on implementation of international standards for
The quality control of polio vaccines including OPV and IPV
31 October- 2 November 2023
Jakarta, Indonesia

Manasi Majumdar

31st of October 2023

WHO Technical Report Series, No. 1045, 2023: Annex 2: MAPREC Assay

- For type 3 (472-C) content in test sample is normalized to 472-C content in IS DNA standard, the ratio should not exceed 1.0 by a statistically significant margin (t-test).
- The limits for types 1 and 2 should be approved by the NRA. Levels of mutations obtained by manufacturers who have implemented the test for types 1 and 2 virus have been less than 2.0 for type 1 Sabin (for the sum of both mutations 480-A and 525 C) and less than 1.5 for type 2 Sabin (481-G).
- These mutations can lead to increased neurovirulence when present in high proportions in the viral population, or possibly acting with other mutations within the viral genome. However, no correlation with virulence in monkeys has been established when these mutations are present at levels typically found in vaccine batches. Therefore, the MAPREC test for Type 1 and 2 OPV has been developed to measure the consistency of vaccine production.
- If a filtered monovalent bulk fails the MAPREC assay, it cannot be used in the manufacturing of finished product, and an evaluation of the manufacturing process (including the suitability of the virus working seed) should be undertaken and discussed with the NRA. Filtered monovalent bulks that pass the MAPREC assay should be tested subsequently for in vivo neurovirulence.
- The MAPREC assay for type 3 is highly predictive of in vivo neurovirulence in animal models. No such correlation exists for types 1 and 2 at the level of revertants present in vaccine bulks. For these types, the MAPREC assay results provide a measure of consistency.

WHO Technical Report Series, No. 1045, 2023: Annex 2: High throughput sequencing (HTS) Assay

The major issues addressed in the revised Recommendations include:

- The use of HTS in quality control of OPV as an alternative to the MAPREC assay as a preferred in vitro test
- Analysis of whole genome mutational profiles generated by HTS as a possible future replacement of the MNVT and TgmNVT for routine lot release once manufacturing consistency has been established – practical experience in these areas is currently limited and further guidance will be provided in due course.
- Further developmental work needs to be completed before HTS can be introduced for general regulatory purposes.
- 2019, the WHO Expert Committee on Biological Standardization recommended that a study be performed to explore the utility of HTS technology for the quality control of OPV made from Sabin strains.
- Study results indicated that HTS could accurately quantify 472-C mutants in monovalent bulks of OPV3
- A second phase of the same study showed that HTS could also accurately quantify mutations of 480-A/525-C and 481-G for OPV1 and OPV2, respectively
- The results generated by HTS and MAPREC methods were very well correlated indicating that HTS could in principle be used as an alternative to MAPREC, providing an appropriate test format and analytical processes for establishing assay validity and pass/fail criteria were agreed with the NRA.

WHO Technical Report Series, No. 1045, 2023: Annex 2: HTS Assay

- HTS makes it possible to conduct whole-genome sequencing on a routine basis.

- The degree of sequence heterogeneity expressed in terms of the number of SNPs at nucleotide positions in the genome not necessarily linked to any tangible biological properties, provides a unique molecular “fingerprint” for a particular virus preparation.

- HTS is thus ideally suited to generating quantitative whole genome SNP profiles of individual vaccine lots that can be used to identify types of polio seed virus and monitor consistency of manufacture.

- After appropriate validation and the establishment of manufacturing consistency, quantitative whole-genome SNP profiles of OPV lots could be used for routine lot release instead of the MNVT or TgmNVT. In all cases, appropriate acceptance criteria would need to be approved by the NRA.

Developing SOP, WHO International Standards and Reference Reagents for HTS

Aim of the Study:

- The primary aim of this study is to establish reference reagents to be used in HTS methods to monitor the consistency of production of OPV and the characterization of virus bulks used for the manufacture of sIPV prior to virus inactivation.
- The objective will be to establish reference reagents suitable for measuring neurovirulent domain V mutations and/or whole genome sequence analysis.
- The study will also focus on providing appropriate test formats and bioinformatics analytical processes for establishing assay validity and pass/fail criteria.
- Overall, the study will provide further scientific assessment of NGS as a replacement test of animal NVTs for vaccine lot release.

Study Samples

- MHRA-NIBSC will prepare and provide samples for the study. Up to ten monovalent OPV coded samples of each of the three poliovirus serotypes will be sent. Due to containment measures, participation will also be extended to labs that can handle only PV1 and PV3.

- The CS panel for each serotype will include:

  - 0% mutation cDNA-derived control virus grown at MHRA
  - 1 candidate at the Just pass % MAPREC mutation limit from FDA
  - 1 candidate at the Just pass % MAPREC mutation limit from MHRA
  - Low Mutant Reference Virus (MAPREC assay)
  - High Mutant Reference Virus (MAPREC assay)
  - 1 failed vaccine
  - 1 vaccine from manufacturer
  - 1 historical reference vaccine suitable for WGS analysis (containing mutations at different % across the genome)
  - Blind duplicate sample

  Manufacturers and NCLs have already received CS SOP and have expressed interest of participation. If you think you want to take part and have not been contacted, please reach out to Manasi Majumdar/Javier Martin.

WHO CS on HTS workflow:

PV1 ~ n=10

PV2 ~ n=10

PV3 ~ n=10

Due to the limited availability of vaccine samples, only one vial (approximately 0.5 ml) per sample will be sent to the laboratories.

→ **RNA Extraction** →

Simulating MAPREC assay design

WG PCR product 5 replicates per vaccine sample

↓

Sequencing library preparation (Illumina MiSeq)

Random enzymatic fragmentation using DNA Prep kit and indexed using Nextera DNA Unique Dual Indexes

Libraries pooled in equimolar concentrations and sequenced with 250 bp paired-end reads on MiSeq v2 (500 cycles) kit

Initial demultiplexing is performed on-board by the MiSeq Reporter software

↓

Send Raw Data to Study Coordinators

← Quality trimming ←

Generation of sequence contigs

↓

Single Nucleotide Polymorphism analysis – Variant calling

↓

Calculation of average SNP per nucleotide position →

Sending in-house whole genome SNP results to Study co-ordinators

Partners: MHRA, CEBER-FDA, Haifa University, PATH

Summary: Whole genome HTS for OPV

- Comparison of whole-genome SNP profiles reveals differences between batches made from different seed lots and by different manufacturers but there is high consistency within manufacturer and product

- NGS seems to provide a sensitive tool for monitoring consistency of production and identifying outliers

- Close similarity of SNP profiles with historical data is a proof of consistency and suggests that biological properties (neurovirulence) are also very similar

Caveats:

- Inconsistency of molecular profiles does not necessarily mean that a vaccine lot is unacceptable
- It suggests that conditions of virus growth have changes, which is a red flag and may require investigation

Path forward:

- Accumulation of SNP profiles of historical vaccine lots that were successfully released and used
- Development of an algorithm to make pass-fail decisions

A proposed future scheme for routine OPV lot release

- A series of consistency lots of monovalent OPV is tested by WG-HTS analysis to establish the range of variations of SNP profile.

- Lots are passed with known MNVT, TgmNVT and/or MAPREC results.

- **After the consistency of manufacture is established:**

  - Only HTS can be used to test for conformity of molecular composition of each new batch of OPV to 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 NVT
  - If a new lot falls outside of these criteria, an Investigation is conducted, possibly including performing NVT
  - If the outcome of the investigation is favorable, the historical SNP database can be updated

Thank you for your kind attention

Stay connected

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Copyright information

November 2023

Establishing HTS (a.k.a. NGS) for quality control of novel OPV2

John Konz

Global Head of Polio Projects,
Center for Vaccine Innovation and Access

PATH

biofarma
Member of Biofarma Group

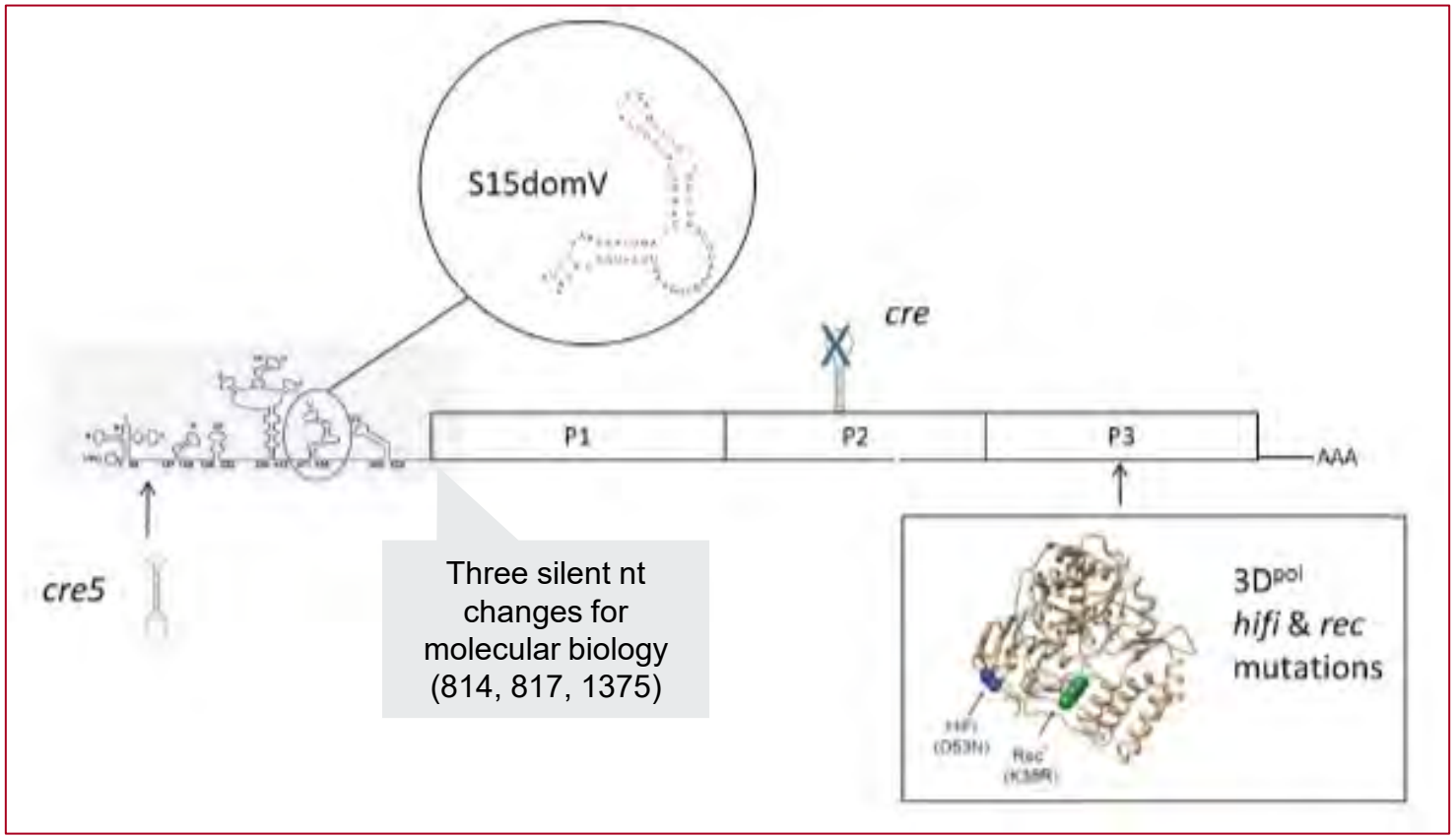
Terima Kasih!

PATH

Agenda

- Background/context: nOPV2 development and HTS method/history
- Defining variations of interest
- Multiple roles of HTS assay in drug substance release
- Assay validation considerations
- Assay controls and control qualification
- Advanced topics
    - Co-location of variations
    - Alternative positive controls

Novel OPV2 design and attributes



- Sabin 2 backbone, with cre5 insertion in 5' UTR and 34 other nucleotide changes
- 50% paralytic dose in Tg mice >100-fold higher than Sabin 2

References of interest

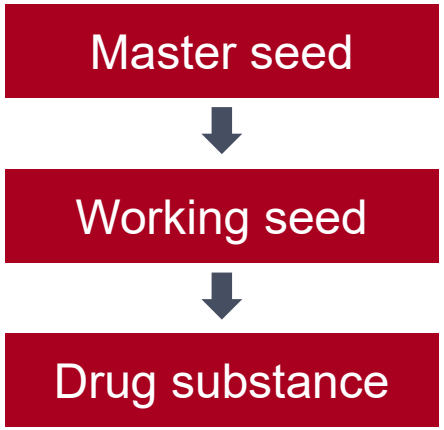
Yeh *et al.,* CHOM, 27(5) 736, 2020. https://doi.org/10.1016/j.chom.2020.04.003

Van Damme et al., The Lancet 394(10193) 148, 2019. https://doi.org/10.1016/S0140-6736(19)31279-6

Sáez-Llorens et al. The Lancet 397 (10268) 27, 2020. https://doi.org/10.1016/S0140-6736(20)32540-X

Uses of HTS in nOPV2 development

Characterization of seeds and virus bulks



Key questions

- Phenotypic effect of mutations present?
- Are the genetic modifications preserved?

Understanding of genetic evolution in humans

mOPV2 or nOPV2 dosing → PCR/ $CCID_{50}$ to identify last analyzable shed virus → Mouse neurovirulence and HTS analyses

Key questions

- Does the S15 domV evolve in ways which reduce attenuation?
- What variations are commonly observed in shed virus? Impact on neurovirulence?
- Are the mutations present in bulks selected for/against?

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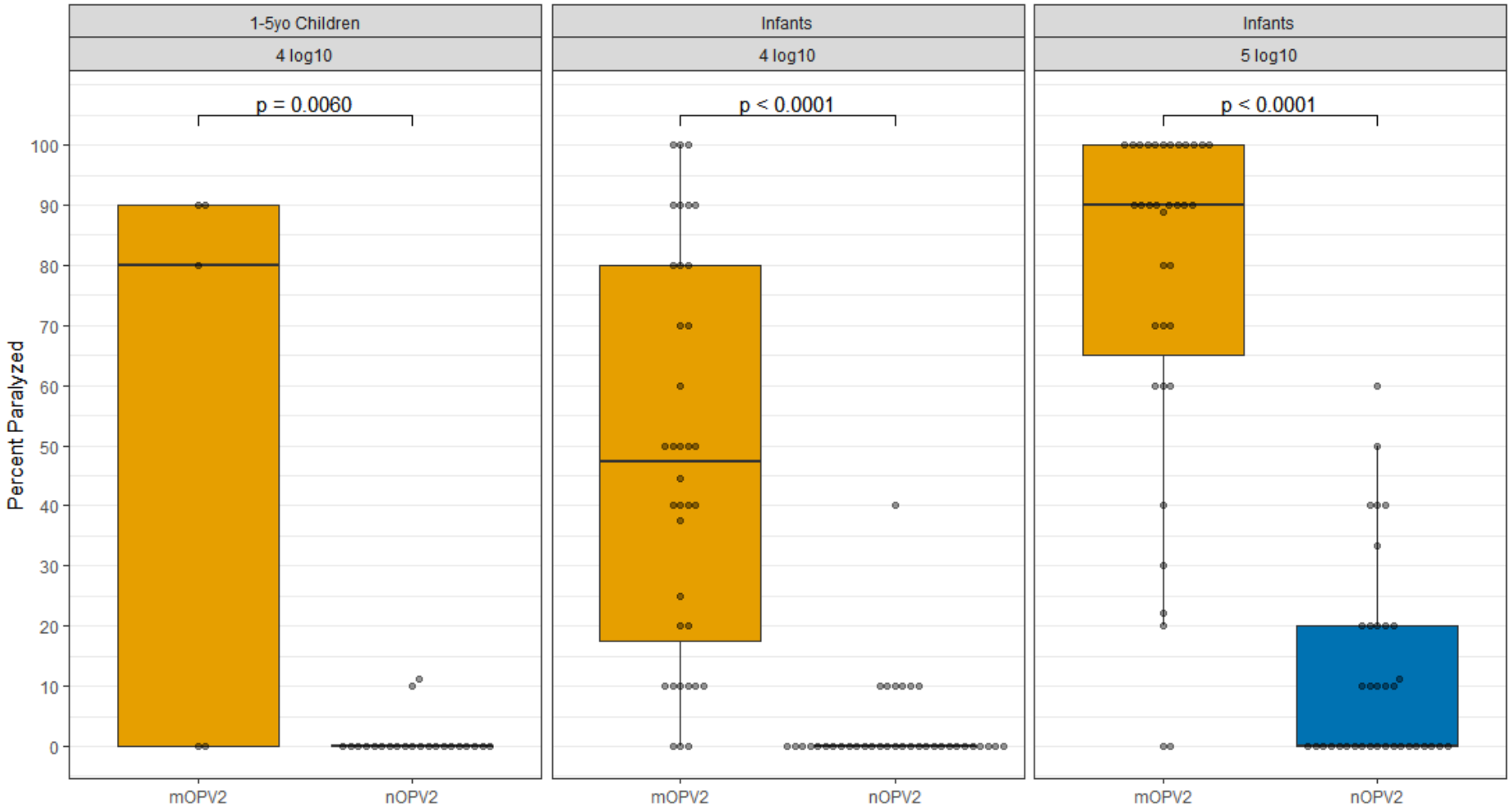
Shed nOPV2 virus has lower neurovirulence in mice than shed Sabin OPV2 virus – results from trials in Panama

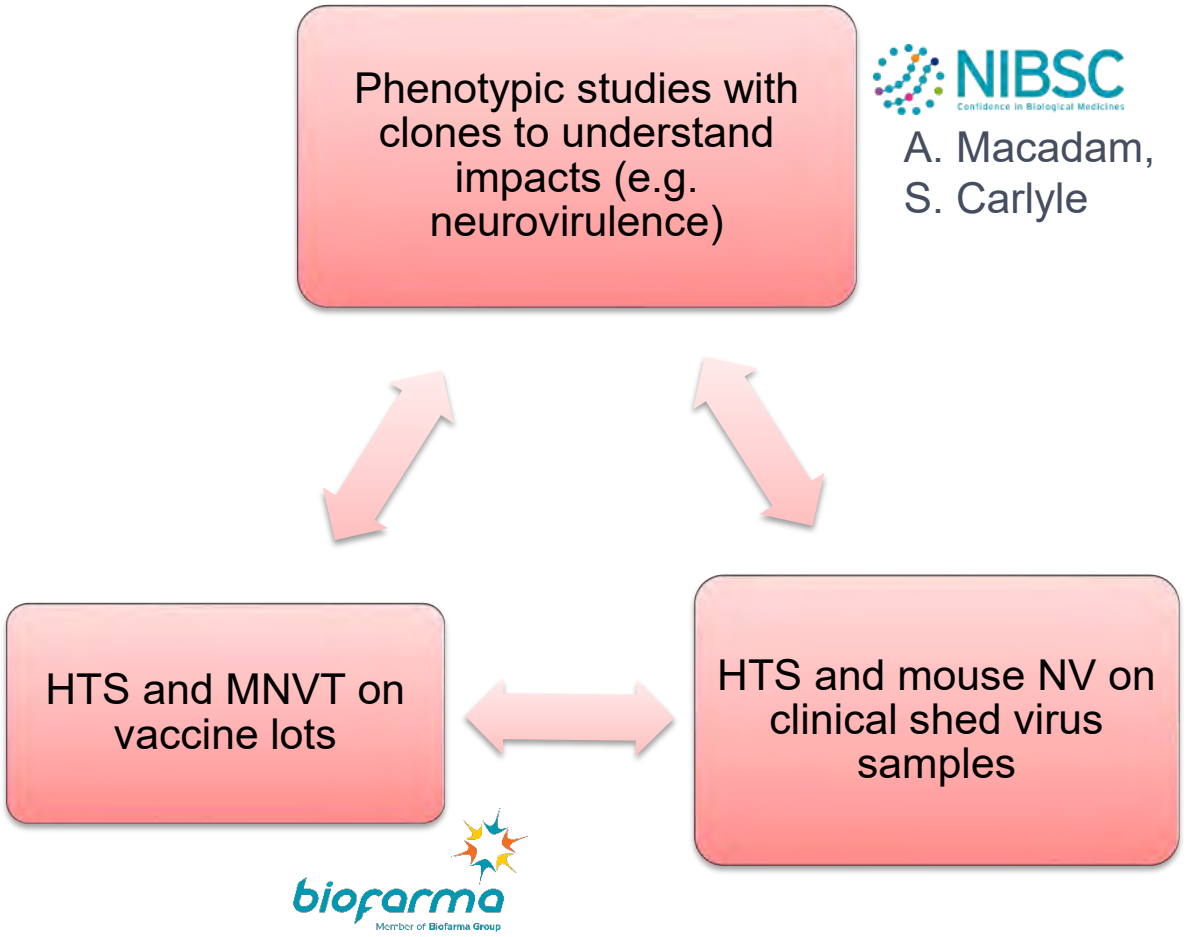


Logistic regression analyses

- Sabin-2 NV increase driven by A481G reversion (domain V)
- Limited nOPV2 NV increases driven by cre5 strengthening and VP1-143 reversion

Reference: Wahid et al, 2022, Lancet microbe; https://doi.org/10.1016/S2666-5247(22)00254-3

Collaborating to understand impact of variations



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Preliminary sketch of genetic evolution of Sabin-2 and nOPV2 in humans and impact on neurovirulence in mice

Log_{10} PD_{50} of molecular clones in tg66 mice (NIBSC)

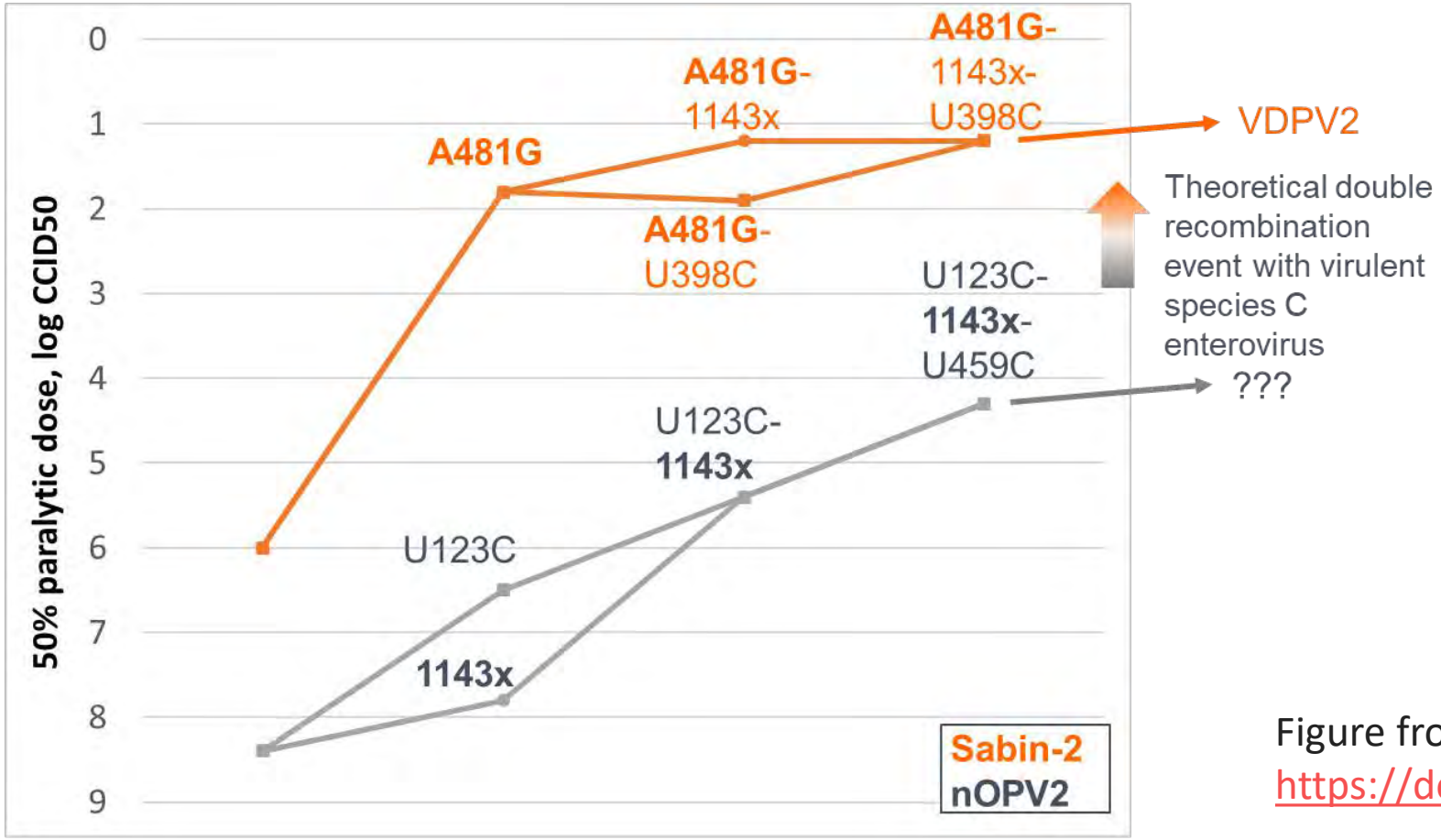

Figure from Wahid et al, 2022 npj Vaccines - https://doi.org/10.1038/s41541-022-00437-5

Bold mutations are present in monovalent bulk
1143x refers to mutations in VP1-143 (e.g. I to T or V)

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HTS role in nOPV2 drug substance release

Negotiated during EUL review

Attribute	Approach
1. “Genetic stability” - Confirming unique modifications	188 nucleotides in the modified regions of the virus (relocated cre, domain V, cre knockout, polymerase mutations) assessed to confirm no variations Specific criteria: no variations detected at any position in modified regions above 1% reporting threshold = +/- limit test
2. Confirming identity	Same as 1.
3. Confirming absence of Sabin-2 contamination	Same as 1. Sabin-2 contamination appears as variations in the modified regions when reads are mapped against nOPV2 reference.
4. Limit testing three SNPs of interest present in bulks (Variations of Interest – VOI)	One-sided threshold for each SNP = “quantitative” limit test

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Mutations in nOPV2 bulks

Variant	Level (%)	Impact
T92C	<9	Not anticipated to have substantial impact; not part of any of the 5' UTR RNA structures
VP4-A41V	<5	Associated with low temperature passaging and has been previously reported to be present in commercial vaccine preparations made on Vero cells
VP1-S33G	<5	Located in a region mediating inter-subunit interactions, and may therefore also result from low temperature adaptation
VP1-A101D	<10	In an antigenic site; has not been associated with virulence
VP1-I143T*	<2.2	Variant in the known secondary attenuation site for Sabin2 (increases NV). Further selected in most trial participants.
VP3-E234K	<46	No significant impact on virulence or fitness.
VP1-N171D*	<36	Variant shows higher virulence than the parental nOPV2 but less virulence than unreverted Sabin-2 strain
VP1-E295K*	<44	Variant negatively impacts immunogenicity in a permissive mouse model and growth in culture at physiological temperatures; may decrease neurovirulence in the transgenic mouse model.

**Selected as "variation of interest (VOI)"; monitored as part of bulk release*

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Considerations for validation from ICH Q2(R2)

Type of measured product attribute / Analytical Procedure Performance Characteristics to be demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY content/potency
		Quantitative	Limit	Other quantitative measurements (1)
Specificity (3)				
Specificity Test	+	+	+	+
Working Range				
Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4)				
Accuracy Test	-	+	-	+
Precision (4)				
Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

Impurity category was best fit for considering validation requirements

- Variations of interest (VOI) can be considered quantitative impurities
- Exclusion of mutations in modified regions is a limit test
- Exclusion of Sabin-2 contamination is a limit test

Validation study parameters:
Precision, linearity over range, LLOD

PATH

Validation study design considerations

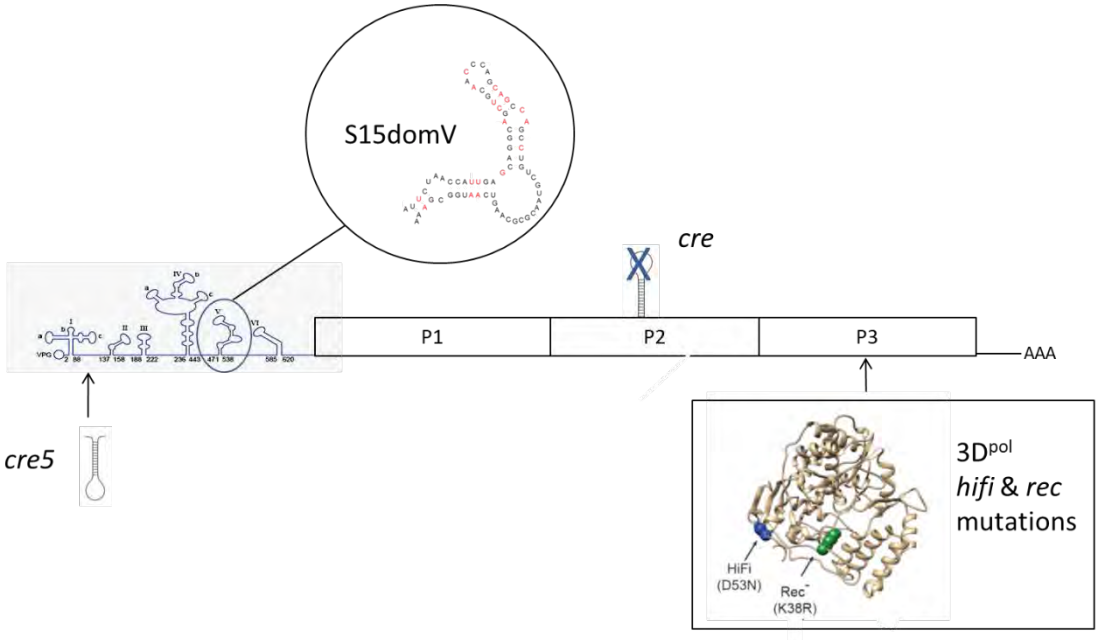
Assay is formally assessing:

- 3 quantified VOI
- 188 x 3 nucleotide substitutions excluded in modified regions

= **567 tests in one!**

Intractably complex to independently validate each measurement

Compromise approach: Evaluate spikes of a Sabin-2 lot into a nOPV2 bulk with 3 VOI



Allowed interrogation of cre5 insertion, 34 nucleotide substitutions across genome and 8 mutations unique to nOPV2 bulk

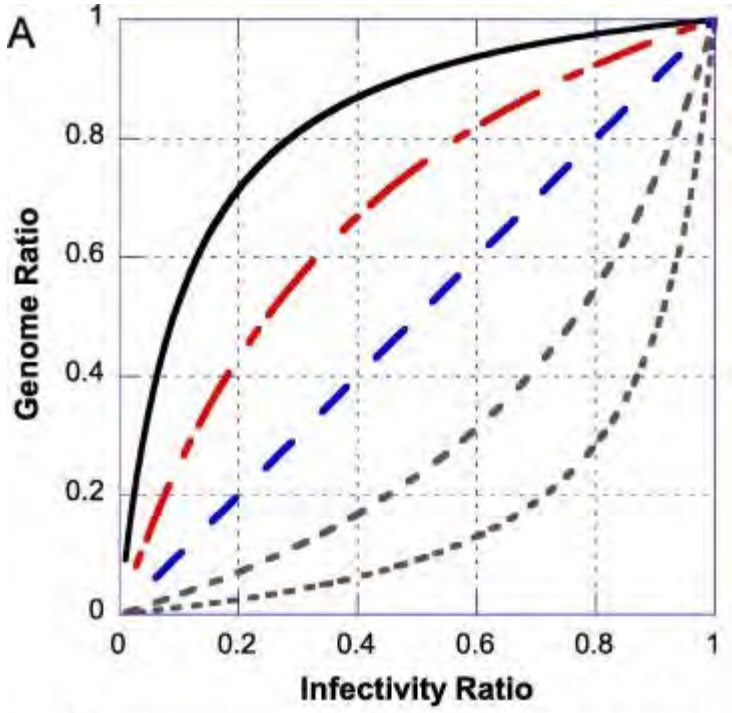
- Sabin-2-specific nucleotides increase with spike
- nOPV2 mutations decrease with spike

Study described in Konz *et al.* 2021 Vaccine:X
https://doi.org/10.1016/j.jvacx.2021.100102

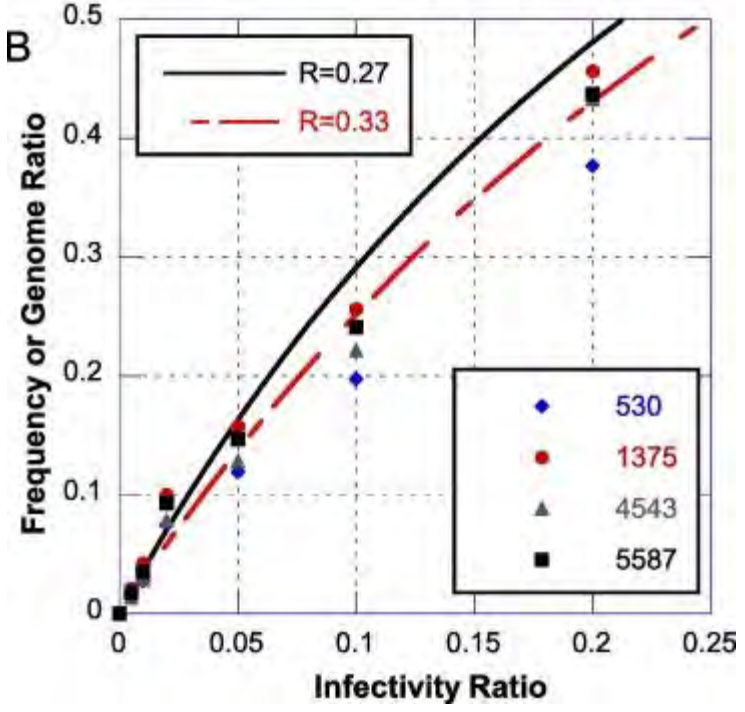
PATH

Validation highlights: spike preparation considerations

- Different preparations of a strain may have varied genome to infectious unit ratios (G:IU)
- Different strains used in mixtures in spiking studies are likely to have significant differences in G:IU ratio
- Need orthogonal assay (e.g. RT-qPCR) or an HTS pre-study to define genome ratios for mixtures



Relationship between genome and infectivity ratios for mixture of two virus preparations with ratio of G:IU ratios ranging from 0.1 to 10

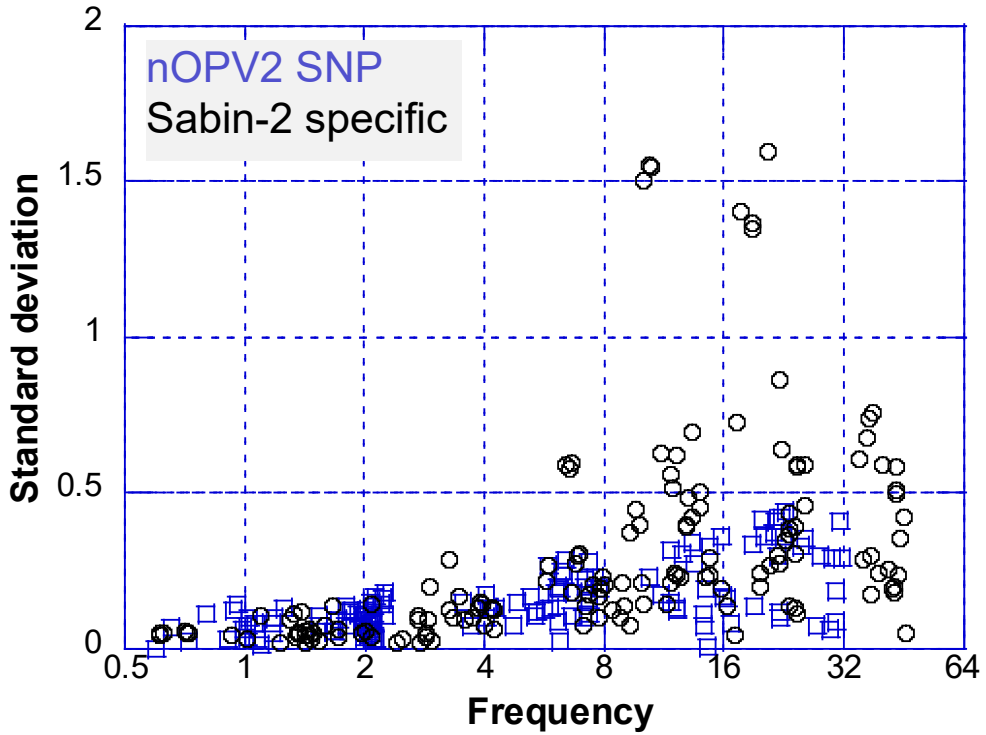


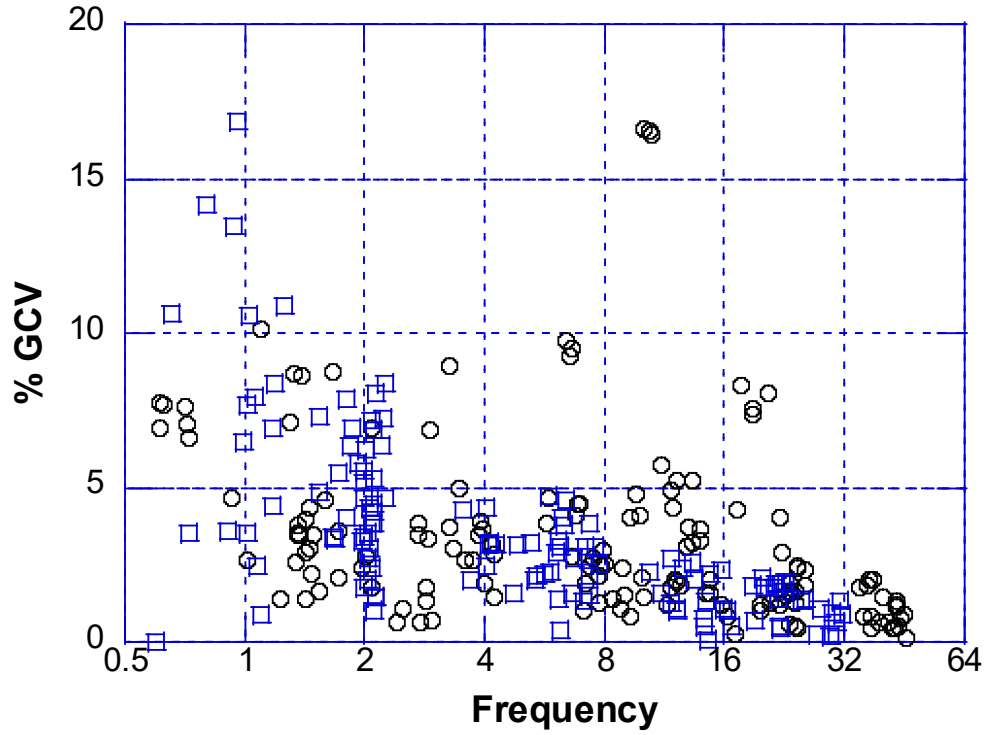
Estimation of ratio of G:IU ratios using RT-qPCR and HTS pre-study

PATH

Validation highlights: precision

42 positions formally evaluated: 34 from Sabin-2 spike, 8 SNPs in nOPV2 lot



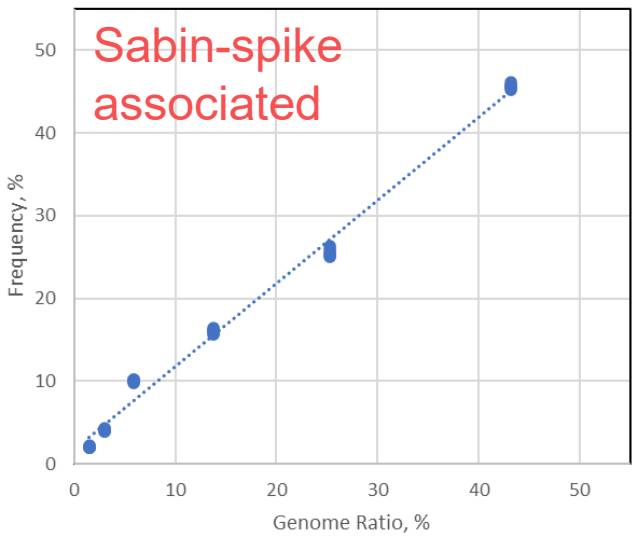


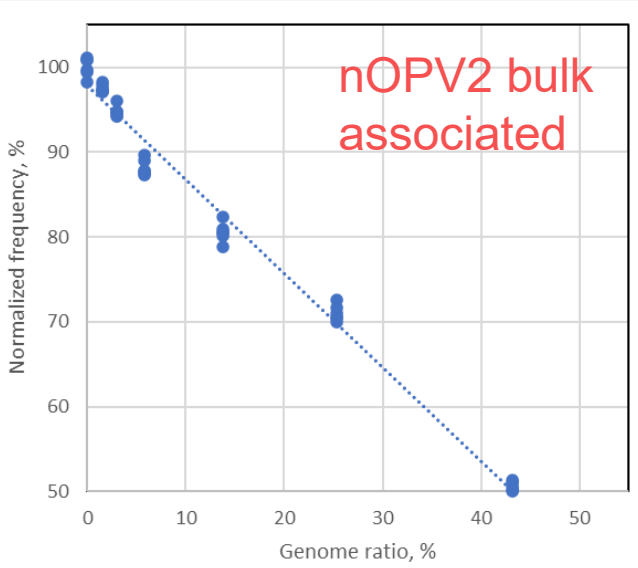
ACCEPTANCE CRITERIA: Repeatability <15%, Intermediate precision <20% relative standard deviation (RSD, or equivalent with log transformation), for the three critical SNPs T2970C, A3053G, and G3425A (VOI); report results for other variants or groups.

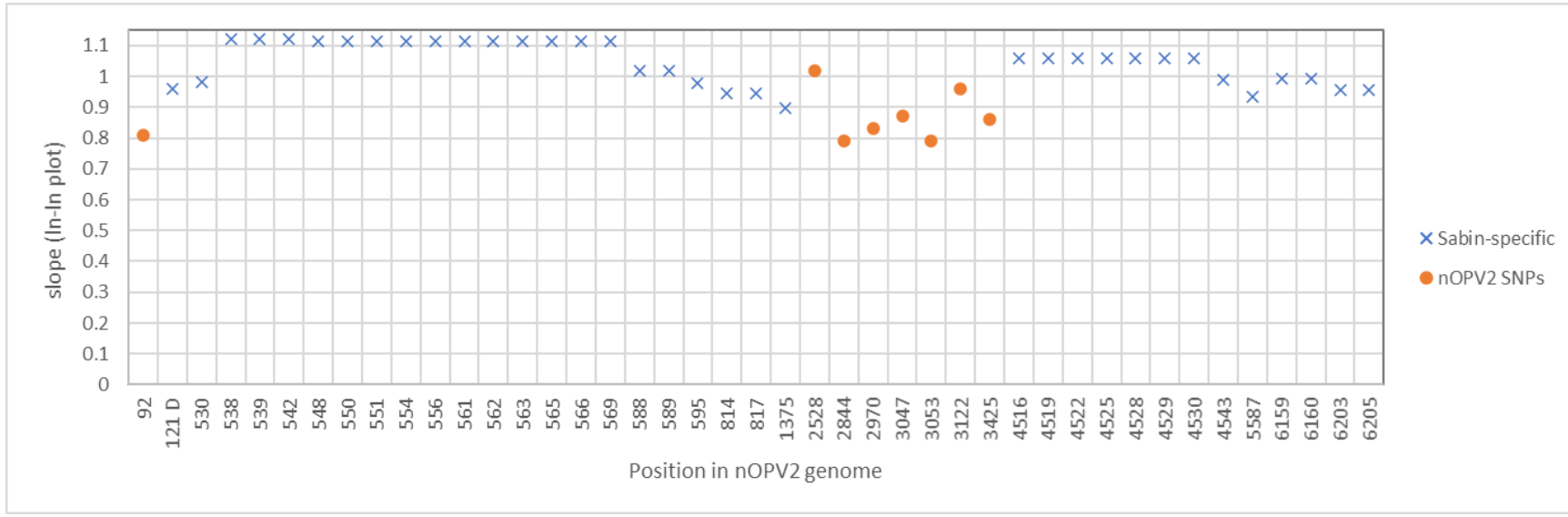
Criteria met for 3 VOI. Results for 34 Sabin-2 positions also readily met criteria.

PATH

Validation highlights: linearity







ACCEPTANCE CRITERIA: Individual slopes of log-log plot for Sabin-spike level versus frequency detected should be between 0.5 and 1.5. Pooled slopes for groups should be between 0.8 and 1.2.

- Criteria met for Sabin-spike positions
- Dilution behavior of nOPV2 bulk mutations also met criteria

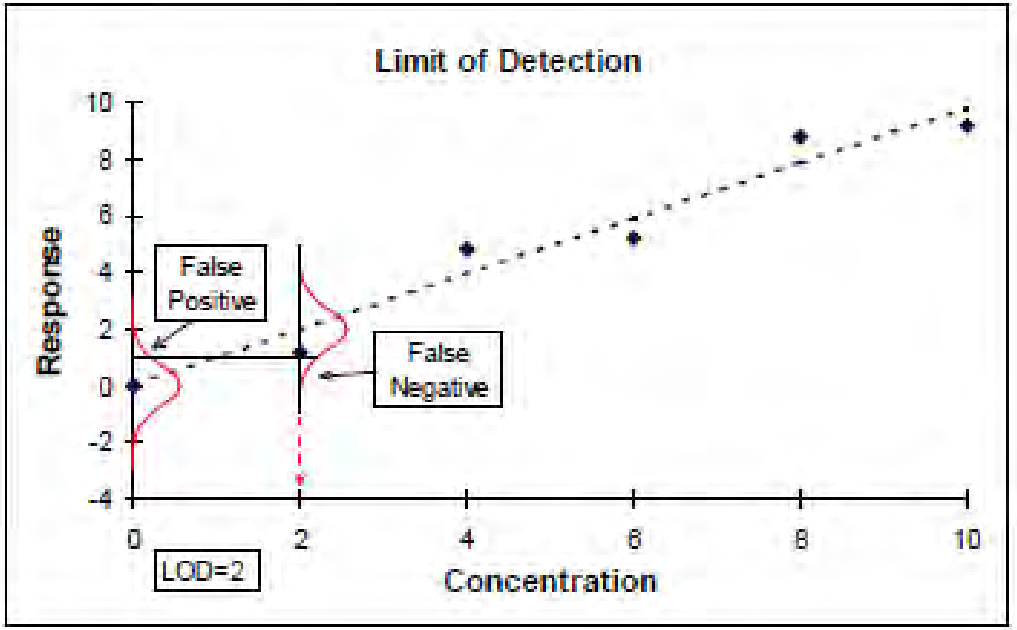
Validation Highlights: Lower Limit of Detection (LLoD)

APPROACH

- Determine LLoD focusing on 13 of the Sabin-2-spike associated variations
- LLoD determined by calculating the minimum spike genome fraction which excludes selected false positive and negative rates, considering the observed variability observed in that range of spikes
- For “allowable” false positive and negative rates of 1%, LLoDs ranged from **0.5-0.7%**

CONCLUSION

- For the chosen variant reporting threshold of 1%, we have high confidence that variants identified are valid



From SCHOFIELD, T.L. Chapter on “*Assay Validation*”. Encyclopedia of Biopharmaceutical Statistics. Edited by Shein-Chung Chow for Marcel Dekker, Inc., New York, Published 8/99.

PATH

Confirmation of sensitivity/specificity (informal)

- For all spikes levels, all 34 Sabin-specific nucleotides were detected in all replicates (1224/1224 above 1% cutoff)
- For replicates with Sabin-spike, 10 pre-specified positions near the Sabin-specific nucleotides confirmed anticipated negativity (0/360 above 1% cutoff)
- Sabin-specific nucleotides were not observed in pure nOPV2 replicates (0/210 above 1% cutoff)

HTS viral controls

To be run in parallel with each batch of samples

Control	Purpose	Proposed test validity criteria
Negative [custom low passage nOPV2 lot from NIBSC]	Ensure NGS background is not unusually high in run (i.e., potentially resulting in false positives)	No detected variations in modified regions or three variants of interest (2970, 3053, 3425)
Positive [Sabin-2 spiked into typical nOPV2 lot]	1. Ensure Sabin-2 contamination would be detectable 2. Ensure mutations in modified regions would be detectable 3. Ensure method is accurate (trending of quantitative value for three variants of interest)	1. Selected spike-associated positions in modifications are detected 2. Three variants of interest are detected; frequencies within trend

PATH

Qualification of virus controls

Reporting thresholds relaxed for study to avoid data censuring and maximize understanding of variations which might be detected

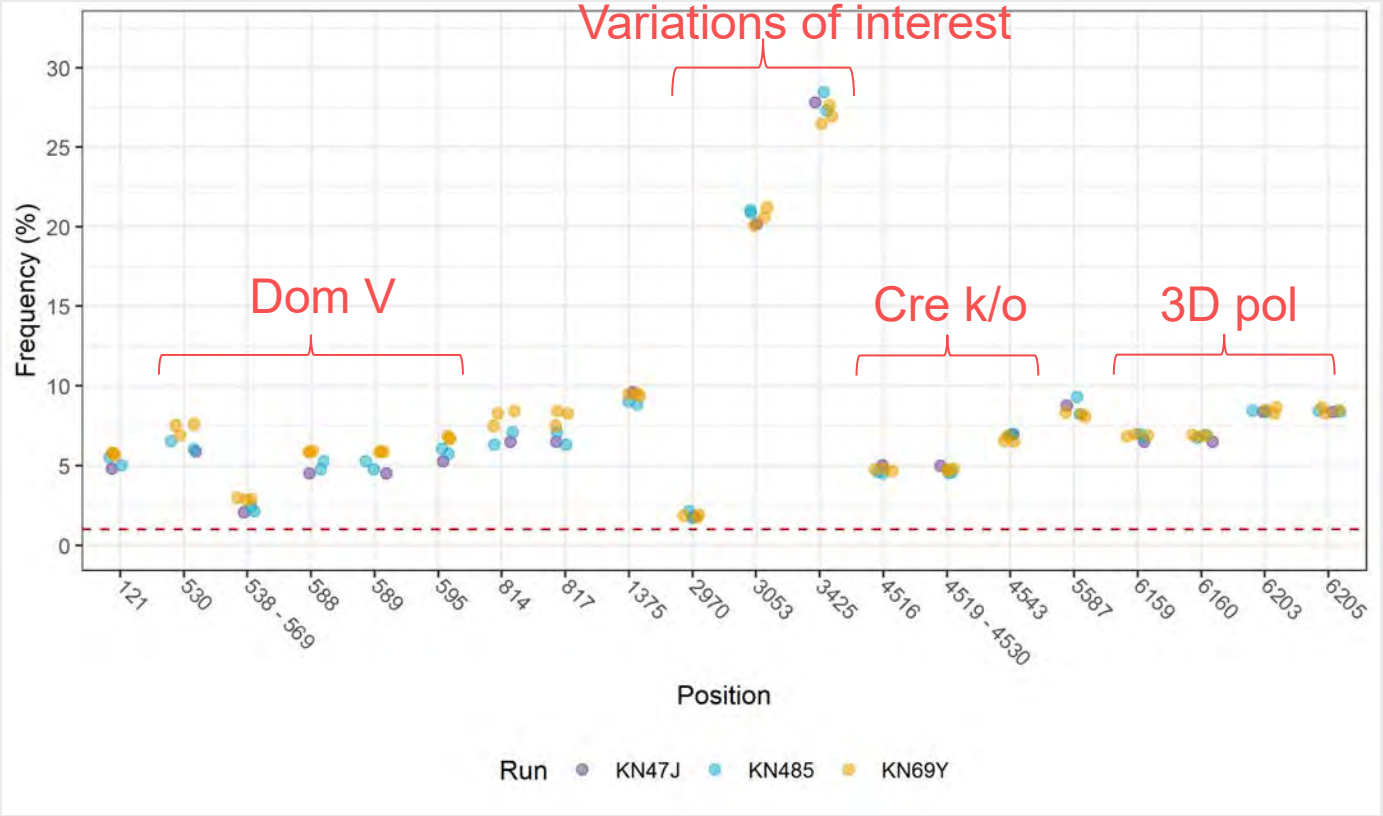
- Frequency threshold: 0.5% vs 1% routine
- Quality thresholds: 20 vs 30 routine

Negative Control

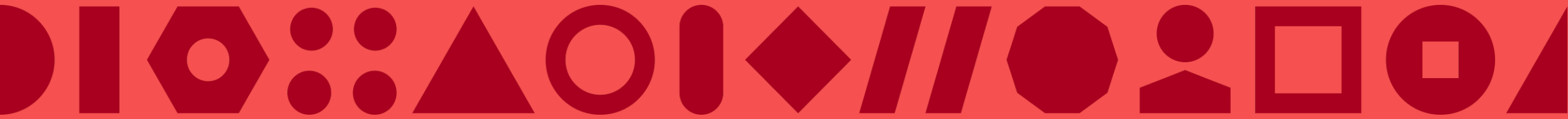
- No detection of variations in any of the nOPV2 modified regions
- Three VOI not detected

Positive Control

- Spike-specific “polymorphisms” from nOPV2 modified regions reliably detected in 6 replicates spread over 3 runs
- Three variations-of-interest (VOI) reliably detected; these will be trended over time

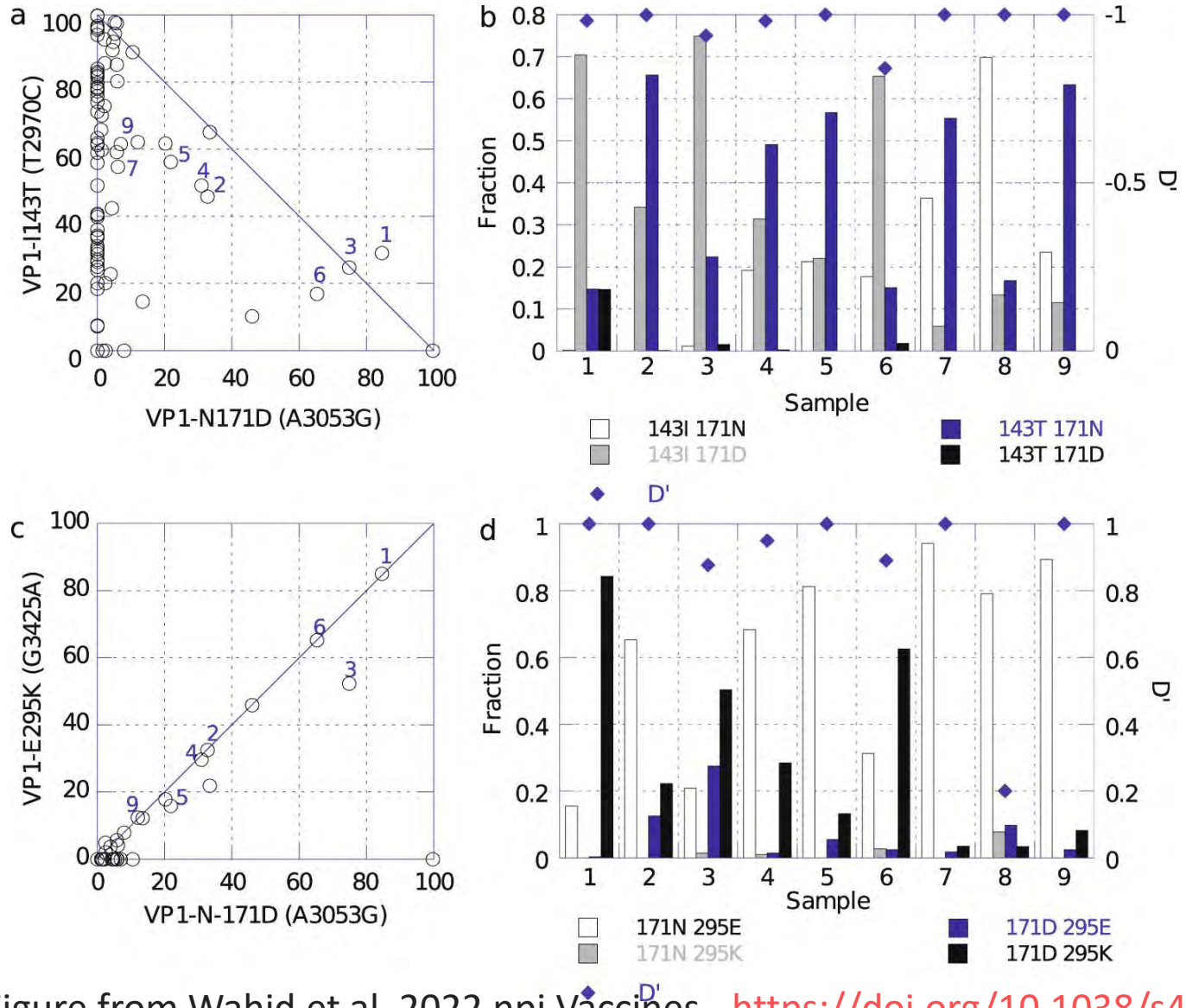


Advanced topics



Topic 1. Mutations may not be independent

observations from shed virus



A. Two virulence-increasing VOI are rarely present in sum greater than 100%

B. Custom co-location analysis shows they are generally not on common genomes

C. VP1-E295K mutation (decreases fitness at 37 C and immunogenicity in mice) never observed at levels exceeding VP1-N171D

D. Custom co-location analysis shows that VP1-E295K is almost always on genomes with VP1-N171D

Figure from Wahid et al, 2022 npj Vaccines - https://doi.org/10.1038/s41541-022-00437-5

PATH

Characterization of molecular clones

Clone	50% paralytic dose, log CCID50	Impact on growth in culture at 37C, HEp-2c	Impact on growth in culture at 37C, Vero
Sabin 2	5.9	-	-
Sabin 2/A481G	1.9		
Sabin 2/VP1-I143V	3.5	-	-
Sabin 2/VP1-N171D	4.1	-	-
Sabin 2/VP1-E295K	>8.1 (3/8)	+	++
Sabin 2/VP1-I143V, VP1-N171D	3.4	-	-
Sabin 2/VP1-N171D, VP1-E295K	6.4	-	-

Double mutant has parental-like properties

Similar results were obtained from nOPV2 clones, but with significantly lower neurovirulence levels

From Konz *et al.* 2021 Vaccine:X https://doi.org/10.1016/j.jvacx.2021.100102

Understanding colocation can change perspective on risk: consistency study lots

		Standard HTS analysis			Co-location analysis on nt 3053 /3425			
Lot	**Bulk lot**	**T2970C VP1-I143T**	**A3053G VP1-N171D**	**G3425A VP1-E295K**	**Parental 171N/ 295E**	**Double 171D/ 295K**	**171D alone**	**295K alone**
1	B0320	1.03	21.2	25.1	74	16	2	9
2	B0420	1.25	27.6	34.3	60	26	2	12
3	B0520	1.49	35.3	43.9	54	33	4	8
						normal NV normal fitness	increased NV normal fitness	decreased NV decreased fitness

Initial understanding from standard HTS analysis

- Up to 37% of virus has slightly elevated neurovirulence
- Up to 44% of virus may be less immunogenic

Refined understanding from co-location analysis:

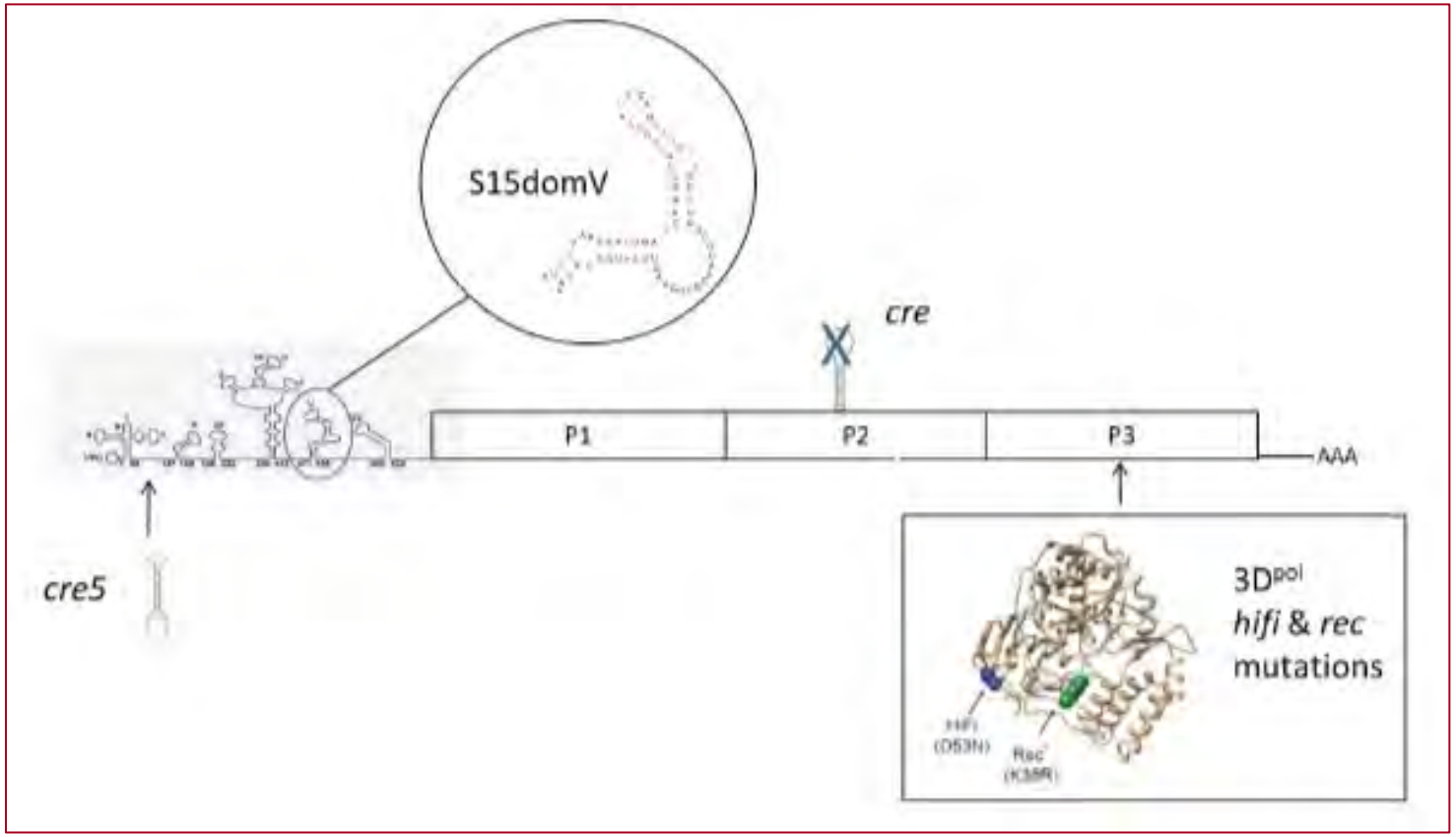
- 3-5% of virus has slightly elevated neurovirulence
- 8-12% of virus may be less immunogenic

PATH

Topic 2. Better and safer viral “positive” controls?

Problem: Use of a Sabin-spike complicates method due to containment requirements and bioinformatic artifacts

Opportunity: An nOPV-derived molecular clone which WHO Containment Advisory Group could view as being similar to nOPVs



Example:

Invert base pair in cre5 e.g. U-A to A-U as a surrogate for pair-strengthening SNP

Invert base pair in domain V

Include variations of interest

Include SNP in cre KO that doesn’t restore function

Include silent SNP in 3D pol amino acid substitution(s)

PATH

Summary of HTS development and validation

1. Unlike Sabin OPV2, reversion in the primary attenuation site (domain V) is not detected in nOPV2 vaccine lots nor in shed virus
2. Clinical data and molecular clones were used to establish “variations of interest”
3. Assay validated to quantitate variations of interest and exclude mutations in the modified regions of nOPV2
4. Assessments of precision, linearity, and lower limit of detection met expectations and pre-specified criteria
5. Positive and negative viral controls were developed and qualified
    - Opportunity for improved positive controls which can be handled outside containment
6. Caution is needed when using standard HTS to predict phenotype

PATH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THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

edqm

European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE

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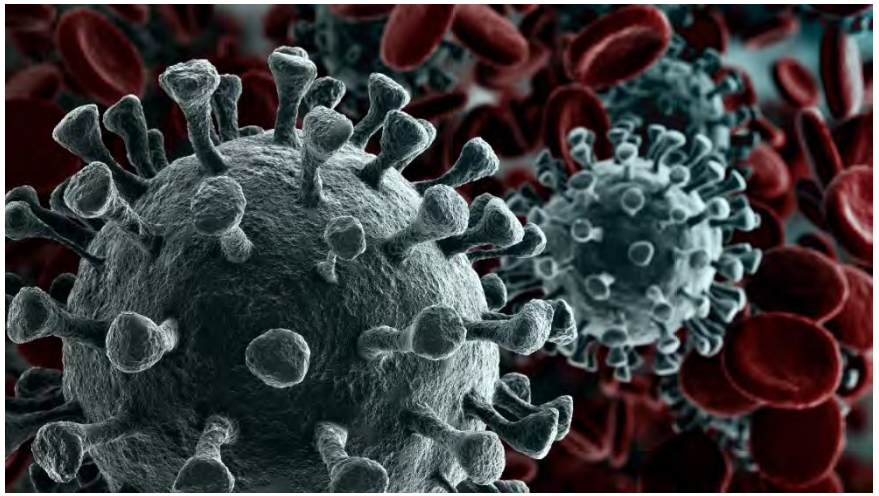
Points to consider in the validation of HTS in context of QC of OPV and IPV



WHO workshop on implementation of international standards for quality control of polio vaccines including OPV and IPV

31 Oct- 2 Nov 2023, Jakarta, Indonesia

Catherine Milne and Laurent Mallet, EDQM

Outline

- Introduction
- Different applications of High Throughput Sequencing (HTS) for Polio vaccines

- Parameters to be considered in the validation
  - For replacement of MAPREC
  - For whole genome molecular consistency
  - For adventitious viruses

- Update on the EDQM/Ph. Eur. Texts related to HTS
- Conclusion

Introduction: High Throughput Sequencing (HTS): What is it?

- Also called Next Generation Sequencing (NGS) or Massive Parallel Sequencing
- Sequencing of nucleic acids with high throughput, scalability and speed
- Different technologies
  - Short reads, long reads
  - Read length from a hundreds of nucleotides to 50+ Kb
- Allows:
  - Sensitive detection of viral sequences
  - Sensitive quantification of viral quasi-species

Different potential applications of HTS for Polio vaccines 1

In the context of absence of neurovirulence/molecular consistency

- sOPV:
  - Replacement of MAPREC test and/or for whole genome molecular consistency (Viral Seed lots*, Single Harvests (*may test*), Monovalent bulks)
    - In vivo test also required (A.3.2.3.3 Seeds: seed plus 3 consecutive monovalent bulks from a new seed, A.4.4.7.2 monovalent bulks: tested but may eventually be omitted if replaced by validated whole genome HTS)
- nOPV
  - Whole genome molecular consistency (Viral Seed lots*, Single Harvests (*may test*), Monovalent bulks)
    - A.3.2.3.4 Seed: in vivo neurovirulence also required but recommend whole genome HTS as replacement; A.4.4.7.3 monovalent bulk: if pre-clinical and clinical data supports in vivo can be omitted and only HTS is performed
- sIPV:
  - Whole genome molecular consistency (Viral Seed lots*, Purified monovalent pools**)
    - A.3.1.3.3 amended, Seeds: WGMC preferred, alternatively MAPREC or in vivo may be used, A.4.4.2.7 amended, Purified monovalent pools: may use WGMC or MAPREC and in vivo

** A.4.4.2.7.1 (amended) molecular consistency as an additional tool on a suitable number of monovalent pools

*and at least three consecutive monovalent pools/bulks

Different potential applications of HTS for Polio vaccines 2

In the context of detection of adventitious viruses

- wIPV, sIPV, nOPV, sOPV:
    - Cell banks,
    - Viral Seed lots
    - Single harvests

Parameters to be considered in the validation (1)

Replacement of MAPREC

- **Quantification** of specific mutations
  - 472-C in type 3 OPV, 480-A and 525-C for type 1, 481-G for type 2
- Corresponds to category of tests called "**Impurity/Other quantitative measurements**" in draft ICHQ2(R2) table
- Validation parameters to be considered:
  - ✓ Specificity
  - ✓ Working range including Quantitation limit,
  - ✓ Accuracy
  - ✓ Precision

Table 1: Typical performance characteristics and related validation tests for measured product attributes

Type of measured product attribute / Analytical Procedure Performance Characteristics to be demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY content/potency Other quantitative measurements (1)
		Quantitative	Limit	
Specificity (3) Specificity Test	+	+	+	+
Working Range Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

Published examples of qualification/validation:

- Sarcey (S) et al, 2017 Journal of Virological methods
  - OPV3: Samples: cDNA plasmids encoding the Sabin 472-T or 472-C revertant; 2 OPV3 bulks (routine and high 472-C), panel with WS (2) and monovalent bulks (19) in normal range, 1st International Low Virus RR (96/572), 1st International High Virus RR (96/578)
- Charlton (C) et. al. 2022 Journal of Infectious diseases
  - OPV3: Samples: cDNA plasmid: T7 RNAP driven, sabin T3 (472-U), 11 historic OPV3 bulks with known MAPREC, MNVT, TgmNVT data
- Mujumdar et. al. (M) – WHO/BS/2022.2438. OPV1 bulks (n=8, 6 plus duplicates) and OPV2 bulks (n=7, 6 plus duplicate) with known MAPREC data

Example Approaches from the papers

- Background error:
  - Sequence 472-T(U) plasmid to determine % 472-C (direct and PCR amplified) (C,S)
- Linearity/Specificity
  - Gradient mixtures 472-T with increasing 472-C content up to 1.5% (0.25% increments) – observed value compared to expected (S)
- Precision – repeatability/intermediate precision
  - 6 runs – with triplicates of a routine level bulk, highest level bulk, 96/572 and 96/578 (S)
  - 5 runs – all samples (C, M)
- Precision - reproducibility
  - Panel tested in 2 different laboratories with different platforms (MiSeq or HiSeq 1500) (S)
  - Panel tested in 8 laboratories with different NGS platforms (C) or 2 laboratories (M)
- Accuracy – correlation MAPREC – NGS
  - Panel of n=21 samples and the 2 International Virus References (S)
  - Panel of 11 OPV bulks (C)
  - Panel of 8 (T1) or 7 (T2) OPV bulks (M)
- Working range including Quantitation limit
  - From data accumulated in the Background error, Linearity and Correlation NGS versus MAPREC (S)

Parameters to be considered in the validation (2)

Whole genome molecular consistency

- **Quantitative** scan of the whole genome for Single Nucleotide Polymorphisms
- Presence/absence of specific adapted sequence (nOPV)
- Not really corresponding to category of tests described in ICHQ2
- **Principles related to "Impurity/Other quantitative measurements"**
  - Specificity
  - Working range including Quantitation limit,
  - Accuracy,
  - Precision

Table 1: Typical performance characteristics and related validation tests for measured product attributes

Type of measured product attribute / Analytical Procedure Performance Characteristics to be demonstrated (2)	Identity	Impurity (Purity) Other quantitative measurements (1)		Assay content/potency Other quantitative measurements (1)
		Quantitative	Limit	
Specificity (3)				
Specificity Test	+	+	+	+
Working Range				
Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4)				
Accuracy Test	-	+	-	+
Precision (4)				
Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

- Published example of qualification/validation: Konz et al, 2021, Vaccine
  - Samples: nOPV2 lot, sOPV2 reference derived from WHO reference 15/296 and nOPV2 spiked with sOPV2

Example Approaches from the paper

See detailed presentation; Case study: Establishing HTS for QC of nOPV2, J. Konz

Linearity, repeatability and intermediate precision

- Spiking Study:
  - nOPV2 spiked with sOPV2 (0, 0.5, 1, 2, 5, 10, 20, 100%)
  - 3 independent replicates x 2 days on different MiSeq;
  - % frequency of variant at each studied position (34 nOPV/sOPV variants plus 10 negative control sites) – calculated at all positions/spike levels
- 2 bio-informatic approaches compared
- Limit of detection
  - calculation of minimum spike genome fraction –taking into account false positive and negative and the variability
- Specificity (informal)
  - Nucleotides detected in replicates where expected and not detected where not expected according to known sequence of samples

Parameters to be considered in the validation (3)

Detection of adventitious viruses

- **Qualitative** detection of any unwanted viral sequences (agnostic/non-targeted approach) from known and unknown viruses
- Corresponds to category of tests called "**Impurity/Limit tests**" in draft ICHQ2(R2) table
- Validation parameters to be considered:
  - Specificity/breadth of detection
  - Sensitivity/detection limit

Table 1: Typical performance characteristics and related validation tests for measured product attributes

Type of measured product attribute / Analytical Procedure Performance Characteristics to be demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY content/potency
		Quantitative	Limit	Other quantitative measurements (1)
Specificity (3)				
Specificity Test	+	+	+	+
Working Range				
Suitability of Calibration model	-	+	-	+
Lower Range Limit verification	-	QL (DL)	DL	-
Accuracy (4)				
Accuracy Test	-	+	-	+
Precision (4)				
Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

- Published example of qualification/validation: Charlebois et al. 2020, npj Vaccines
  - Samples – 16 NIH model viruses plus 6 viruses of interest spiked into a viral vaccine crude harvest (live yellow fever vaccine) or a Vero cell substrate matrix.
- Development of Ph. Eur. HTS method validation chapter

Example Approaches from the paper

- Specificity/breadth of detection
  - Unequivocal detection of all 22 spiked viruses and lack of detection in negative samples
- Sensitivity/detection limit
  - 3 independent replicates over 2 spiking studies
  - Detection between 10^3 – 10^4 copies/mL for the viral vaccine crude harvest matrix (similar to PCR based methods)
  - At or below 0.01 viral genome copies per cell in the cell substrate matrix

General considerations for regular implementation

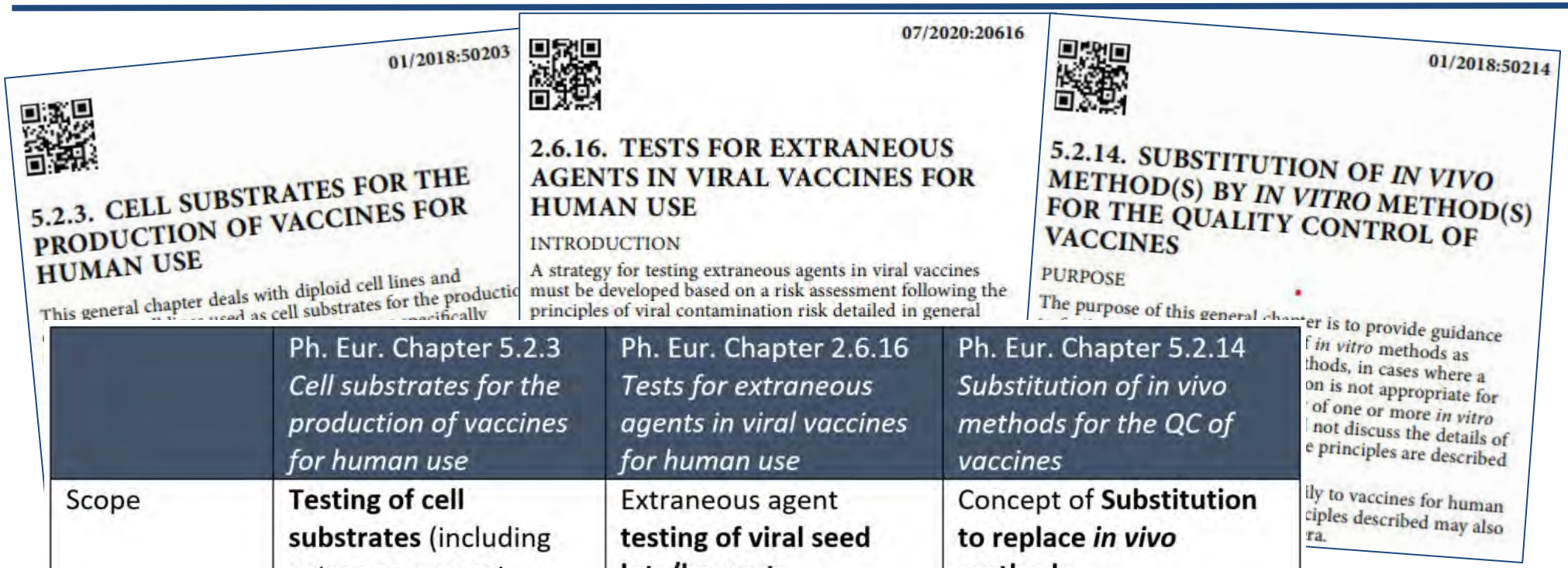
Experimental controls

- **Neurovirulence / MAPREC replacement / molecular consistency**
  - Samples with expected levels of defined signal (+ and -)
  - Controls for sample preparation – Nucleic acid extraction

- **Adventitious agents**
  - WHO Reference material prepared by FDA
    - WHO IRR for Adventitious Virus Detection in Biological Products using HTS (5 live viruses representing a diverse panel of enveloped/non-enveloped dsRNA/ssRNA/DNA) – adopted in October 2020
    - Additional IRR project proposed at ECBS 2023 to make more of the 5 existing and add 2 new viruses to the panel

- Update on the EDQM/Ph. Eur. Texts related to HTS

Evolution of Ph. Eur. chapters for vaccines



01/2018:50203

5.2.3. CELL SUBSTRATES FOR THE PRODUCTION OF VACCINES FOR HUMAN USE

This general chapter deals with diploid cell lines and

07/2020:20616

2.6.16. TESTS FOR EXTRANEOUS AGENTS IN VIRAL VACCINES FOR HUMAN USE

INTRODUCTION

A strategy for testing extraneous agents in viral vaccines must be developed based on a risk assessment following the principles of viral contamination risk detailed in general

01/2018:50214

5.2.14. SUBSTITUTION OF *IN VIVO* METHOD(S) BY *IN VITRO* METHOD(S) FOR THE QUALITY CONTROL OF VACCINES

PURPOSE

The purpose of this general

er is to provide guidance
in vitro methods as
thods, in cases where a
on is not appropriate for
of one or more *in vitro*
not discuss the details of
e principles are described

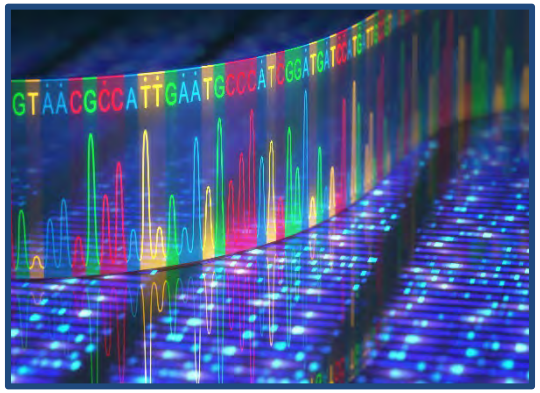
ily to vaccines for human
ciples described may also
ra.

	Ph. Eur. Chapter 5.2.3 *Cell substrates for the production of vaccines for human use*	Ph. Eur. Chapter 2.6.16 *Tests for extraneous agents in viral vaccines for human use*	Ph. Eur. Chapter 5.2.14 *Substitution of in vivo methods for the QC of vaccines*
Scope	**Testing of cell substrates** (including extraneous agent testing)	Extraneous agent **testing of viral seed lots/harvests**	Concept of **Substitution to replace *in vivo* methods**
Year introduced or year of last major update	July 2017 (Ph. Eur. Suppl. 9.3)	July 2017 (Ph. Eur. Suppl. 9.3)	July 2017 (Ph. Eur. Suppl. 9.3)

- Revision of chapters 5.2.3 & 2.6.16
- Elaboration of chapter 5.2.14 (concept of Substitution)

Use of specific and broad molecular methods

	Chapter 5.2.3 (cell substrates)	Chapter 2.6.16 (viral seed lots/harvests)	Chapter 5.2.14 (substitution of *in vivo* assays)
Test and method	**Tests for specific viruses** by NAT (e.g. PCR)		Test for extraneous agents: Considerations for the substitution of *in vivo* methods by broad molecular methods
	Tests for viruses using broad molecular methods (e.g. HTS) - As an alternative to *in vivo* tests and specific NAT, or - In addition/as an alternative to *in vitro* cell culture tests		
Year introduced	NAT, broad molecular methods: 2017		2017



→ The use of molecular methods is foreseen in the Ph. Eur.!

Perspectives on HTS

- Ph. Eur. chapters 5.2.3 & 2.6.16 mention HTS and foresee its use as part of the testing strategy for extraneous agents

- However, HTS methods are currently not described in detail in any regulatory document and no guidance for their validation is available

- The availability of regulatory standards including validation guidelines in the Ph. Eur. will serve as a reference for regulators and manufacturers, while:
  - HTS is planned to be introduced in the revised ICH Q5A guideline (*Viral safety evaluation of biotechnology products*)
  - FDA has recently developed panels of viruses as reference preparations for HTS (adopted by WHO ECBS)

Elaboration of a Ph. Eur. chapter on HTS

- *"High Throughput Sequencing for the detection of extraneous agents in biological products* (2.6.41)"
- Non-binding general chapter
- Proposed content: description of the technology/methods and of the HTS workflow, **guidelines for validation of HTS methods**

- Under elaboration by Ph. Eur.'s HTS Working Party

  (international group of regulators, OMCLs and industry from Europe, US, Canada)

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Introduction

- Place of HTS in a consolidated analytical strategy for extraneous agents detection, risk assessment
- Scope / Considerations for specific product classes : human/vet vaccines, gene therapy vectors, cell therapy, recombinant proteins
- Different HTS technologies
- Targeted vs broad detection
- Approaches: genomics, transcriptomics, viromics
- Type of samples / matrices: viral seeds and harvests, cell banks, raw materials, other biological samples

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Part 1: Description of the method(s)

- Sample pre-treatment
- Extraction of nucleic acids
- Enrichment of nucleic acids
- Library preparation
- Sequencing
  - short reads and long reads
- Data analysis (bioinformatics) using databases
- Scientific evaluation of the results (reporting/interpretation)
  - Validity criteria of the run
  - Control samples, suitability test
- Follow-up investigation

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Part 2: Validation (guidelines for method validation)

- General method validation
  - Cannot strictly follow ICH Q2 approach
  - “Modular” approach
  - Validation parameters of the analytical method
    - Breadth of detection (specificity)
    - Sensitivity / LOD
    - Others?
- Product-specific validation

Elaboration of a Ph. Eur. chapter on HTS – draft outline

Part 2: Validation (guidelines for method validation)

- Selection of appropriate spiking material for validation
  - Should be well characterised, relevant to the type of analysis (genomics vs viromics vs transcriptomics)
    - Viruses
    - Nucleic acids
    - Infected cells
- Replacement/substitution of existing assays for extraneous agents

Conclusion

- HTS is a powerful innovative tool
- A diversity of applications are possible
  - Quantifies sNPs
  - Monitors whole genome molecular consistency
  - Detects adventitious viruses
- A few examples of validation approaches for each application
- Increasingly supported by document and physical standards
- Provides an opportunity to apply an advanced technical approach and reduce the use of animal testing

Thank you for your attention

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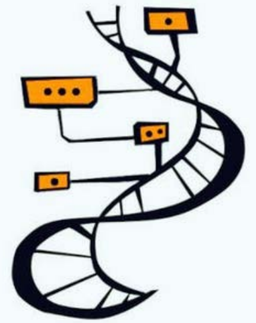
Bioinformatics Considerations for Analysis of HTS data

Julia Panov, PhD

Tauber Bioinformatics Research Center,
University of Haifa, Israel



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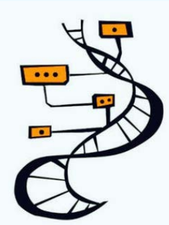
Bioinformatics Considerations for Analysis of HTS data

- Overview of HTS
- Basic steps of analysis: considerations for choosing algorithms and software
- Presentation of a code-free pipeline for analysis of HTS vaccine data



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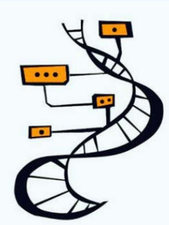
Two uses of HTS data

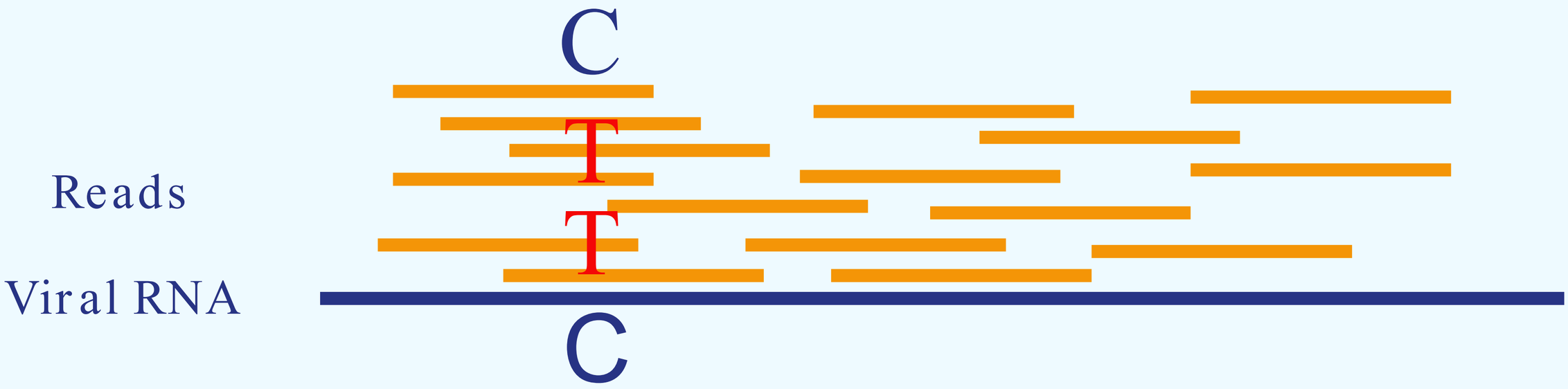
- 5'-UTR mutations → MAPREC
- Whole genome mutation profiles → animal neurovirulence tests



HTS: Library preparation

Sequencing

fastQ


C
T
T
Reads
Viral RNA
C

Mutation Variant	Sample 1	Sample 2	Sample 3
472 T>C			
2943 C>T			
4872 A>G			

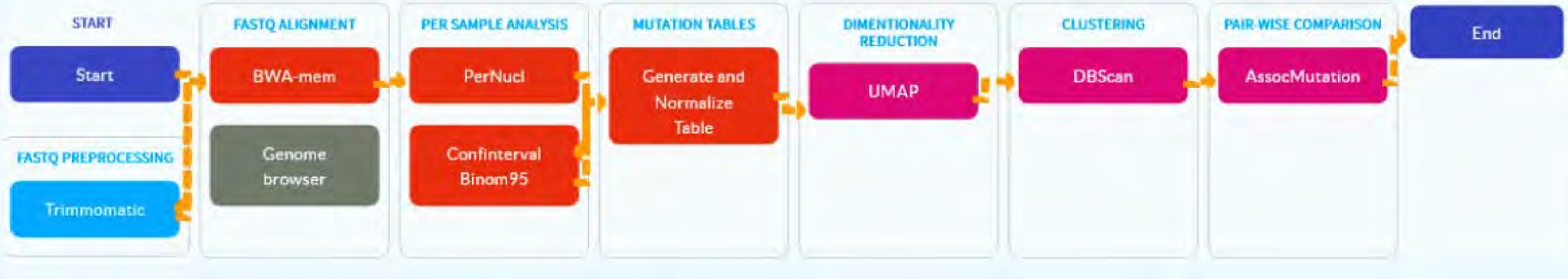
Mutation Variant	Sample 1	Sample 2	Sample 3
472 T>C	0.0002	0.0001	0.00005
2943 C>T	0.1	0.32	0.05
4872 A>G	0.003	0.006	0.01

Steps of Analysis

1. Quality of HTS reads
2. Alignment
3. Variant Calling
4. Statistical Analysis

VacQC

VacMut

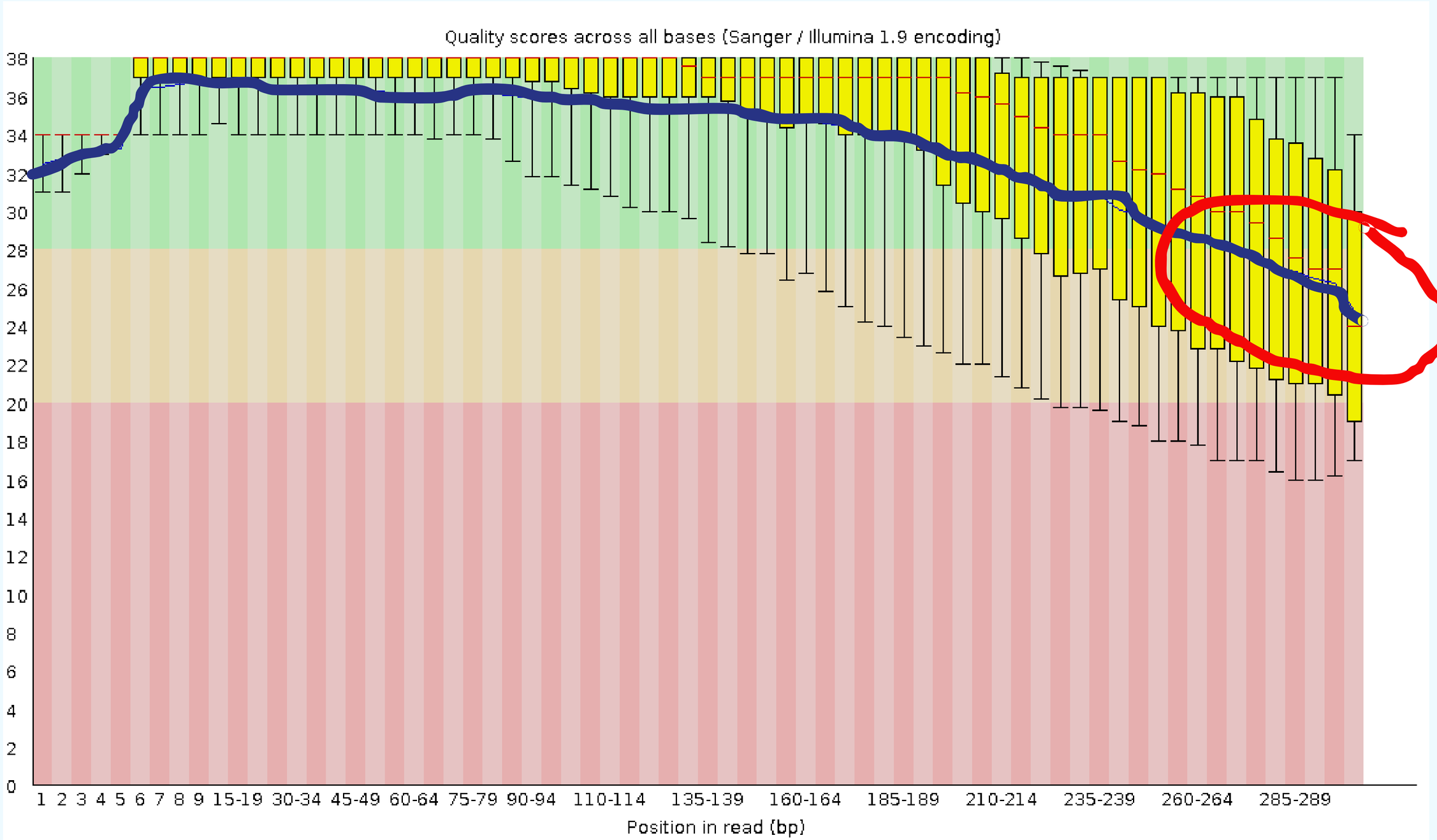


Steps of Analysis

1. Quality of HTS reads

- Visualization
- Cleaning
- Contamination

Visualization of raw reads: FastQC



Cleaning HTS reads: Adapters and low-quality bases

TRIMMOMATIC

cutadapt

BBDuk

Assessing the Impact of Data Preprocessing on Analyzing Next Generation Sequencing Data

Binsheng He[1†], Rongrong Zhu[2†], Huandong Yang[3], Qingqing Lu[4], Weiwei Wang[4], Lei Song[4], Xue Sun[4], Guandong Zhang[4], Shijun Li[5], Jialiang Yang[1,4], Geng Tian[4*], Pingping Bing[1*] and Jidong Lang[4*]*

https://www.frontiersin.org/articles/10.3389/fbioe.2020.00817/full

Contamination

nature biotechnology

PERSPECTIVE

https://doi.org/10.1038/s41587-020-0507-2

Check for updates

Viral contamination in biologic manufacture and implications for emerging therapies

Paul W. Barone[1], Michael E. Wiebe[1], James C. Leung[1], Islam T. M. Hussein[1], Flora J. Keumurian[1], James Bouressa[2,25], Audrey Brussel[3,26], Dayue Chen[4,27], Ming Chong[5], Houman Dehghani[6,28], Lionel Gerentes[7], James Gilbert[8,29], Dan Gold[9], Robert Kiss[10,30], Thomas R. Kreil[11], René Labatut[3], Yuling Li[12,31], Jürgen Müllberg[13], Laurent Mallet[7,32], Christian Menzel[14], Mark Moody[15,33], Serge Monpoeho[16], Marie Murphy[4], Mark Plavsic[17,34], Nathan J. Roth[18], David Roush[19], Michael Ruffing[20], Richard Schicho[21,35], Richard Snyder[22], Daniel Stark[23], Chun Zhang[24,36], Jacqueline Wolfrum[1], Anthony J. Sinskey[1] and Stacy L. Springs[1]✉

Recombinant protein therapeutics, vaccines, and plasma products have a long record of safety. However, the use of cell culture to produce recombinant proteins is still susceptible to contamination with viruses. These contaminations cost millions of dollars to recover from, can lead to patients not receiving therapies, and are very rare, which makes learning from past events difficult. A consortium of biotech companies, together with the Massachusetts Institute of Technology, has convened to collect data on these events. This industry-wide study provides insights into the most common viral contaminants, the source of those contaminants, the cell lines affected, corrective actions, as well as the impact of such events. These results have implications for the safe and effective production of not just current products, but also emerging cell and gene therapies which have shown much therapeutic promise.

https://www.nature.com/articles/s41587-020-0507-2

VirFinder

KrakenUniq

K-Mer stats

Contamination: k-mer based approach

GGCTCAATGCAGTGATGTGCCTACACATCAGTTTTACCCTAGCCTGCACTGGGTTATGGC

GGCTCAATGCAGTGATGTGCCTACACATCAGTTTTACCCTAGCCTGCACTGGGTTATGGCATTTTAGTAGGTGGTAGTTGTAGTAATTATTAGT

Virology | Research Article | 21 April 2021

k-mer-Based Metagenomics Tools Provide a Fast and Sensitive Approach for the Detection of Viral Contaminants in Biopharmaceutical and Vaccine Manufacturing Applications Using Next-Generation Sequencing

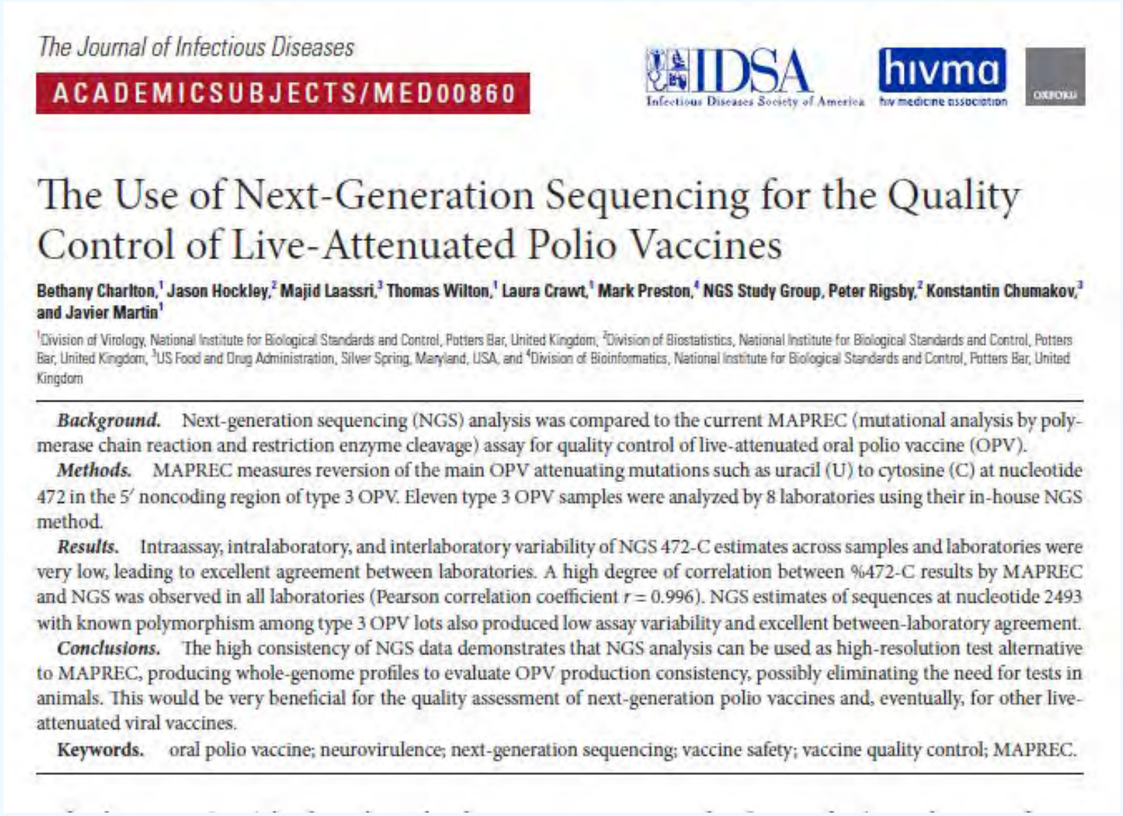
Authors: Madolyn L. MacDonald, Shawn W. Polson, Kelvin H. Lee | AUTHORS INFO & AFFILIATIONS

ABSTRACT

Adventitious agent detection during the production of vaccines and biotechnology-based medicines is of critical importance to ensure the final product is free from any possible viral contamination. Increasing the speed and accuracy of viral detection is beneficial as a means to accelerate development timelines and to ensure patient safety. Here, several rapid viral metagenomics approaches were tested on simulated next-generation sequencing (NGS) data sets and existing data sets from virus spike-in studies done in CHO-K1 and HeLa cell lines. It was observed that these rapid methods had comparable sensitivity to full-read alignment methods used for NGS viral detection for these data sets, but their specificity could be improved. A method that first filters host reads using KrakenUniq and then selects the virus classification tool based on the number of remaining reads is suggested as the preferred approach among those tested to detect nonlatent and nonendogenous viruses. Such an approach shows reasonable sensitivity and specificity for the data sets examined and requires less time and memory as full-read alignment methods.

https://journals.asm.org/doi/10.1128/msphere.01336-20

Data



The Journal of Infectious Diseases

ACADEMICSUBJECTS/MED00860

The Use of Next-Generation Sequencing for the Quality Control of Live-Attenuated Polio Vaccines

Bethany Charlton,[1] Jason Hockley,[2] Majid Laassri,[3] Thomas Wilton,[1] Laura Crawt,[1] Mark Preston,[4] NGS Study Group, Peter Rigsby,[2] Konstantin Chumakov,[3] and Javier Martin[1]

[1]Division of Virology, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [2]Division of Biostatistics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom, [3]US Food and Drug Administration, Silver Spring, Maryland, USA, and [4]Division of Bioinformatics, National Institute for Biological Standards and Control, Potters Bar, United Kingdom

Background. Next-generation sequencing (NGS) analysis was compared to the current MAPREC (mutational analysis by polymerase chain reaction and restriction enzyme cleavage) assay for quality control of live-attenuated oral polio vaccine (OPV).

Methods. MAPREC measures reversion of the main OPV attenuating mutations such as uracil (U) to cytosine (C) at nucleotide 472 in the 5′ noncoding region of type 3 OPV. Eleven type 3 OPV samples were analyzed by 8 laboratories using their in-house NGS method.

Results. Intraassay, intralaboratory, and interlaboratory variability of NGS 472-C estimates across samples and laboratories were very low, leading to excellent agreement between laboratories. A high degree of correlation between %472-C results by MAPREC and NGS was observed in all laboratories (Pearson correlation coefficient $r = 0.996$). NGS estimates of sequences at nucleotide 2493 with known polymorphism among type 3 OPV lots also produced low assay variability and excellent between-laboratory agreement.

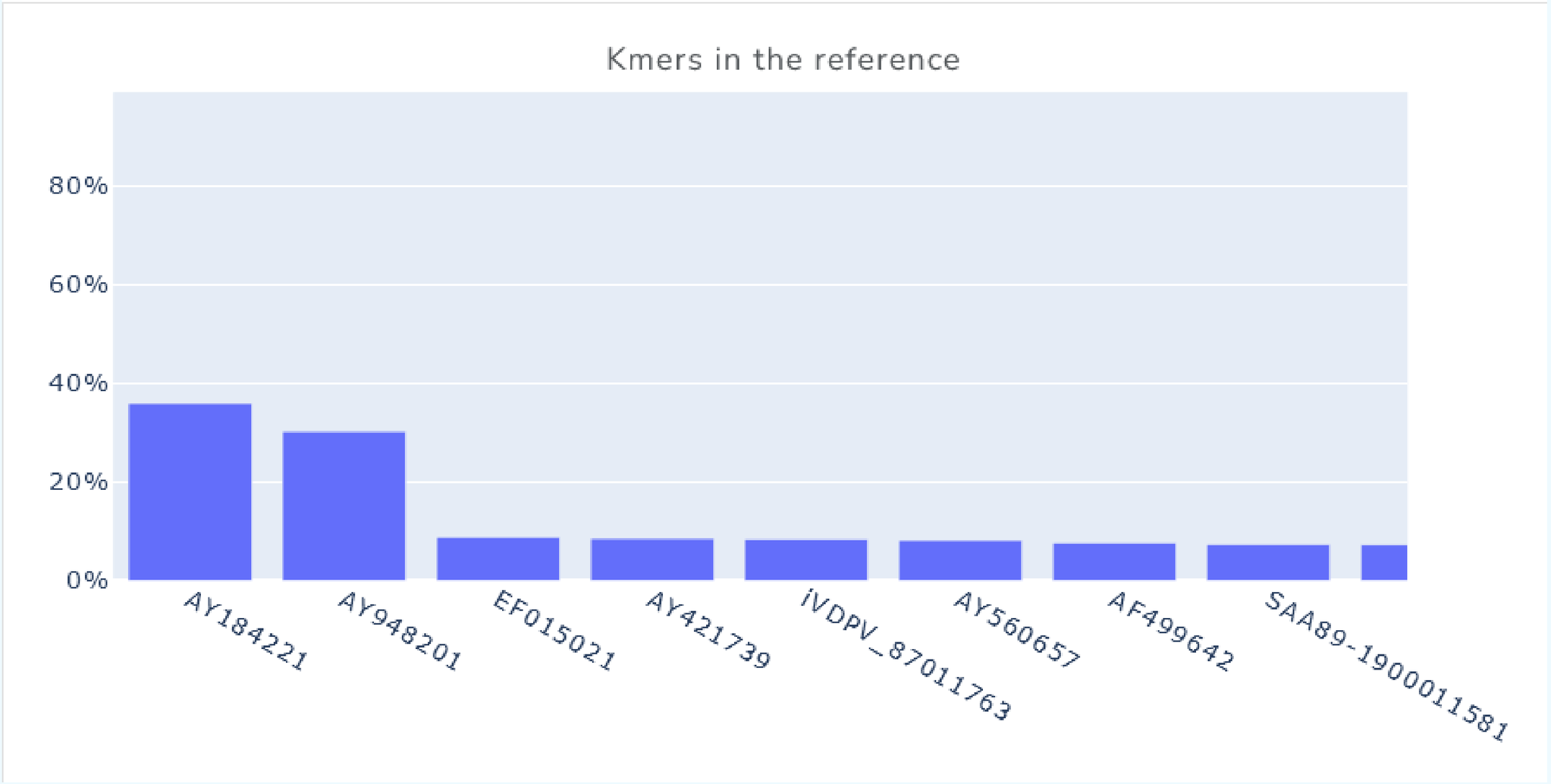
Conclusions. The high consistency of NGS data demonstrates that NGS analysis can be used as high-resolution test alternative to MAPREC, producing whole-genome profiles to evaluate OPV production consistency, possibly eliminating the need for tests in animals. This would be very beneficial for the quality assessment of next-generation polio vaccines and, eventually, for other live-attenuated viral vaccines.

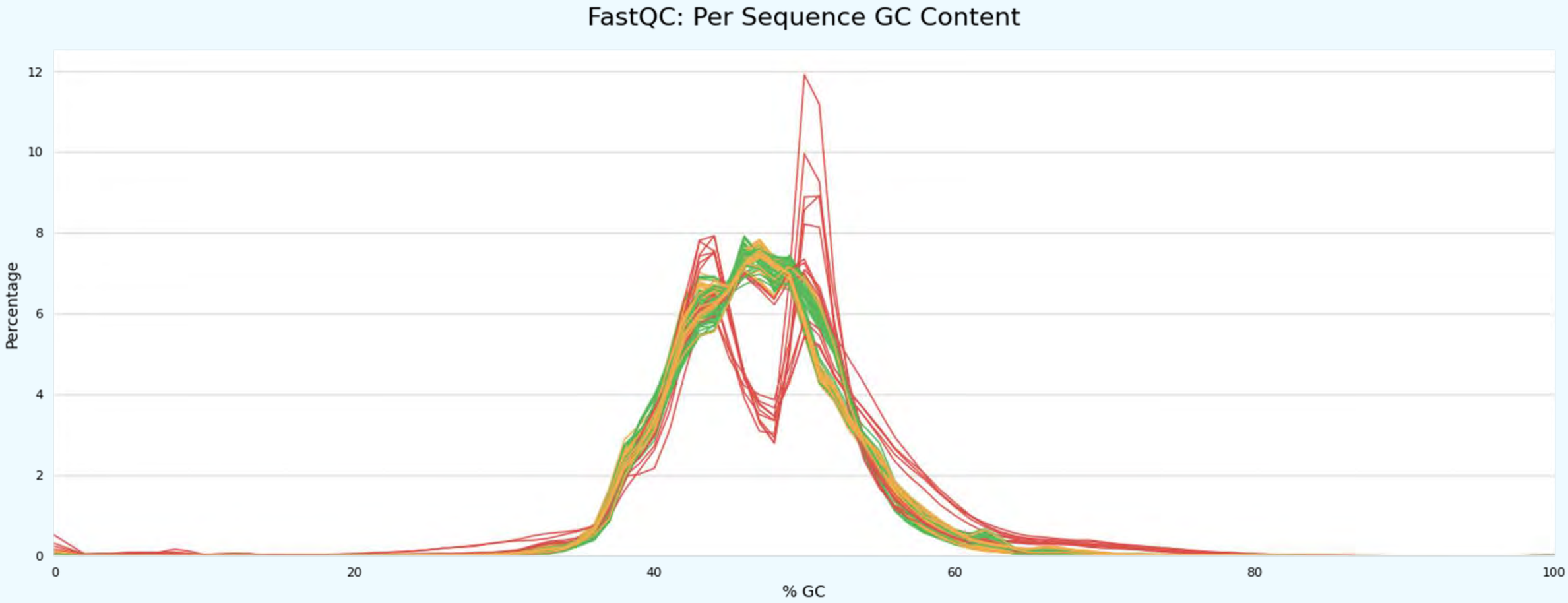
Keywords. oral polio vaccine; neurovirulence; next-generation sequencing; vaccine safety; vaccine quality control; MAPREC.

https://academic.oup.com/jid/article/222/11/1920/5850979

Sample Code	Description	Result		
		MAPREC	MNVT	TgmNVT
3A	SO+3	Pass	Pass	Pass
3B	High MAPREC ref	-	-	-
3C	SO+3	Fail	Fail	Fail
3D	SO+3	Pass	Pass	Pass
3E	Low MAPREC ref	-	-	-
3F	RSO3	Fail	Fail	Pass
3G	RSO3	Pass	Pass	Pass
3H	RSO3	Pass	Pass	Pass
3I	High MAPREC ref	-	-	-
3J	SO+3	Fail	Fail	Fail
3K	cDNA-derived	Pass	Pass	Pass

3G_5_GGAGGAAT_L007_1



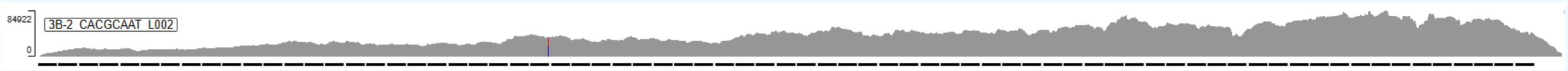

FastQC: Per Sequence GC Content
Percentage
0
2
4
6
8
10
12
0
20
40
60
80
100
% GC

Example: Alignment on genome

https://igv.tauberbioinformatics.org/igv/3625566-e1453ece224a29b1

Sample 3G

Sample 3B



Sample 3A

Steps of Analysis

VacQC

1. Quality of HTS reads

- Visualization
- Cleaning
- Contamination

VacQC

OUTPUT FILES

DOWNLOAD PIPELINE OUTPUT FILES

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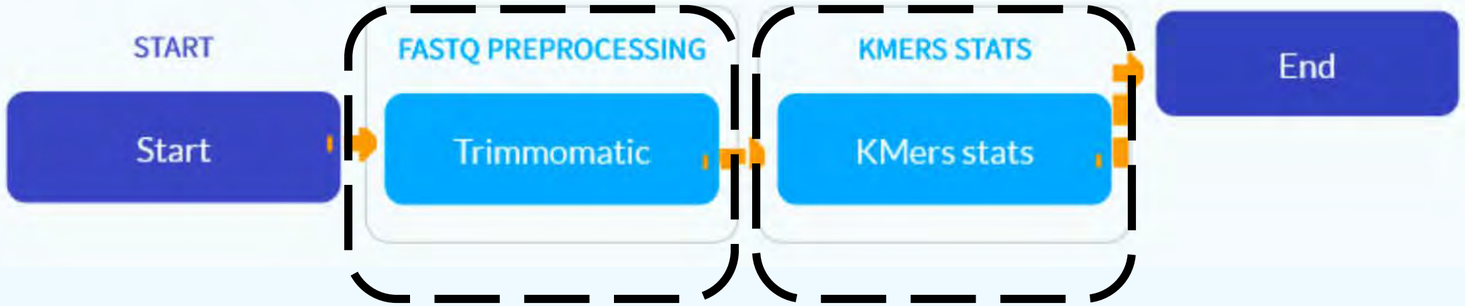
MultiQC Report

Dashboard

PIPELINE GRAPH

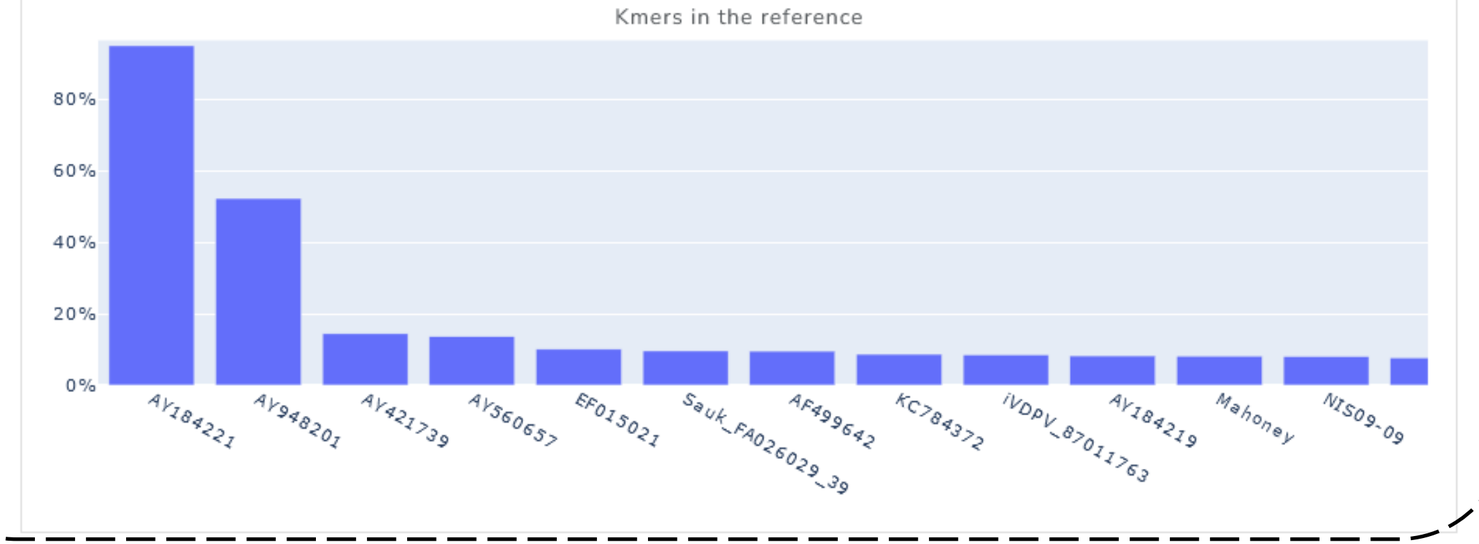


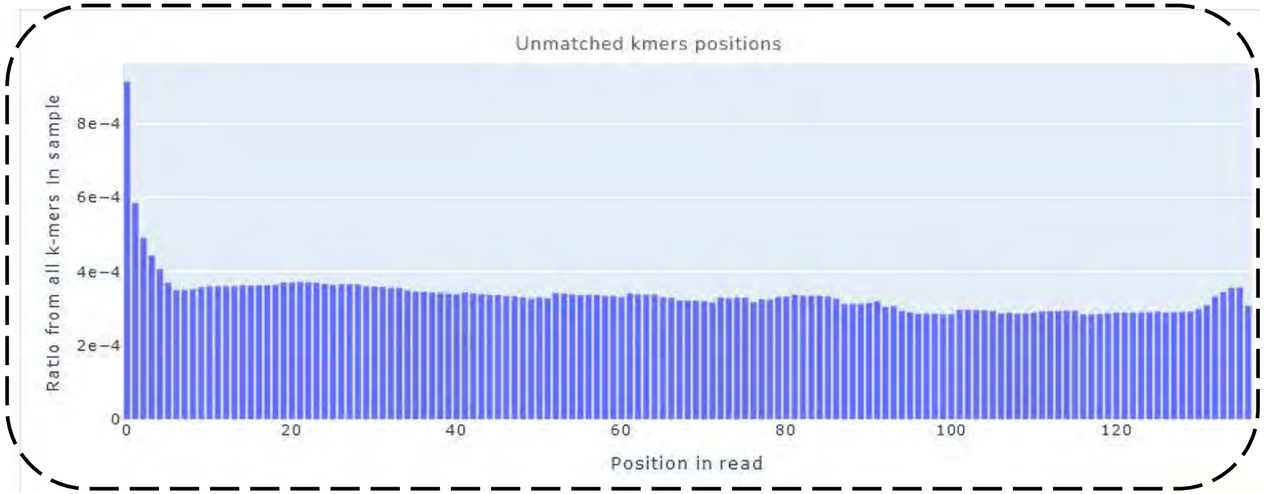
VacQC Dashboard

KMers Statistics

Download results

3A-1_CAACTCCA_L001_1

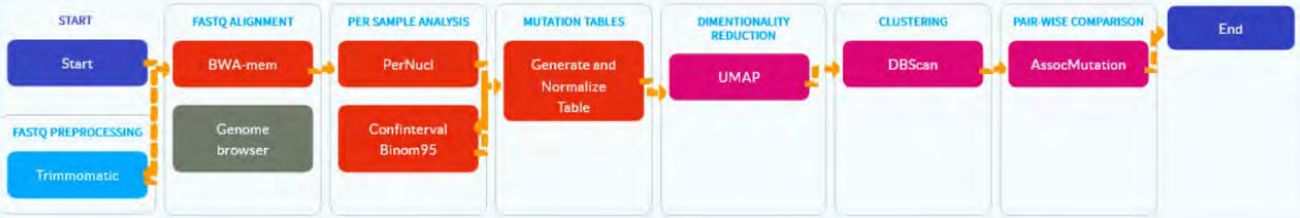




Steps of Analysis

1. Quality of HTS reads

VacMut



2. Alignment

3. Variant Calling

4. Statistical Analysis

Alignment

BWA-mem

Bowtie2

Sailfish

Alignment

Original Article | Published: 16 June 2021

New evaluation methods of read mapping by 17 aligners on simulated and empirical NGS data: an updated comparison of DNA- and RNA-Seq data from Illumina and Ion Torrent technologies

Luigi Donato, Concetta Scimone, Carmela Rinaldi, Rosalia D'Angelo ✉ & Antonina Sidoti

Neural Computing and Applications **33**, 15669–15692 (2021) | Cite this article

https://link.springer.com/article/10.1007/s00521-021-06188-z

Variant Calling

Variant Calling

Variant Calling

GATK

VarScan

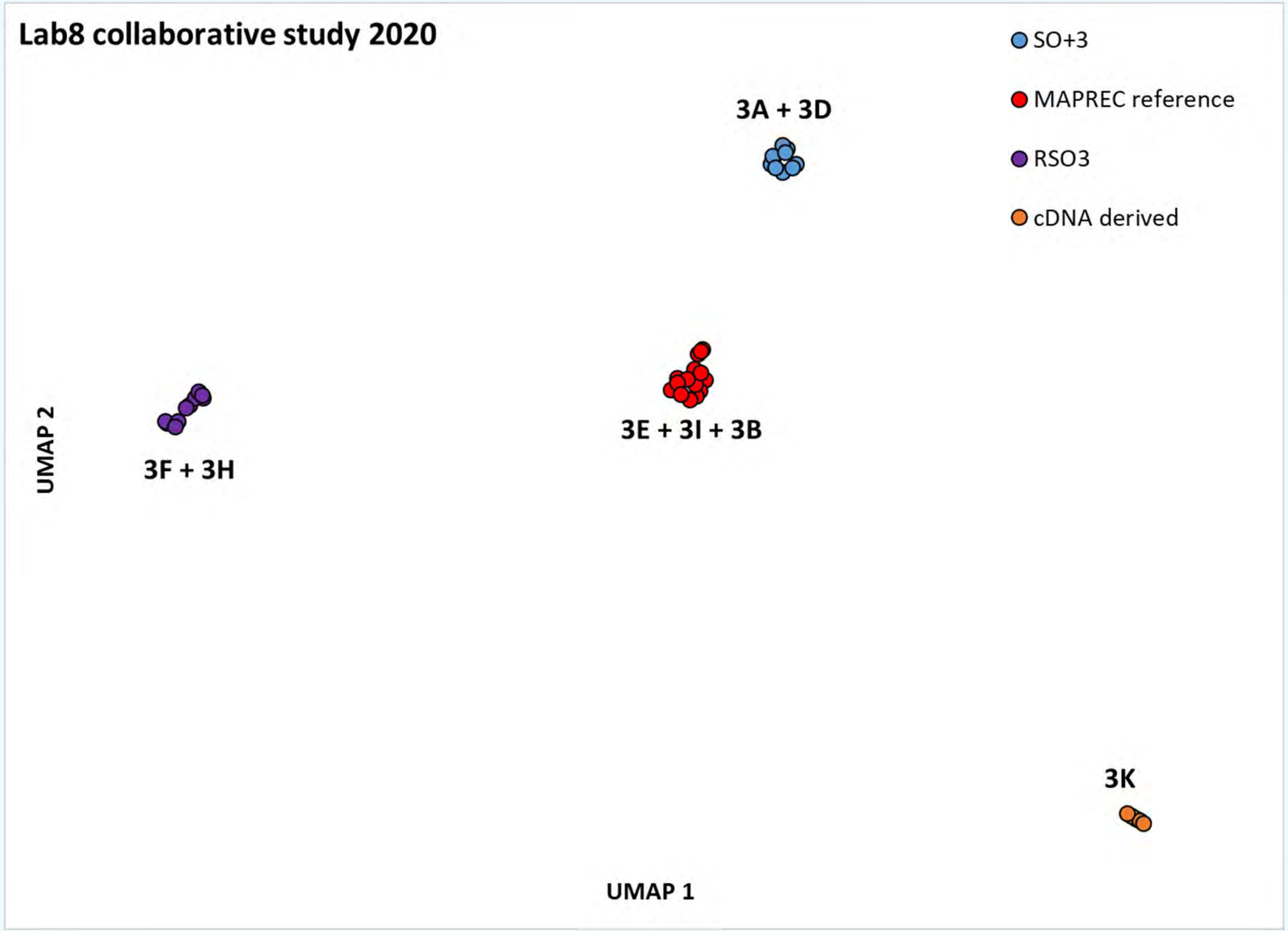
PerNucl

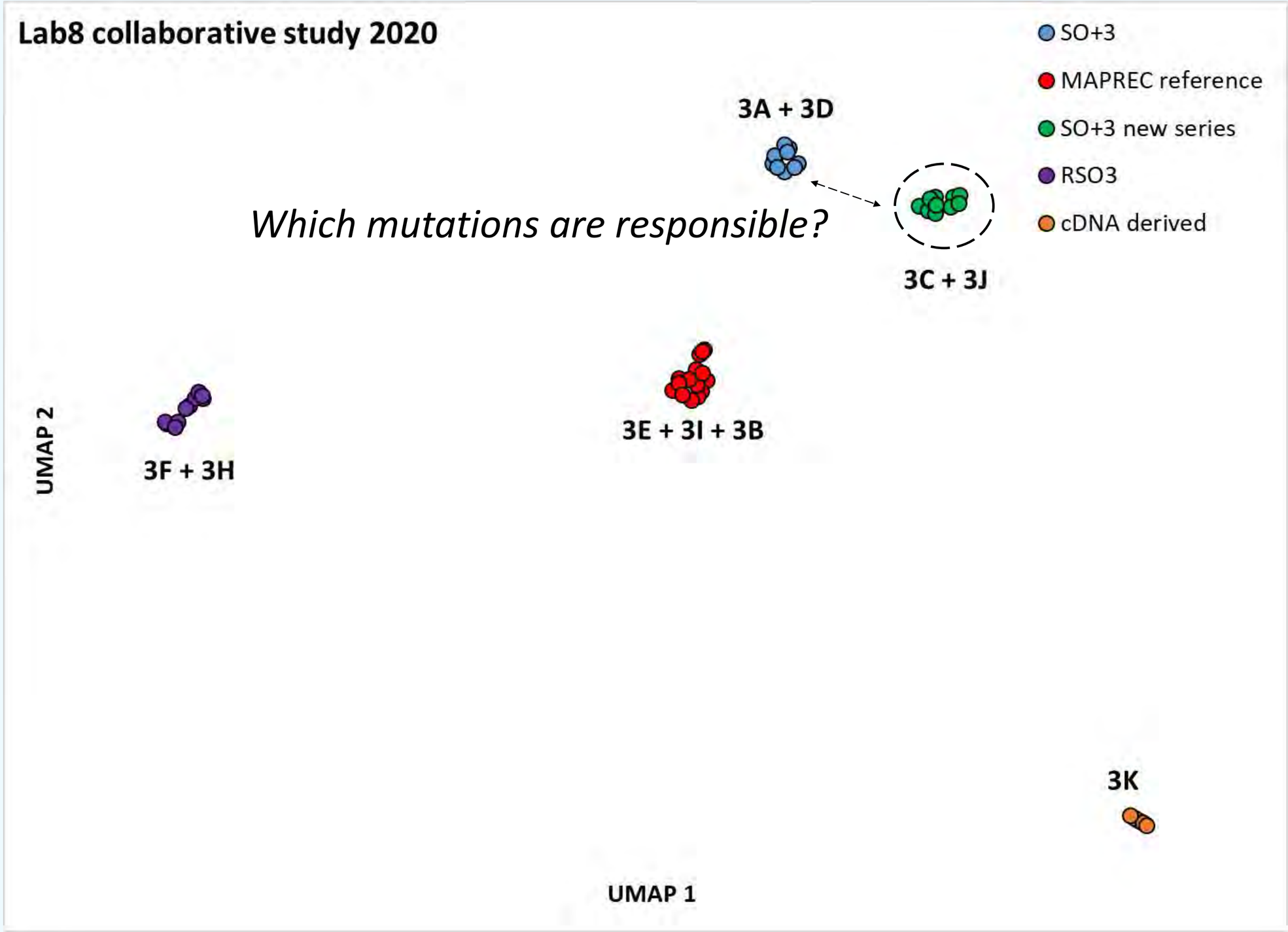
https://www.medrxiv.org/content/10.1101/2022.06.06.22275866v2.full

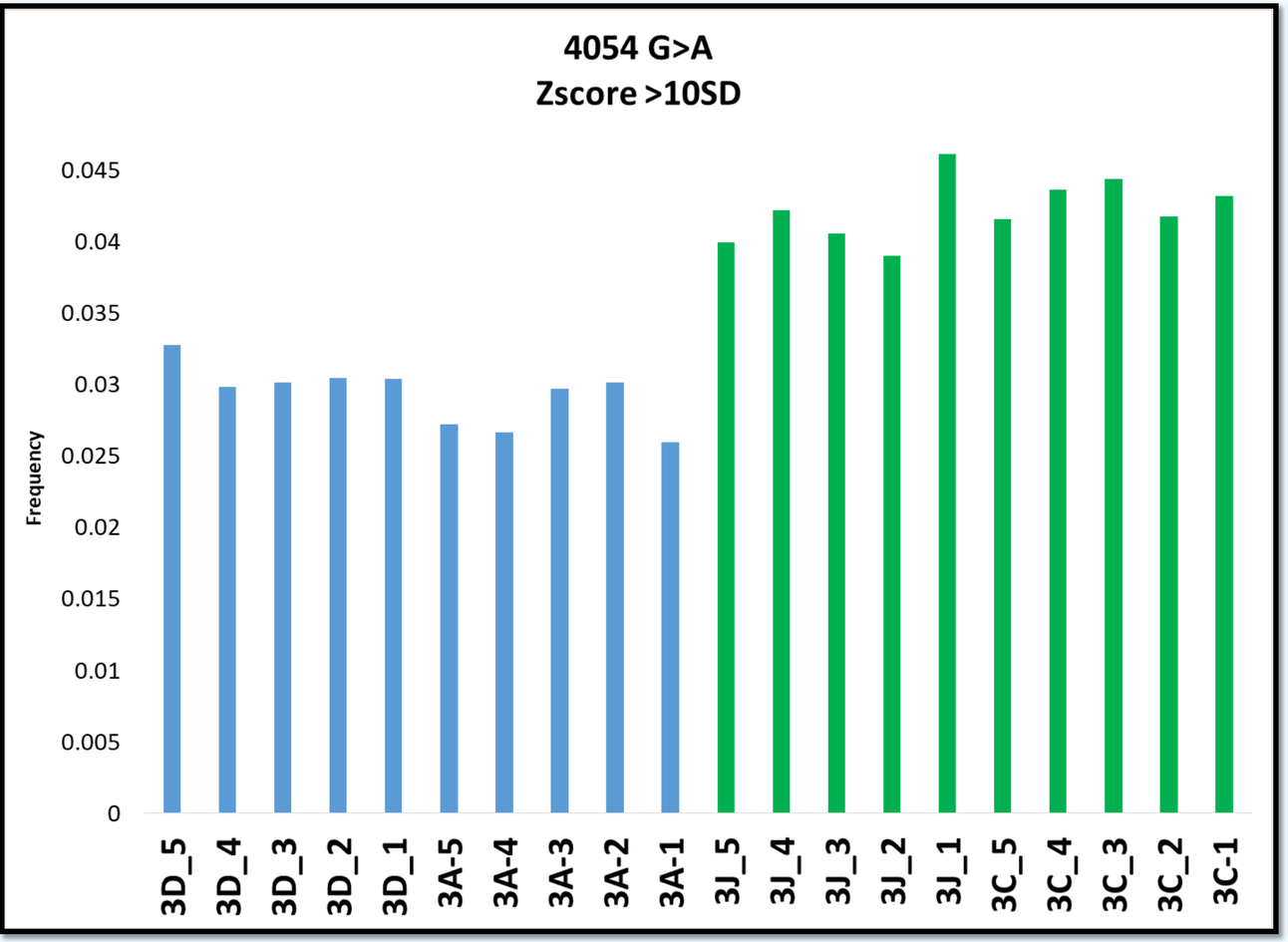
Variant Calling

Variant	Sample 1	Sample 2	Sample 3
472 T>C	0.0002	0.0001	0.00005
2943 C>T	0.1	0.32	0.05
4872 A>G	0.003	0.006	0.01

Statistical Analysis




Lab8 collaborative study 2020
SO+3
MAPREC reference
SO+3 new series
RSO3
cDNA derived
3A + 3D
Which mutations are responsible?
3C + 3J
3E + 3I + 3B
3F + 3H
3K
UMAP 2
UMAP 1


4054 G>A
Zscore >10SD
Frequency
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
3D_5
3D_4
3D_3
3D_2
3D_1
3A-5
3A-4
3A-3
3A-2
3A-1
3J_5
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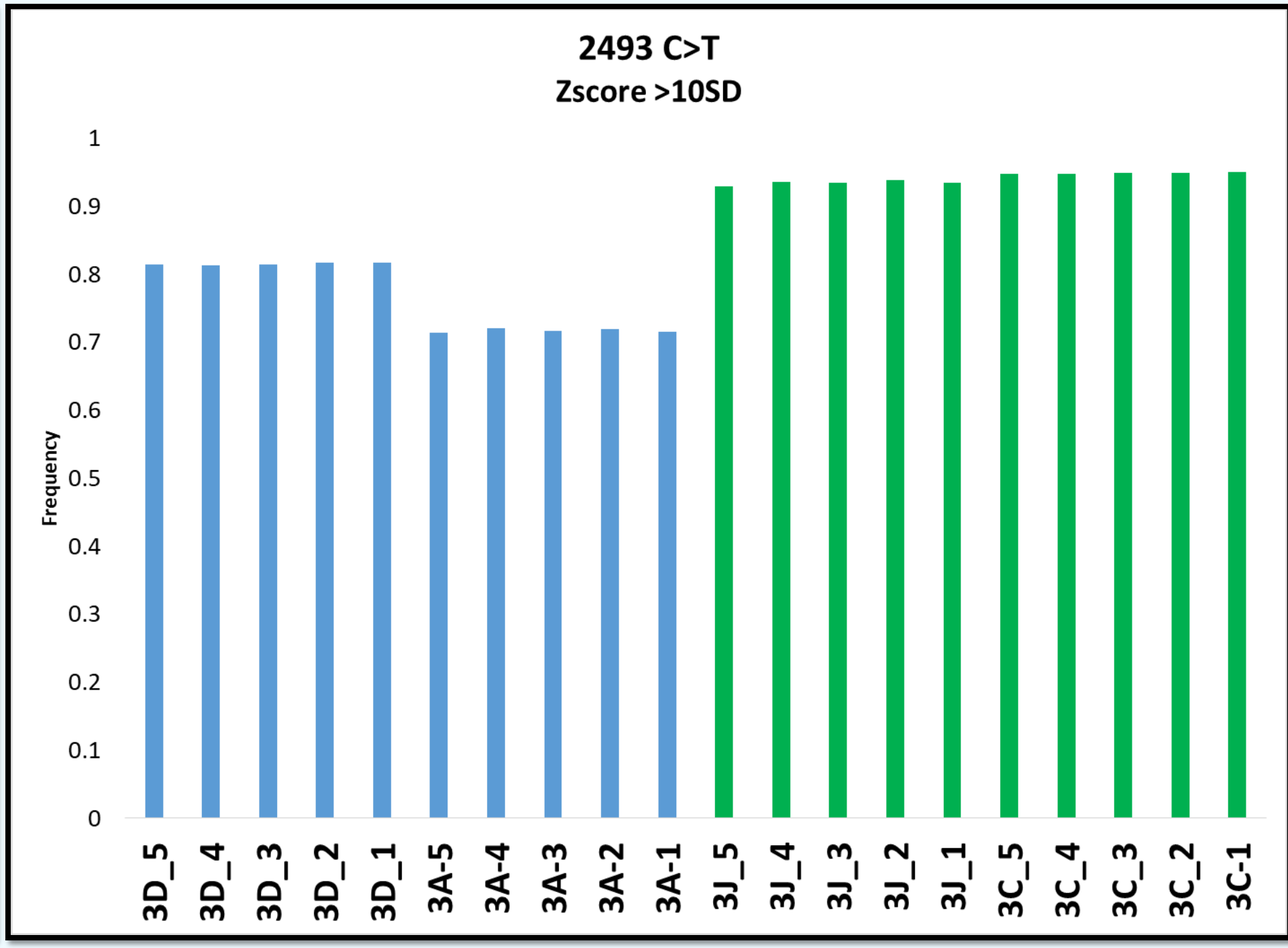
3J_2
3J_1
3C_5
3C_4
3C_3
3C_2
3C-1


2493 C>T
Zscore >10SD
Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3D_5
3D_4
3D_3
3D_2
3D_1
3A-5
3A-4
3A-3
3A-2
3A-1
3J_5
3J_4
3J_3
3J_2
3J_1
3C_5
3C_4
3C_3
3C_2
3C-1

VacMut

OUTPUT FILES

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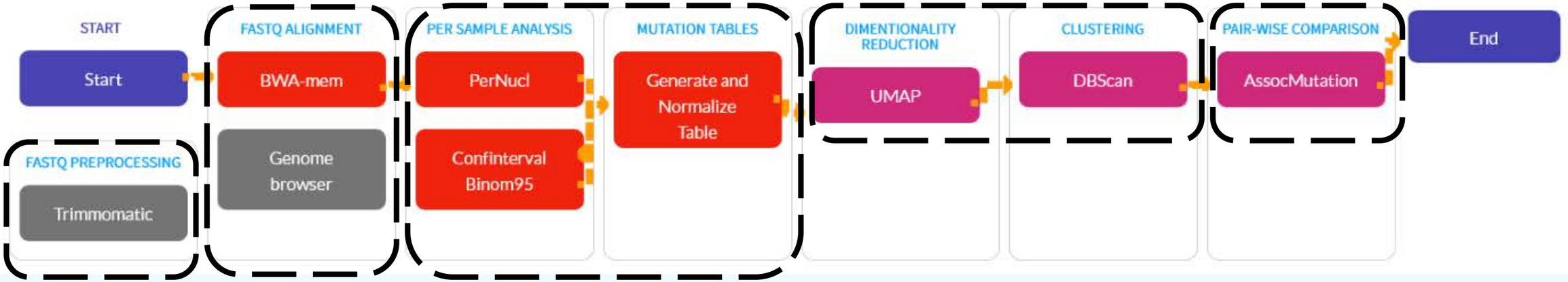
DOWNLOAD JOBS TEMPORARY FILES

DOWNLOAD ALL FILES

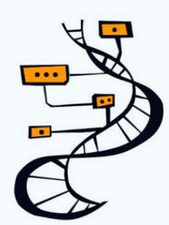
Dashboard

PIPELINE GRAPH



SUMMARY

- Cleaning of raw reads
- Alignment on genome
- Variant calling
- Clustering of samples
- Identifying differential mutations between clusters



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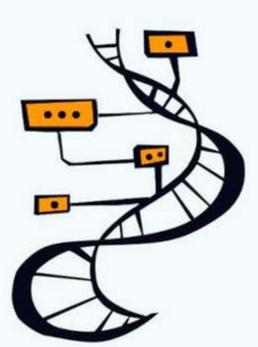
Pipeline demonstration



IMPORTANT LINKS

T-BioInfo Platform: http://tauber-data2.haifa.ac.il:3000/

Login: PATH
Password: PATH-2023

VacMut: http://tauber-data2.haifa.ac.il:3000/pipelinesunsupervisedsimilarityanalysis/

VacQC: http://tauber-data2.haifa.ac.il:3000/pipelinespathpreprocessing/

Instructions: https:/ / path.tauberbioinformatics.org/ assets/ instructions/ mut_pipeline.pdf

https:/ / path.tauberbioinformatics.org/ assets/ instructions/ qc_pipeline.pdf

Data (demo): https:/ / drive.google.com/ drive/ folders/ 1r-Prn0Z1r625Q63sUBUXvvo5xpRzZZaw?usp=sharing

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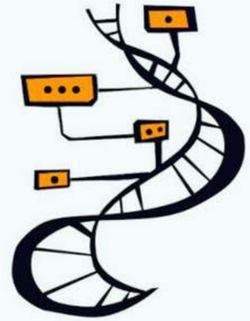
Thank you

Questions



IMPORTANT LINKS

T-BioInfo Platform: http://tauber-data2.haifa.ac.il:3000/

Login: jpanov@univ.haifa.ac.il
Password: PATH-2023

VacMut: http://tauber-data2.haifa.ac.il:3000/pipelinesunsupervisedsimilarityanalysis/

VacQC: http://tauber-data2.haifa.ac.il:3000/pipelinespathpreprocessing/

Instructions: https://path.tauberbioinformatics.org/assets/instructions/mut_pipeline.pdf

https://path.tauberbioinformatics.org/assets/instructions/qc_pipeline.pdf

Data (demo): https://drive.google.com/drive/folders/1r-Prn0Z1r625Q63sUBUXvvo5xpRzZZaw?usp=sharing

Session IV. Standardization of potency tests

Medicines & Healthcare products Regulatory Agency

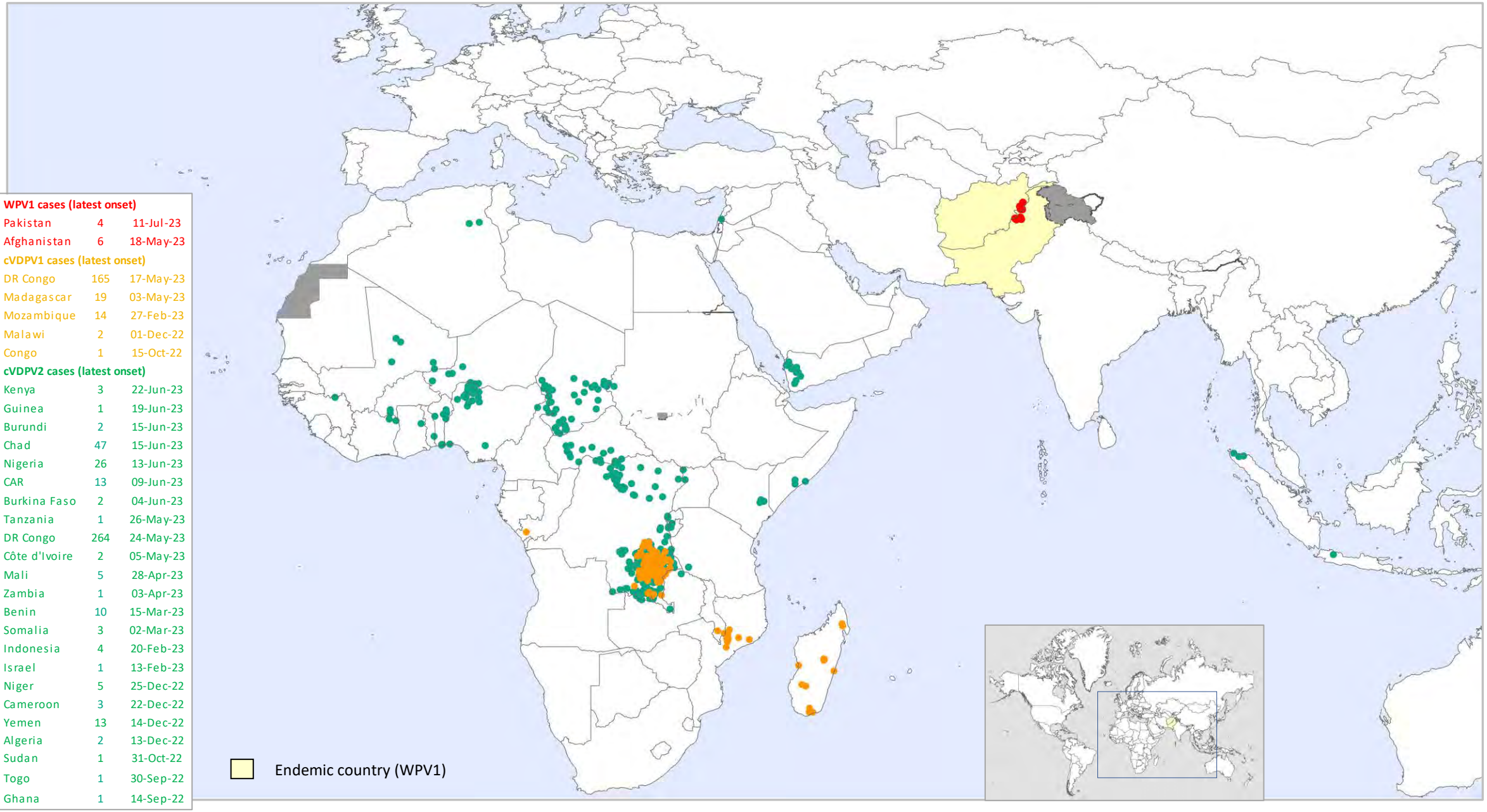
Overview of current potency tests for OPV and IPV

WHO workshop on implementation of international standards for
The quality control of polio vaccines including OPV and IPV
31 October- 2 November 2023
Jakarta, Indonesia

Javier Martin

Global WPV1 & cVDPV Cases[1], Previous 12 Months[2]

World Health Organization



[1]Excludes viruses detected from environmental surveillance; [2]Onset of paralysis: 23 Aug. 2022 to 22 Aug. 2023

Data in WHO HQ as of 22 Aug. 2023

Polio Vaccines and the Polio Eradication endgame

- Polio vaccines will be required for the foreseeable future
- Polio vaccines will need to be produced safely
- Polio vaccines will need to be assessed and released safely
- Changes in vaccination strategies will occur
- This might involve the use of new vaccines

Polio vaccines for polio endgame

- OPV: mOPV1, mOPV2, mOPV3, bOPV, tOPV
- Novel OPV: nOPV1, nOPV2, anOPV3, (t-nOPV)
- Conventional IPV
- IPV based on Sabin strains
- IPV based on new hyper-attenuated strains
- Virus-like particles
- RNA vaccines

Two efficient vaccines against polio

Live-attenuated oral polio vaccine (OPV)

Developed by serial cell-passage of wild poliovirus strains. It protects from disease and is efficient at stopping virus transmission, but it has been associated with paralytic cases (1:2x10^6 doses). OPV is used in monovalent, bivalent and trivalent forms.

Inactivated polio vaccine (IPV)

Prepared by treatment of virus with formaldehyde to kill virus infectivity. It is not infectious and protects from disease, but it is inefficient at stopping virus transmission. IPV is mostly produced using Mahoney, MEF-1 and Saukett wild poliovirus strains but products exist based on Sabin OPV strains

Production of Polio Vaccines

Master Seed (Sabin strains)

↓

Working Seed

↓

Monovalent Single Harvest

↓

Monovalent Pooled Harvest

↓

Final Bulk Vaccine (mOPV, bOPV, tOPV)

Master Seed (wt/Sabin strains)

↓

Working Seed

↓

Monovalent Single Harvest

↓

Final Bulk Vaccine

↓

Inactivated Bulk Vaccine

Quality Control testing of OPV

Identity/Potency Assay

Virus titration in tissue culture (MAbs)

Requires the use of live poliovirus

Attenuation/Consistency of production

MNVT, TgmNVT, MAPREC, rct40, NGS

Requires the use of live poliovirus, nucleic acids

Quality Control testing of IPV

Identity/infectivity

Virus titration in tissue culture

Requires the use of live poliovirus

In vitro Potency Assay

ELISA

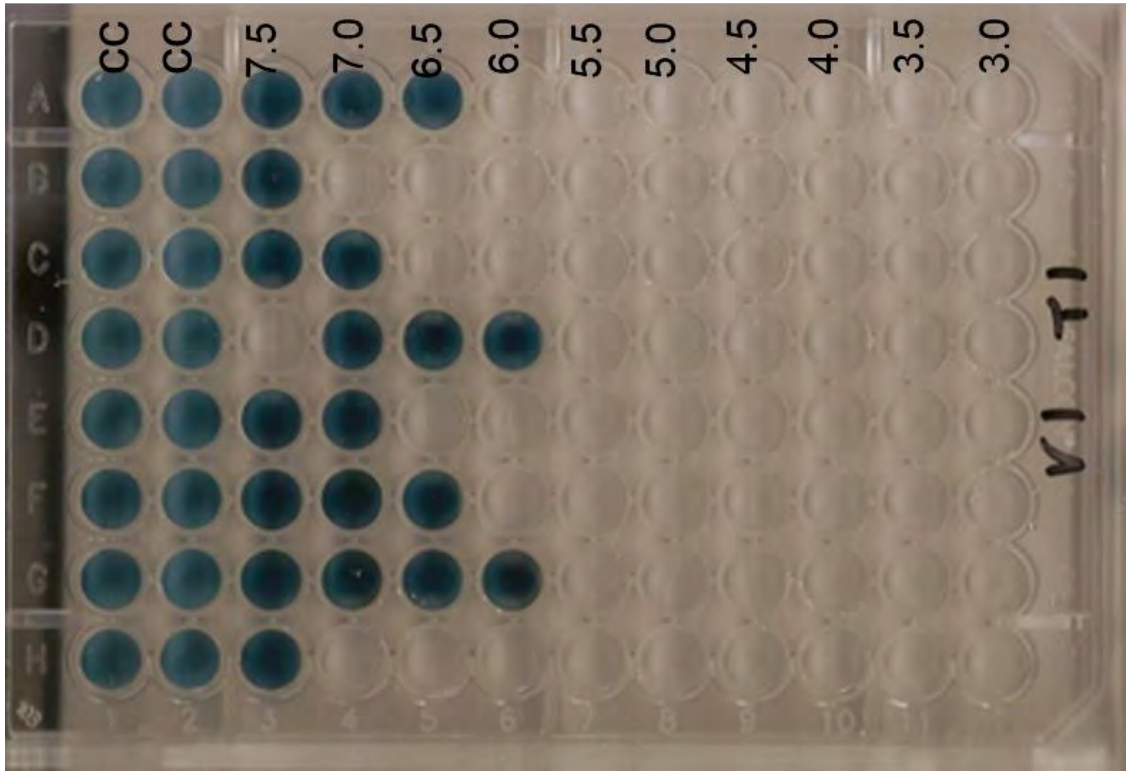
In vivo Potency Assay

Rat, transgenic mouse

Requires the use of live poliovirus (virulent)

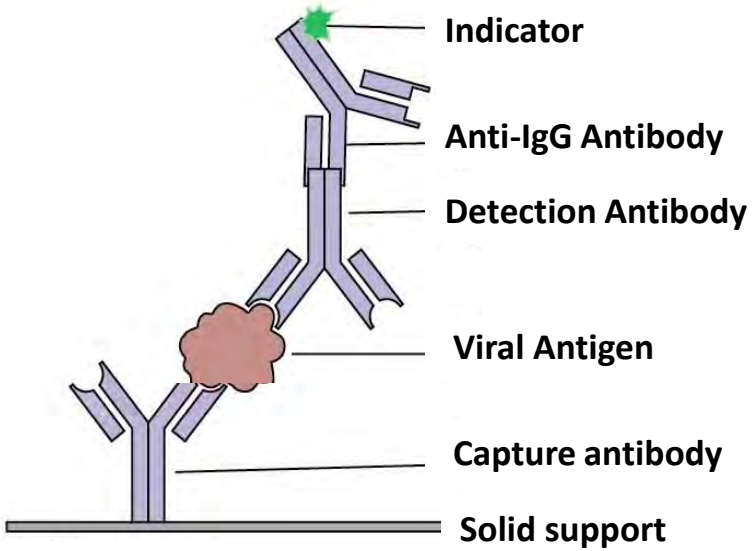
In vitro potency OPV: virus titration

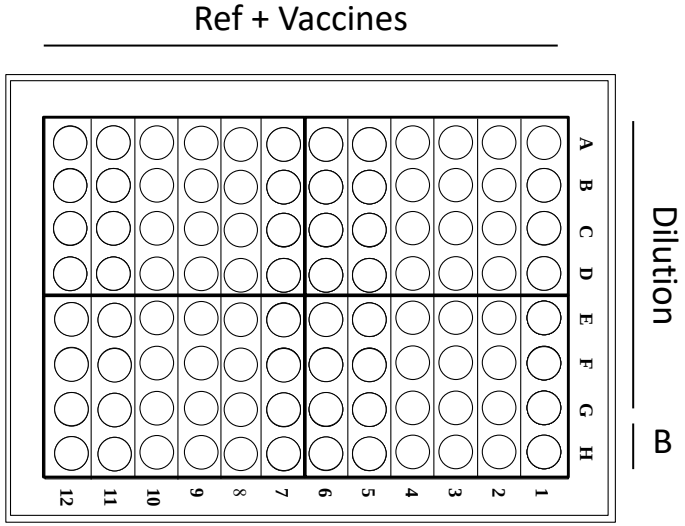
Quantification of live PV



In vitro potency IPV: D-Ag ELISA

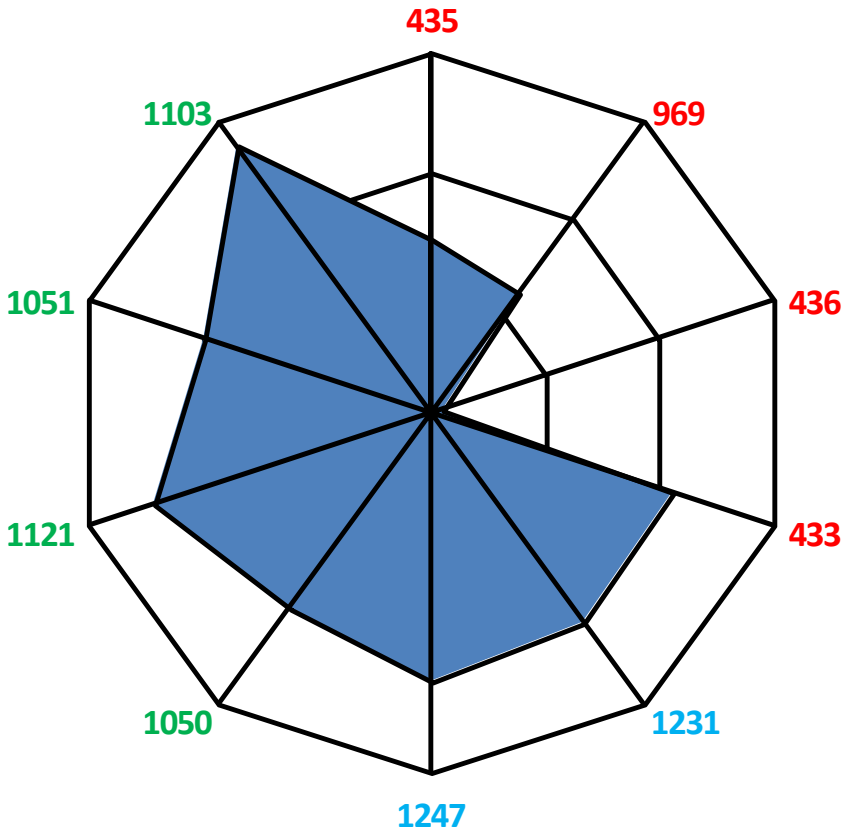
Quantification of PV antigen





IPV - Antigenic Structure

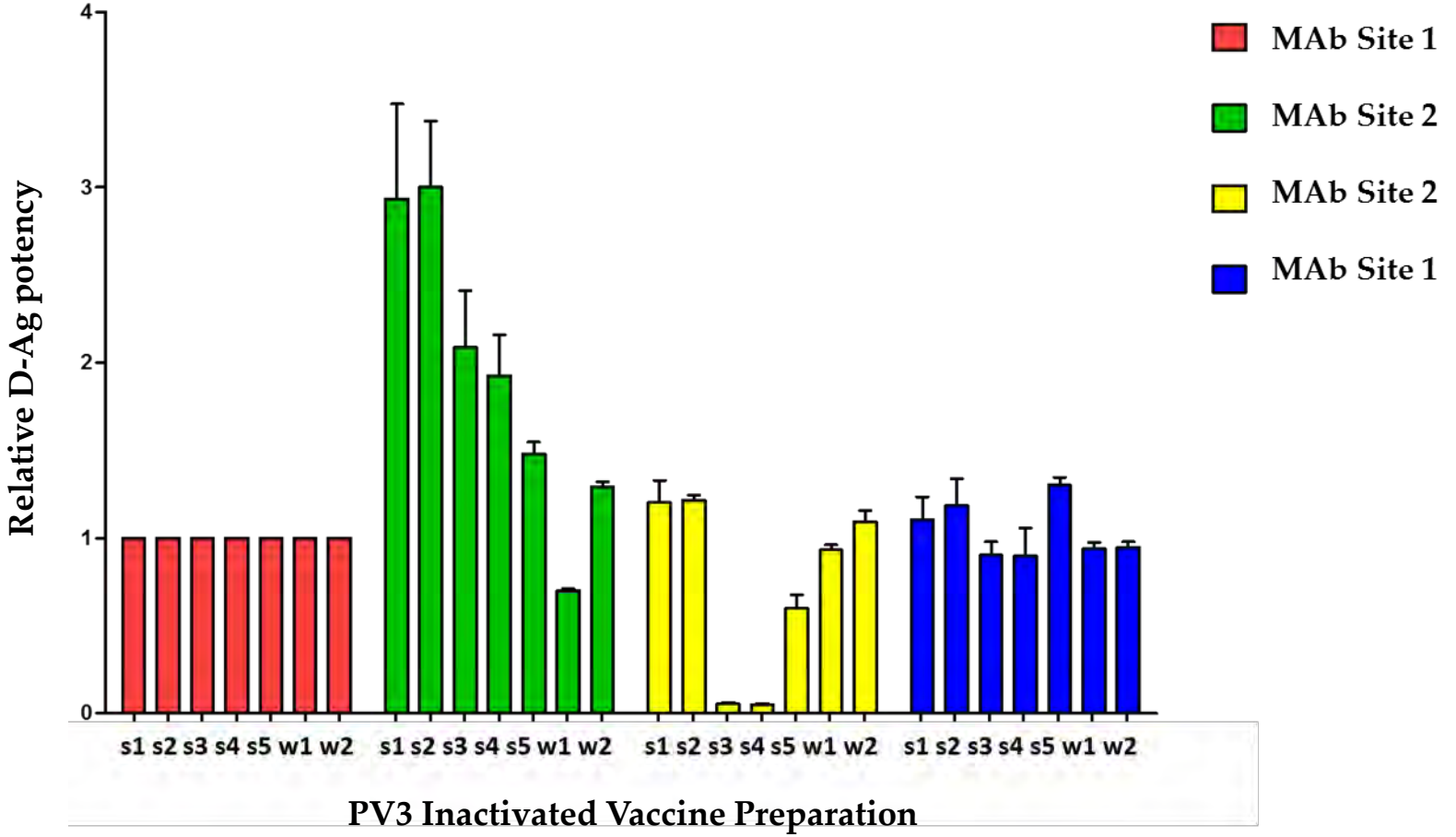
Incubation of PV with inactivation during vaccine production can have an effect on virus antigenicity which might vary between products from different manufacturers



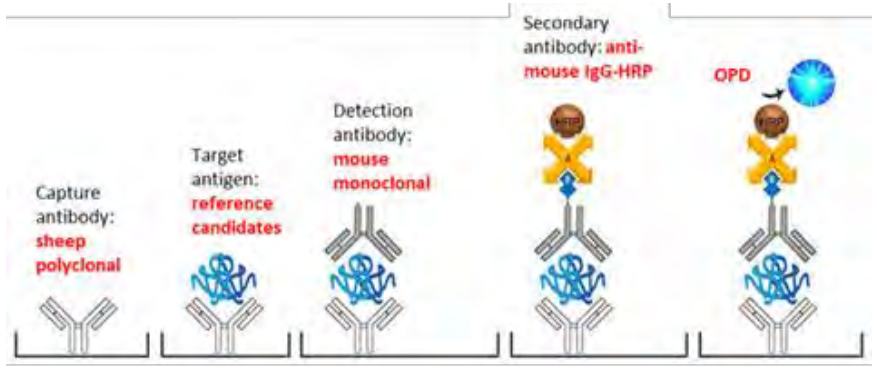
Loss of reaction with anti-PV MAbs live vs inactivated PV: site 1, 2 and 3

Ferguson M et al. J Gen Virol. 1993
Rezapkin G et al. 2005
Tano Y et al. Vaccine. 2007

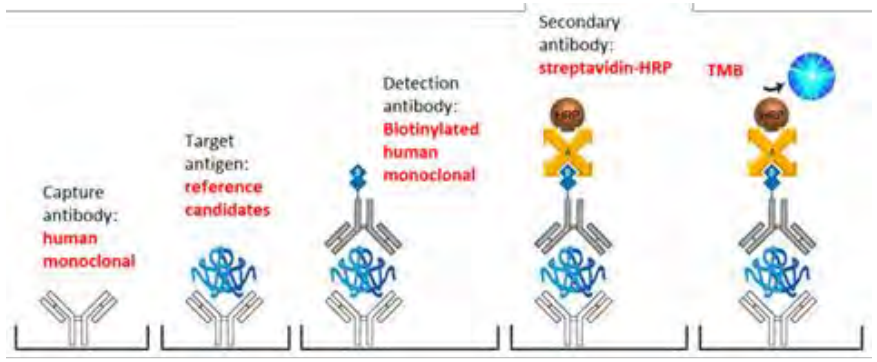
D-Ag potency with different MAbs



Standardization of Sabin-IPV *in vitro* potency assays



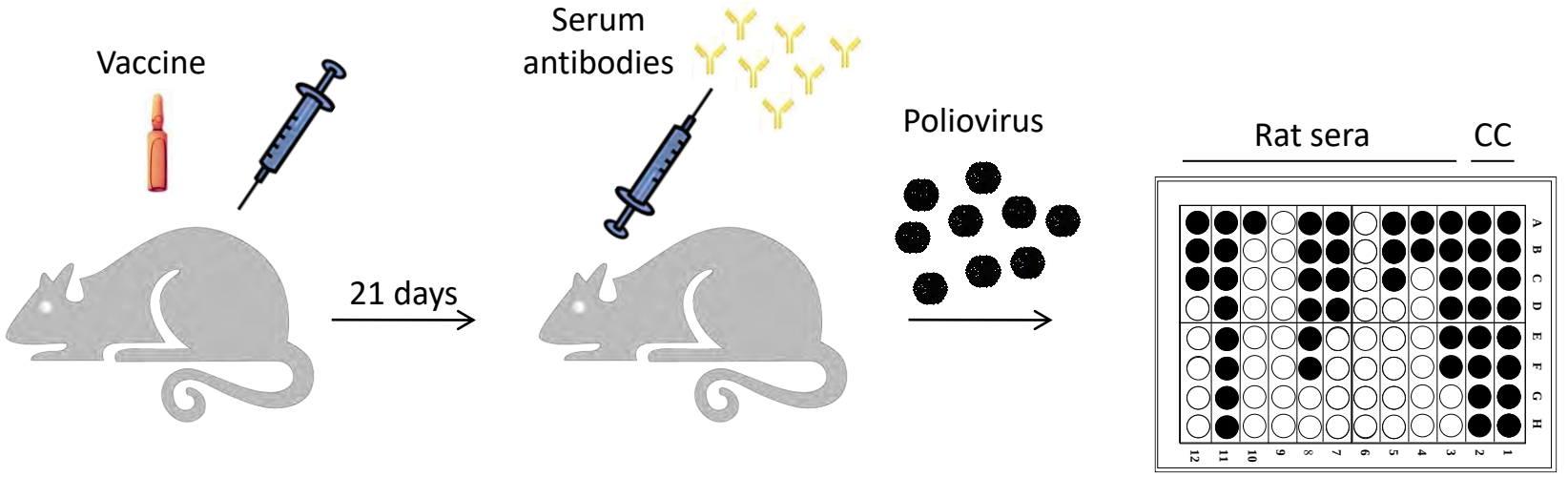
Mouse MAbs



Human MAbs

In vivo potency IPV: Rat Assay

Quantification of PV immunogen

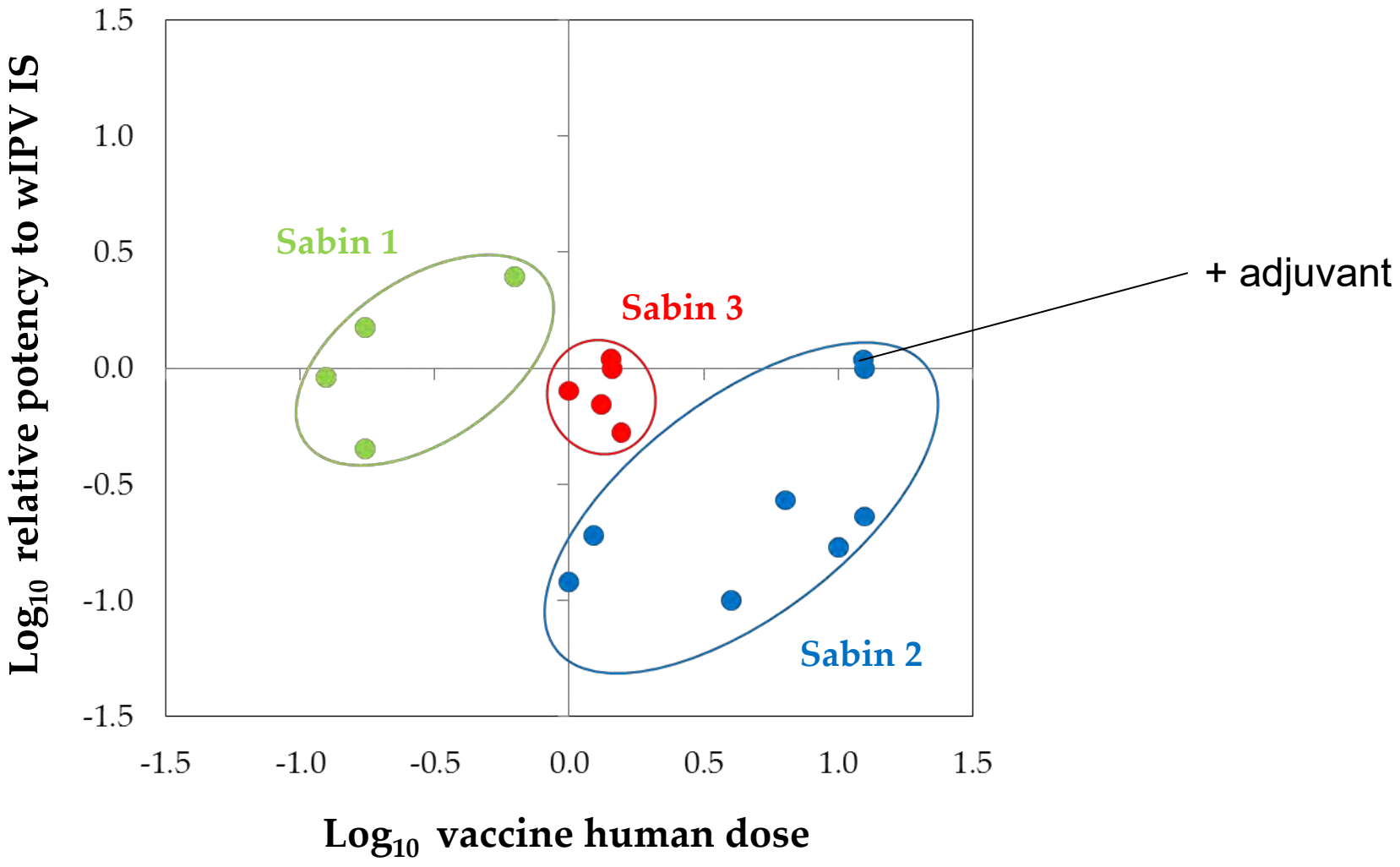


Immunisation

Bleeding

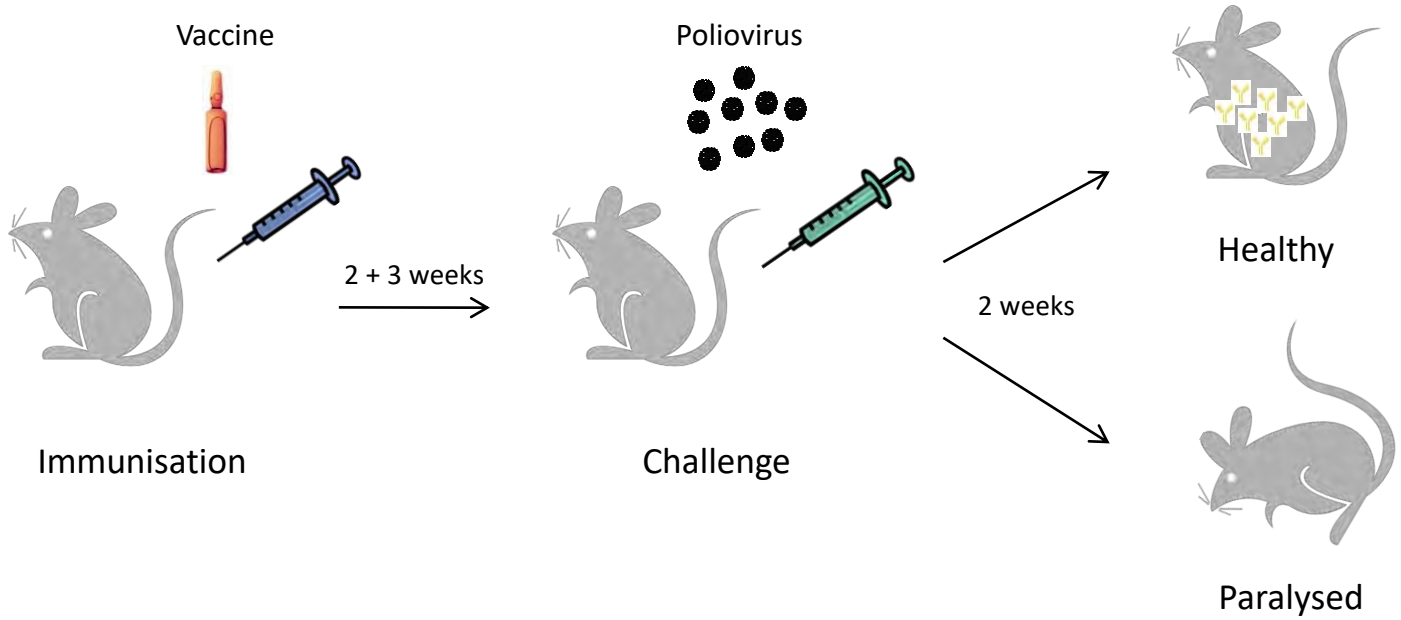
Neutralization assay

In vivo potency of sIPV

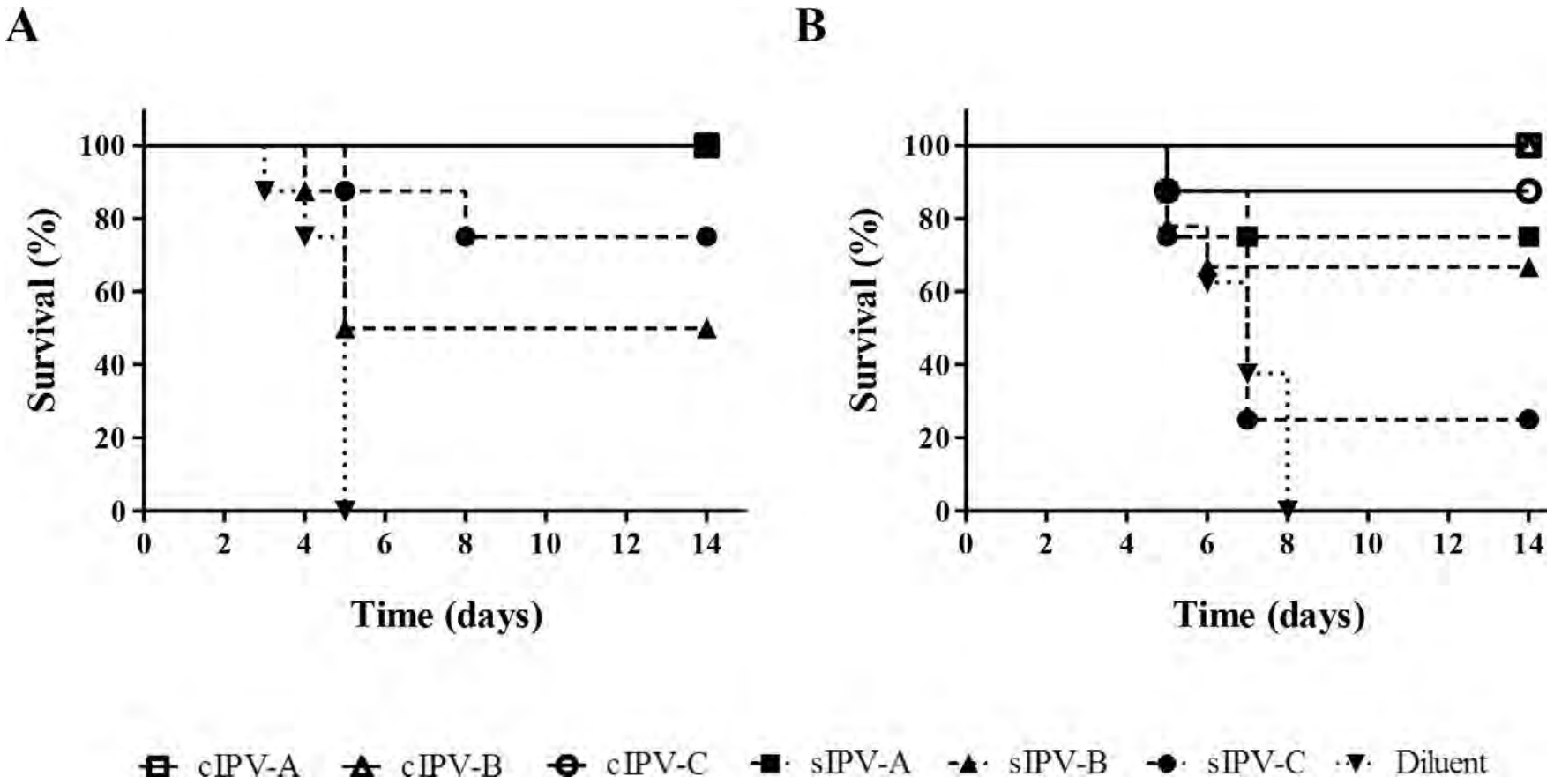


In vivo immunisation-challenge assay

Assessment of protection against PV infection



Survival of Tg21-bx mice immunised with IPV and challenged with paralytic doses of poliovirus



Dunn G, Klapsa D, Wilton T, Stone L, Minor PD, et al. (2015) Twenty-Eight Years of Poliovirus Replication in an Immunodeficient Individual: Impact on the Global Polio Eradication Initiative. PLOS Pathogens 11(8): e1005114. https://doi.org/10.1371/journal.ppat.1005114
http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1005114

International Reference Standards

- Set up laboratory assays
- Compare results from different laboratories
- Compare vaccines from different manufacturers and vaccines from different batches
- Establish consistency of vaccine production
- Set up batch release process
- Calibrate regional, national, in-house reference reagents
- Develop and characterise new vaccines

Acknowledgments

Alison Tedcastle	FDA, USA
Elaine Pegg	Lankenau Institute, USA
Gill Cooper	WHO, Switzerland
Laura Crawt	Vaccine manufacturers
Manasi Majumdar	NRAs
Marylin Quinlan	BMGF, USA
Peter Rigsby	PATH, USA
Helen Fox	Collaborating laboratories
Sarah Carlyle	
Andrew Macadam	

Medicines & Healthcare products
Regulatory Agency

Standardisation of potency test for OPV

WHO workshop on implementation of international standards for
The quality control of polio vaccines including OPV and IPV
31 October- 2 November 2023
Jakarta, Indonesia

Manasi Majumdar

1st of Nov 2023

WHO Technical Report Series, No. 1045, 2023: Annex 2: Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (oral, live, attenuated)

- At least three final containers should be selected at random from each final lot and should be individually tested in a single assay.
- When the vaccine contains more than one poliovirus type, each type should be titrated separately by using appropriate type-specific antiserum to neutralize each of the other types present.
- The amount of poliovirus of each serotype present in the vaccine, and its total poliovirus content, should be determined. The assay should include a reference material.

Dose-ranging studies

- At the time of publication of this document, all licensed Sabin OPV formulations (monovalent, bivalent and trivalent) contained the recommended dose for each poliovirus type (not less than $10^{6.0}$ CCID50 for type 1, $10^{5.0}$ CCID50 for type 2 and $10^{5.5}$ CCID50 for type 3).

- Development of nOPV or novel formulations with improved stability (through the addition of stabilizers/ excipients) or improved immunogenicity (use of an adjuvant) may require dose-ranging studies to determine the minimum dose of virus required in CCID50 to provide adequate immune responses.

- Because nOPV strains have different properties to the classical Sabin strains with respect to optimal growth conditions, the production and quality control of vaccines made from them may differ from those made using classical Sabin strains. Such differences could include growth and titration properties, optimal temperature of growth, dose required, thermal stability and other parameters.

- Proof-of-concept nonclinical studies based on type-specific serum neutralizing antibody titres may also assist in the selection of the doses to be tested in the clinical dose-finding studies.

Thermal Stability

- Thermal stability should be considered as a vaccine characteristic that provides an indicator of production consistency.
- The thermal stability test is not designed to provide a predictive value of real-time stability but rather to evaluate whether the product complies with a defined stability specification.
- Three final containers of each final lot should be incubated at 37 °C for 48 hours.
- The total virus content in both exposed and unexposed containers should be determined concurrently with that of a suitable validated reference preparation.
- The loss of potency on exposure should be within the limit approved by the NRA.
- For tOPV prepared from Sabin strain, the vaccine passes the test when the loss on exposure is not greater than a factor of 0.5 log10 CCID50 per dose.
- Several OPV manufacturers have demonstrated that the thermal stability test specification applied to tOPV formulations is not applicable to some mOPVs and bOPVs.
- Some manufacturers have shown that mOPV formulations that failed the current tOPV specification of 0.5 log 10 have an acceptable stability profile throughout the product shelf-life. Therefore, a specification of 0.6 log 10 has been accepted by the NRAs and by the WHO Prequalification Programme in those cases.
- Suitable thermal stability test for nOPV should be established and validated.

WHO Technical Report Series, No. 1045, 2023: Appendix 4: Cell-culture techniques for determining the virus content of poliomyelitis vaccines (oral, live, attenuated):

- This test quantifies and identifies the 3 serotypes of poliovirus in oral poliomyelitis vaccine.
- Viruses are titrated by a 50% end point technique using Hep2C cells.
- Virus potency is assayed, and each virus is identified after neutralising the other two virus serotypes with mixtures of serotype specific monoclonal antibodies.
- To assess thermal stability total virus potency (titre in the absence of antibodies) is compared to total virus in samples heated at 37°C for 2 days.

Points discussed:

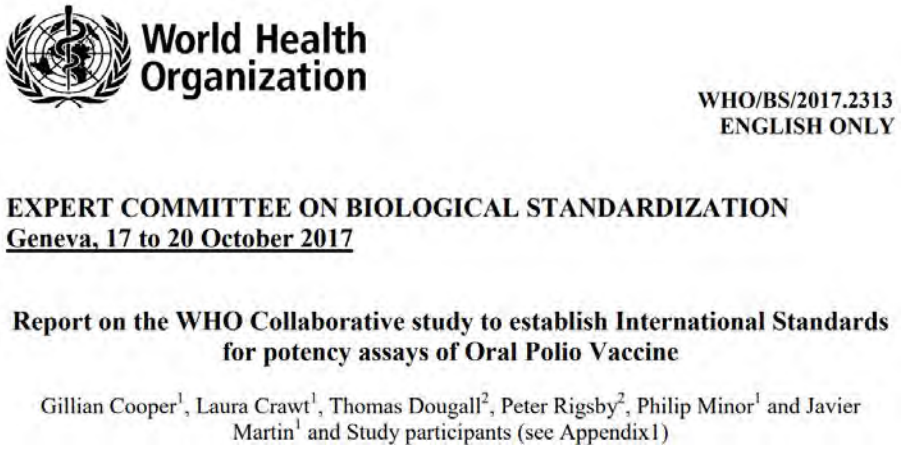
- Available WHO International Standards (IS) for performing potency assays
- Overview of methodology
- Generating results
- Defining validity criteria
- Data monitoring
- Further development

Polio laboratory manual
4th edition, 2004
World Health Organization

Collaborative Study to establish International Standards for potency assays of OPV

- The 2nd International Standard (IS) for the potency estimation of tOPV (NIBSC Catalogue number: 02/306) was established in 2004. Due to the raised containment requirements for poliovirus type 2, this standard was no longer suitable for the testing of bOPV at lower containment levels.
- A collaborative study was conducted with an aim to establish the 1st IS for monovalent (mOPV) and bivalent Polio (bOPV) vaccines. Candidate samples for bOPV 1+3, mOPV1, mOPV2 and mOPV3 were made from well characterised monovalent Polio bulks.
- The candidates were tested by a panel of global laboratories which included seven OPV manufacturers and seven NCLs.
- Laboratories used both their validated methods using their in-house antisera for the bOPV 1+3 (8Labs) and/or the NIBSC monoclonal antibodies freeze dried preparations provided (12 labs)

- The overall levels of within assay variation, within laboratory variation for all the candidates, demonstrated high level of consistency for testing of OPV. This is supported by the range of values obtained between laboratories for all the candidates were within 0.5 log10 TCID50 of the mean, this indicated that the methods are well standardised.
- No differences were observed with the NIBSC monoclonal antibodies or in-house antisera.



World Health Organization

WHO/BS/2017.2313
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 17 to 20 October 2017

Report on the WHO Collaborative study to establish International Standards for potency assays of Oral Polio Vaccine

Gillian Cooper[1], Laura Crawt[1], Thomas Dougall[2], Peter Rigsby[2], Philip Minor[1] and Javier Martin[1] and Study participants (see Appendix1)

Available WHO International Standards (IS) for performing potency assays

Given the suitability of the candidates and the consistency of the study it is recommended that each of the candidates serve as 1st International Standards with the following assigned potencies:

- bOPV 1+3 NIBSC code 16/164 - 7.19, 6.36 and 7.32 log10 TCID50/ml for type 1, 3 and Total Virus content
  mOPV1 NIBSC code 16/196 - 7.32 log10 TCID50/ ml
- mOPV2 NIBSC code 15/296 - 6.74 log10 TCID50/ ml
- mOPV3 NIBSC code 16/202 - 6.66 log10 TCID50/ ml

NIBSC monoclonal antibodies available:

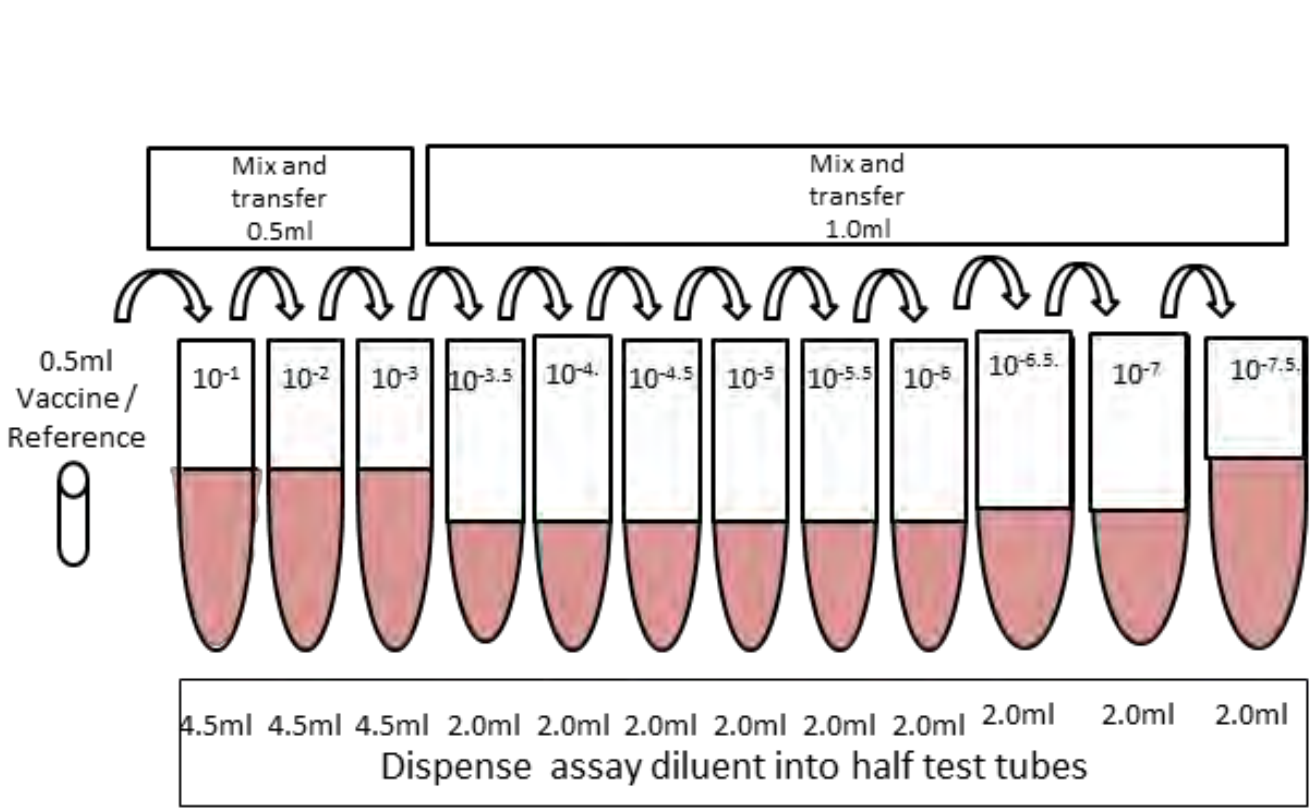
Polio type 1 monoclonal antibody serum 05/184 (NIBSC batch number 425)

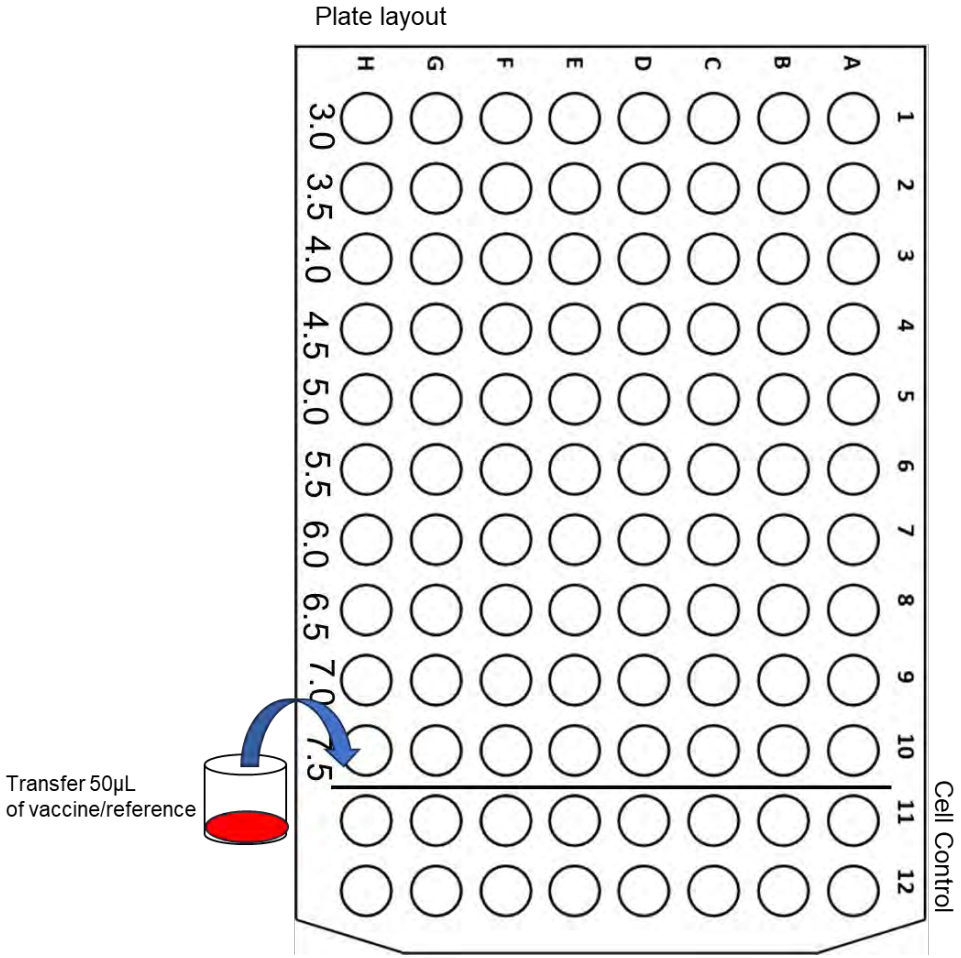
Polio type 2 monoclonal antibody serum 05/186 (NIBSC batch number 267)

Polio type 3 monoclonal antibody serum 05/188 (NIBSC batch number 495)

Methodology: Vaccine virus/Reference samples titration

Example of virus dilution and plating





Selecting type specific neutralizing antibodies for the TCID50 assay for bOPV and tOPV vaccines

For testing bOPV vaccines prepare appropriate volume of antibody			
Monoclonal antibody details	**Volume**	**Assay diluent Volume**	**Virus detected**
Mab I 234/(425)	10 ul	9990 ul	Type 3 PV
Mab III 495	10 ul	9990 ul	Type 1 PV
For testing tOPV vaccines prepare appropriate volume of antibody			
Monoclonal antibody details	**Volume**	**Assay diluent Volume**	**Virus detected**
Mixture I 234/(425) +II 267	10ul+10ul	9980 ul	Type 3 PV
Mixture II 267 + III 495	10ul+10ul	9980 ul	Type 1 PV
Mixture III 495 + I 234/(425)	10ul+10ul	9980 ul	Type 2 PV

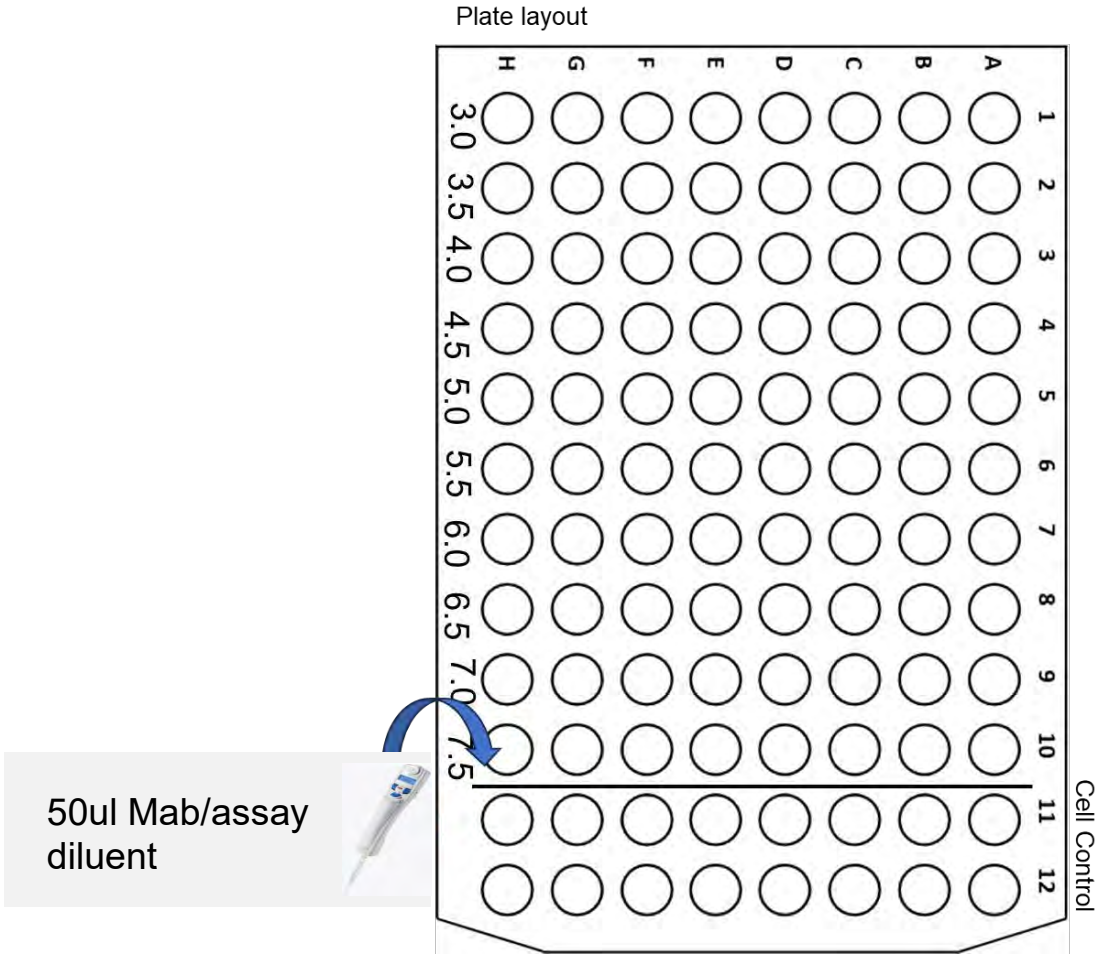
Antibody addition and incubation step

- Using a calibrated multidrop dispensing machine or multichannel pipette transfer 50µLof each monoclonal antibody mixture into each well of appropriately labelled plates.

- Monovalent OPV and nOPV 2 potency testing using TCID 50 method will not require addition of any neutralizing antibodies. To match up the volume add 50 ul of assay diluent.

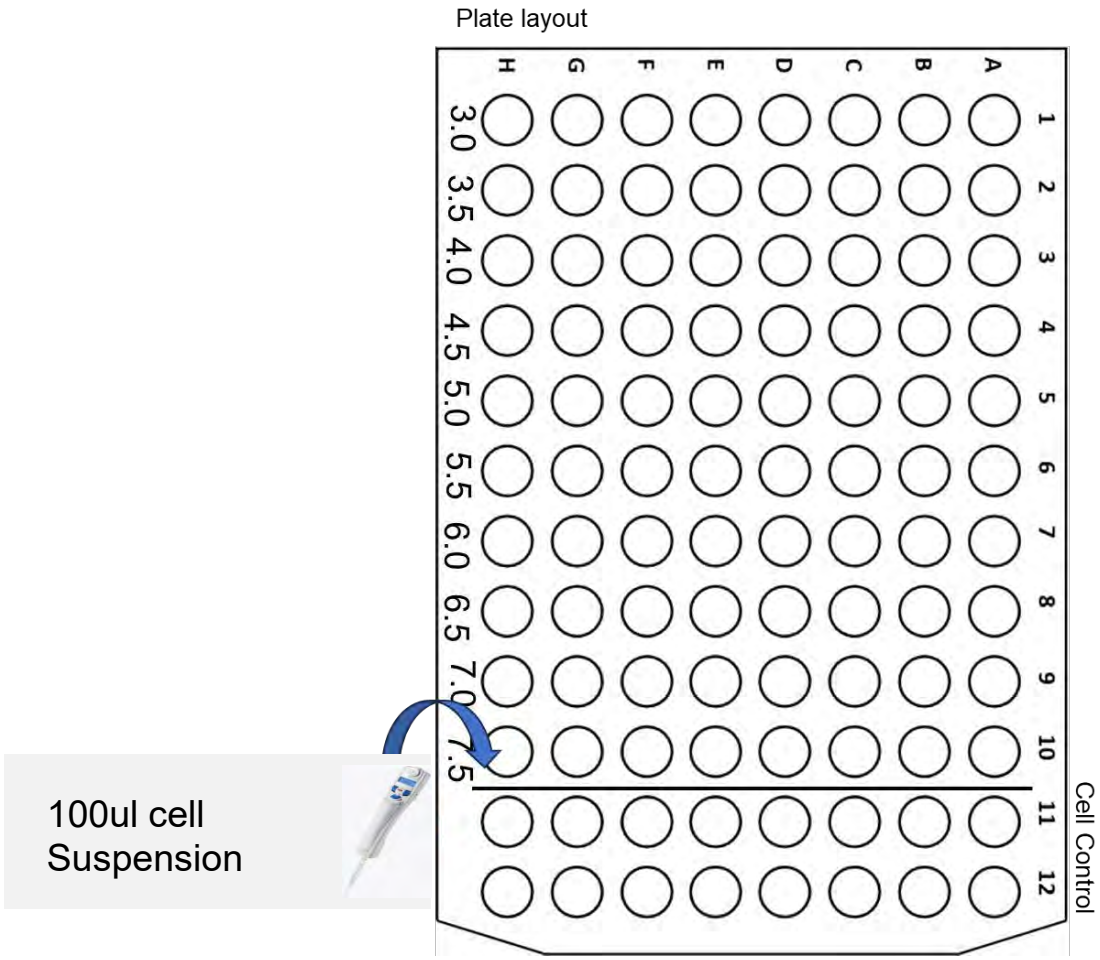
- Use a CO2 incubator at 35°C ± 1°C and ≥ 85% humidity to incubate the bOPV and tOPV vaccine plates for at least 3 hours. tOPV assay should be carried out in CL3 facility because of the Type 2 component of the vaccine.

- **This 3 hours incubation step is not required when testing mOPV or nOPV vaccines.**



Adding cell suspension and incubation

- Prepare sufficient Hep-2C cell suspension for the number of plates
- Add 100µL of cell suspension into each well in all plates. (As only 10rows have been inoculated use the remaining two rows for cell controls).
- Seal the plates thoroughly with pressure sensitive film (PSF).
- Use a CO2 incubator at 35°C ± 1°C and ≥ 85% humidity to incubate the bOPV and tOPV vaccine plates for 5-7 days. tOPV assay should be carried out in CL3 facility because of the Type 2 component of the vaccine.
- Use a CO2 incubator at 33°C ± 1°C and ≥ 85% humidity to incubate the nOPV2 vaccine plates, for 5-7 days.



Fixing and Staining Cells

- After incubation check plates visually for condition of cells and for the absence of bacterial contamination.
- Discard medium from wells into 1% Virkon/ validated disinfectant.
- Uncontaminated plates are then fixed and stained using naphthalene black for at least 3 hour
- Plates are rinsed well and dried
- Plates scanned and results recorded in a Lab book
- Clear wells indicate presence of virus and therefore marked (+)
- Blue wells indicates absence of virus and therefore marked (-)
- Cell controls, Blue wells, normal cell growth

Testing parameters and setting validity criteria

- Titrate the vaccine for infectious virus using **no fewer than three separate containers of vaccine**
- Titrate one container of an appropriate **virus reference preparation in triplicate to validate each assay**
- If the vaccine contains more than one type of poliovirus, **titration of the individual serotypes is undertaken separately**, using mixtures of appropriate type-specific antiserum (or preferably a monoclonal antibody) to neutralize each of the other types present.

Following validity criteria can be set for the assay:

- The estimated virus concentration for the reference preparation is ±0.5 log10 CCID50 of the established value for this preparation
- The confidence interval (P = 0.95) of the estimated virus concentration of the three replicates of the reference preparation is not greater than ±0.3 log10 CCID50.
- The assay is repeated, and results are averaged if: the confidence interval (P = 0.95) of the combined virus concentration of the vaccine is greater than ±0.3 log10 CCID50.

Appendix 4 WHO Technical Report Series, No. 1045, 2023

Cell-culture techniques for determining the virus content of poliomyelitis vaccines (oral, live, attenuated)

Data Monitoring

- The virus titre of the reference preparation is monitored using a control chart, and a titre is established using historical data at each laboratory.
- What is Cusum: Accumulative difference over time that allows us to see change

For example:

With a Mean titre = 5.69TCID50/50ul

Assay 1 titre of 5.9 (5.69 – 5.9) =-0.21

Assay 2 titre of 6.1 (5.69 – 6.1) =-0.41 add 2 to 1

Plot assay 1 -0.21, assay 2 -0. 62

See where changes occur and investigate

Ongoing activity regarding standardization of nOPV potency assay

- During March 2023 ECBS a one pager was submitted for preparing 3 different monovalent (nOPV1, 2 and 3) candidates to be developed for standardization of nOPV potency assay.

- The project proposal was endorsed by WHO ECBS and was deemed important to start as soon as possible keeping in view of potential increase in the number of nOPV manufacturers around the globe.

- The work is supported through a funding collaborative agreement with PATH to initiate the work. PATH was awarded a grant by the BMGF (INV-060991, Kutub Mahmood PI) in Oct 2023, for support of this work.

- Vaccine bulk material will be sourced, and the collaborative study candidates will be prepared during 2024, with a tentative CS initiation date of Q1/Q2 2025.

- Expression of interest for participation in the study will be sent soon. Please contact Javier Martin for further details.

Thank you for your kind attention

Stay connected

Medicines & Healthcare products Regulatory Agency

Copyright information

Standardization of D-antigen ELISA potency test for sIPV: use of new WHO International Standard for sIPV

Kostya Chumakov

George Washington University

Presentation plan

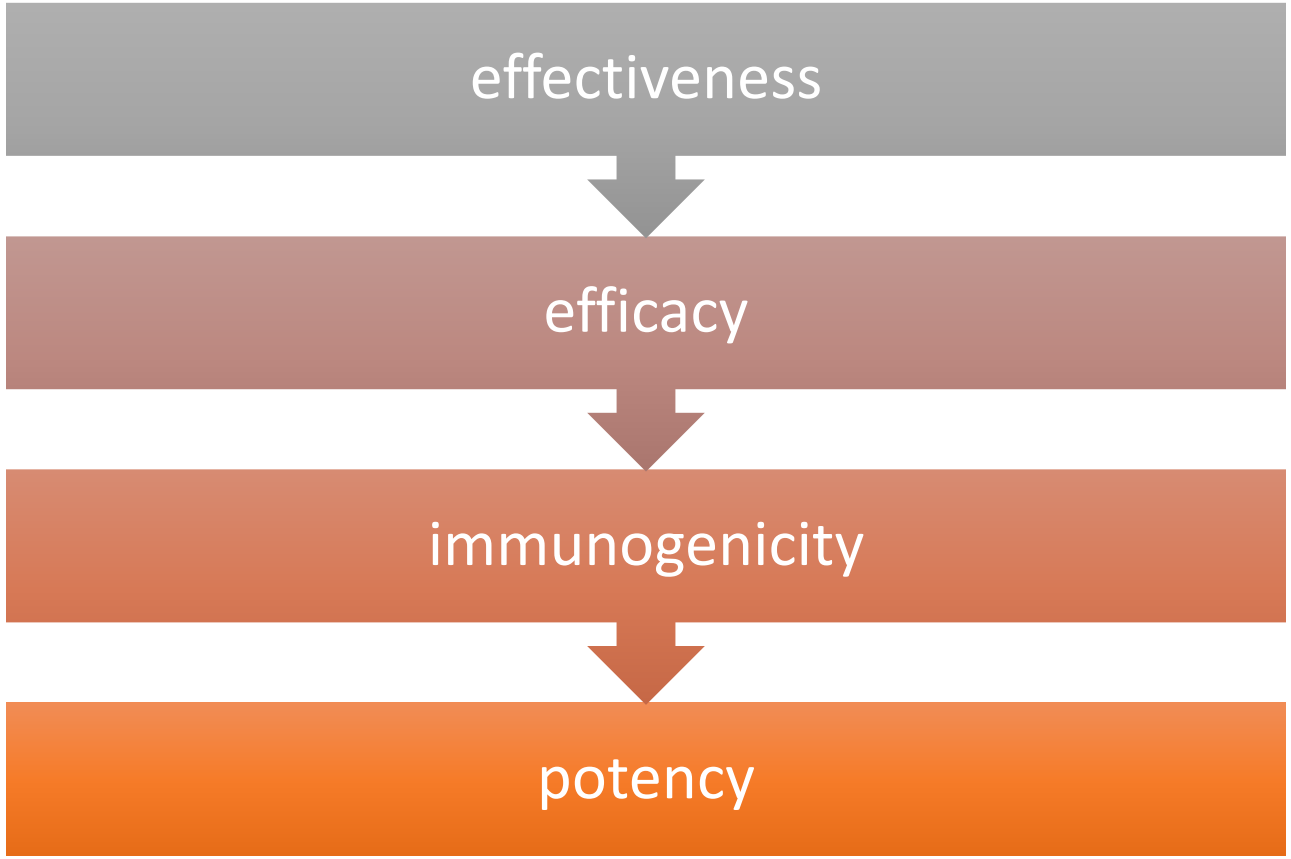
- Definitions of potency, immunogenicity, antigenicity
- History of D-antigen
- Elaborate on the proper use of the sIPV D-antigen International Standard (adopted by ECBS 2018), assignment/meaning of sDU, interpretation of results, example of sDU calculation
- Calibration issues in the case of licensed sIPV before the IS is available

Definitions

- Potency
  - a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity
- Antigenicity
  - the content of protective antigen
- Immunogenicity
  - the ability to induce a humoral and/or cell-mediated immune responses
- Protectivity
  - the ability to induce protection against infectious disease
- Efficacy
  - disease reduction in a vaccinated group compared to an unvaccinated group under the most favorable conditions, e.g. in well-controlled clinical trials
- Effectiveness
  - the reduction of disease upon routine vaccine administration in real-life circumstances

How are they related?

- Public Health
- Clinical evaluation
- Pre-clinical animal studies
- Chemistry-Manufacture-Control



Efficacy vs. Potency

- Vaccines are licensed based on their demonstrated efficacy
- Vaccine dose is determined in dose-ranging studies
  - Antigen content (potency) of one vaccine dose is determined based on optimal clinical efficacy
- Vaccines with similar clinical efficacy may have different *in vitro* potency, and in reverse

Potency and vaccine development process

- Creation of potency assays is a part of analytical development program
- Dose-finding studies
  - For IPV can only be based on immunogenicity
    - Animal immunogenicity
    - Clinical evaluation of immune response
- Specifications for vaccine formulation are determined based on dose-finding studies
  - The optimal antigen content in the new product is expressed in units developed by the analytical studies

Potency tests for cIPV

- In-vivo test
  - Rat immunogenicity test
    - Measures induction of virus-neutralizing antibodies
- In-vitro test
  - D-antigen ELISA test
    - Measures the content of thermo-labile protective antigen

History of IPV potency testing

IMMUNOCHEMICAL STUDIES OF POLIOVIRUS

III. Further Studies on the Immunologic and Physical Properties of Poliovirus Particles Produced in Tissue Culture[1,2]

BERNARD ROIZMAN, MANFRED M. MAYER AND HERBERT J. RAPP

From the Department of Microbiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland

Received for publication June 6, 1958

TABLE I

Fractionation of poliovirus Type 1 (Mahoney strain), preparation JH1B in the density gradient

Fraction	Volume	Total PFU × 10^{-6}	Total CF Antigen (Convalescent)	Ratio PFU/CF (Convalescent) × 10^{-6}	Total CF Antigen (Acute)	CF Antigen Ratio Acute: Convalescent
	ml					
A	3.21	5	<13	>0.4	<30	—
B	1.90	8	11	0.7	300	27
C	2.25	<23	450	<0.05	4500	10
D	3.00	140,000	21,000	7	360	0.02
E	2.48	6,200	870	7	750	0.09

THE D-ANTIGEN CONTENT IN POLIOVACCINE AS A MEASURE OF POTENCY

A. J. BEALE
M.D. Lond., Dip.Bact.
OF THE VIRUS RESEARCH UNIT, GLAXO LABORATORIES LTD.,
SEFTON PARK, STOKE POGES, BUCKS

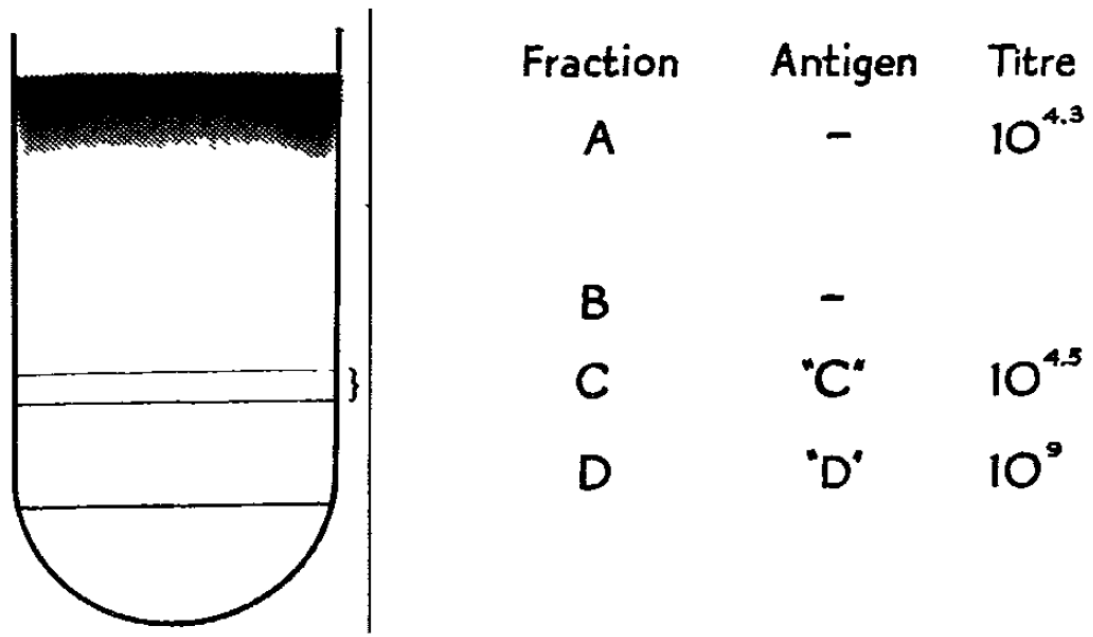
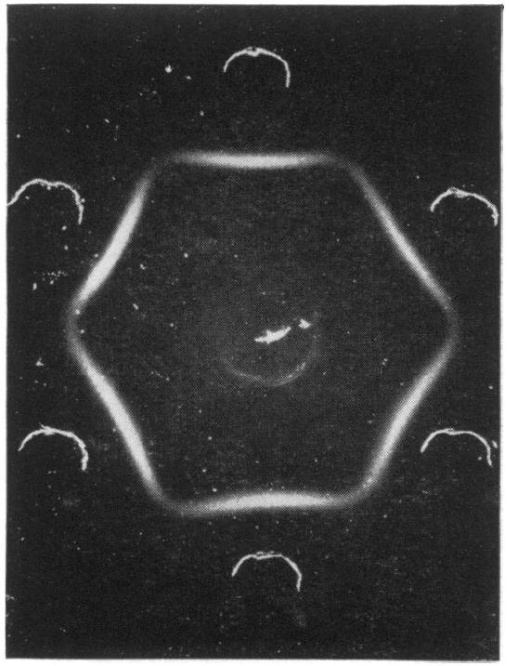
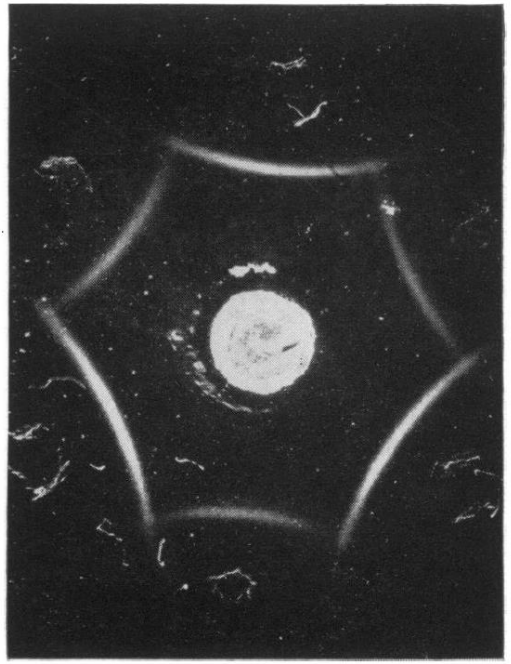
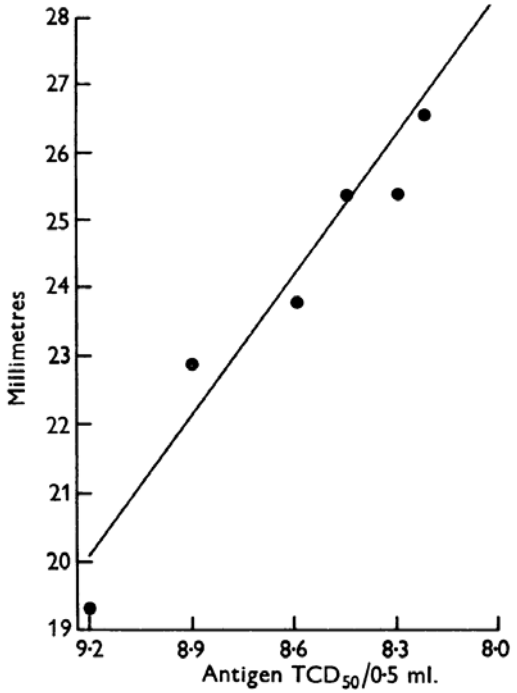


Fig. 1—Sucrose density gradient: poliovirus type $10^{9.5}$.

Agar Precipitin Test







From: Beale and Mason, J. Hyg., Camb., 60: 113-121 (1962)

D-antigen units

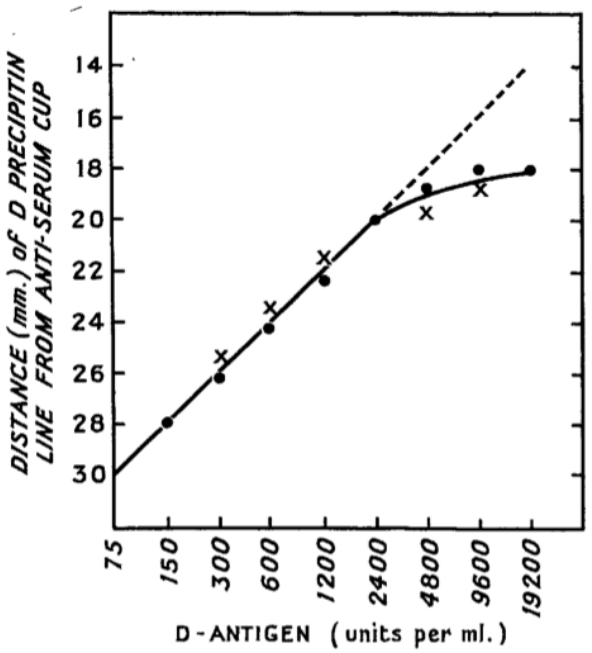


Fig. 1—Relationship between poliovirus type-1 D-antigen concentration and position of precipitin line.

600 units of D-antigen produce precipitin line at 24 mm

Potency of IPV is determined relative to reference vaccine

1 D-antigen unit ≈
- $6*10^6$ infectious units
- 10^9 particles
- ~15 nanogram

From: Beale and Ungar, *Lancet* 280 (7260): 805-808, 1962

ELISA test

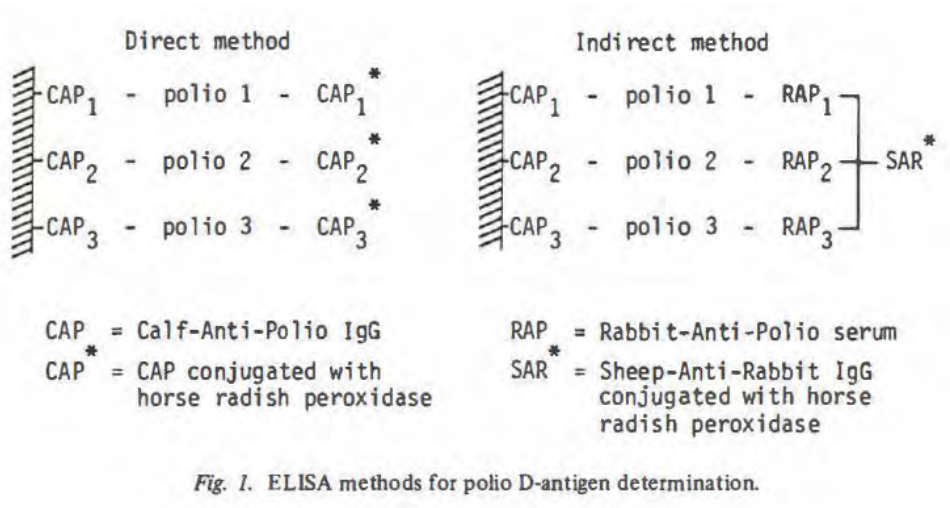
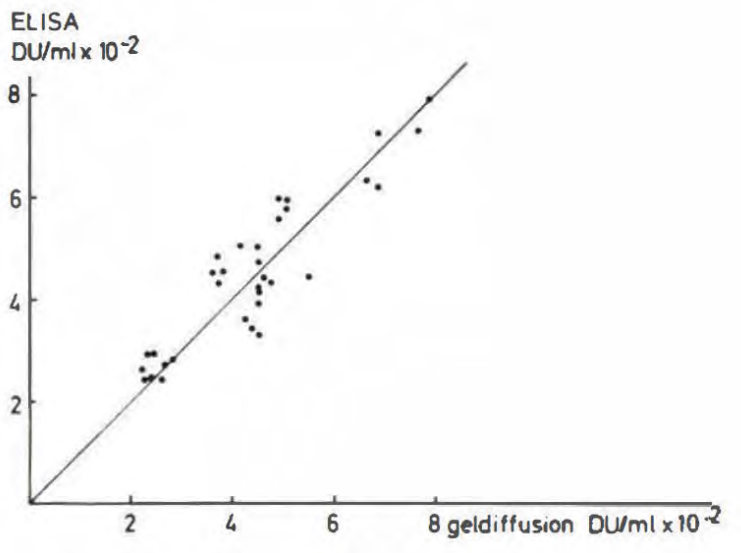


Fig. 1. ELISA methods for polio D-antigen determination.



From: van den Marel et al, *Develop. Biol. Standard.* 47: 101-108 (1981)

D-antigen test: references

- International Standard for potency of IPV
  - 1st International Standard (PU78-02)
  - 2nd International Standard (1994: 91/574, BRP2)
  - 3rd International Standard (2013: 12/104)
- Traceable to a vaccine lot tested in clinical trials
- Used to calibrate in-house references

D-antigen ELISA reagents challenge

- Each lab uses its own set of reagents
  - No two labs use the same reagents and protocol
- Both polyclonal sera and mouse monoclonal antibodies are used
- Replenishment of polyclonal sera requires new validation
- Monoclonal antibodies bind only one epitope and do not necessarily reflect complete antigenic composition of IPV
- Harmonization could help

Sabin IPV Challenge

- Sabin strains differ immunochemically from Salk vaccine strains
  - Sabin 1 *vs.* Mahoney
  - Sabin 2 *vs.* MEF1
  - Sabin 3 *vs.* Saukett
- Reagents used in some labs could not be used with sIPV
- Can existing International Standards be used for sIPV?
- Should a product-specific International Standard be developed for sIPV?
- How to assign potency units to the new IS?
  - Could the existing D-antigen units be used for sIPV?
  - Can scientifically sound bridging tests be conducted?

Should the same units be used to measure potency of cIPV and sIPV?

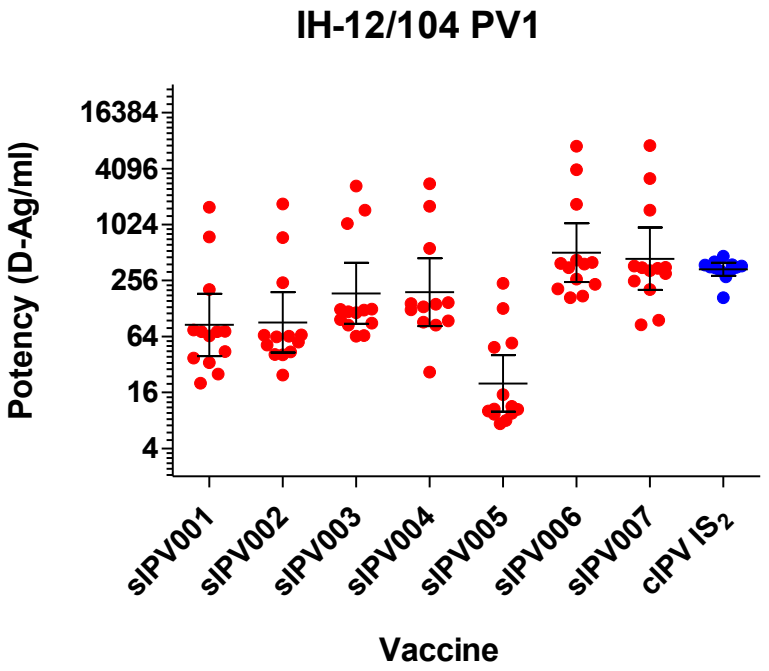
- Original D-antigen units were arbitrary and based on agar immunodiffusion test
  - 600 units producing precipitin line 24 mm in diameter
- The number of D-antigen units in 1 µg of poliovirus of each serotype is different
  - Immunogenicity per µg of each serotype also differs
- No reason to expect that these values for Sabin strains will be the same
  - We know for a fact they are not
- Using the same units will create an illusion that they mean the same and could be compared

Harmonization of IPV potency testing

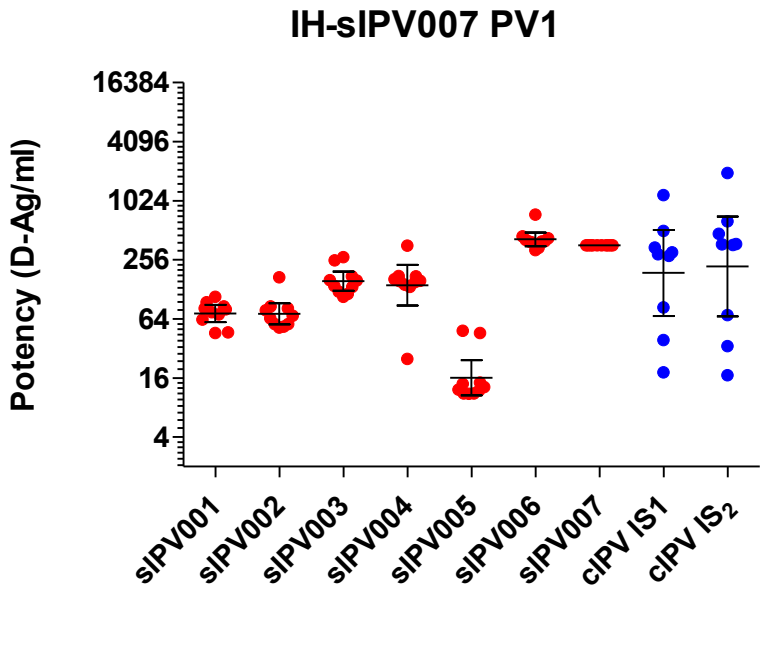
- May 2, 2016 meeting @ BMGF Headquarters in Seattle WA:
  - The need for sIPV-specific reference identified
  - International Collaborative Study led by CBER/FDA and NIBSC, coordinated by PATH
- Phase 1 of the study reviewed at July 11-12, 2018 meeting in Hyderabad, India
  - We proposed that an independent D-antigen potency unit for Sabin IPV be established
  - The same year ECBS established a new potency unit, the sDU
  - The need for universal reagents and protocol identified
- Phase 2 was reviewed at August 24-25, 2022 meeting in Bangkok, Thailand
  - Universal reagents based on human mAbs and the protocol developed at CBER proposed as the International reference for potency testing of both cIPV and sIPV
  - The proposal was approved by ECBS in October 2022. The reagents were established as International Reference Material

Heterologous International Standard results in poor agreement between labs

Salk IPV reference



Sabin IPV reference



Preparation 17/160 was established as International Standard for sIPV

- It should only be used to measure potency of Sabin IPV
  - This reagent was assigned new potency units: Sabin D-antigen units (sDU)
  - The value for each serotype was arbitrarily assigned the value of 100 sDU
- Potency of conventional (Salk) IPV should be measured against 12/104
  - 12/104 reagent SHOULD NOT be used to measure potency of sIPV
  - sDU is completely unrelated to DU used to measure potency of cIPV
- Manufacturers that licensed their sIPV before 17/160 was established and thus use their own in-house Standard and respective potency units can continue using them
  - In parallel they can start using 17/160 and the newly established sDU to measure potency of their product

D-antigen ELISA reagents challenge

- Each lab uses their own ELISA reagents
  - Polyclonal antibodies: polyclonal rabbit IgG
    - Broad reactivity
    - Problems with standardization
    - Difficulties in replenishment of stocks
  - Monoclonal antibodies
    - Standard reagents
    - Only select epitopes
    - Varying reactivity with different strains
- Reagents used in some labs work well with cIPV but not sIPV
- Should there be separate sets of reagents for cIPV and sIPV, or a universal D-antigen ELISA reagents can be prepared?

Human Monoclonal Antibodies

- Collaboration with Dr. Scott Dessain, Lankenau Institute for Medical Research
- Modified mouse myeloma cultures expressing human telomerase gene and IL6
- Results in highly stable human hybridomas
- Surface expression of immunoglobulin molecules enables high-throughput screening using cell sorter

Type 1

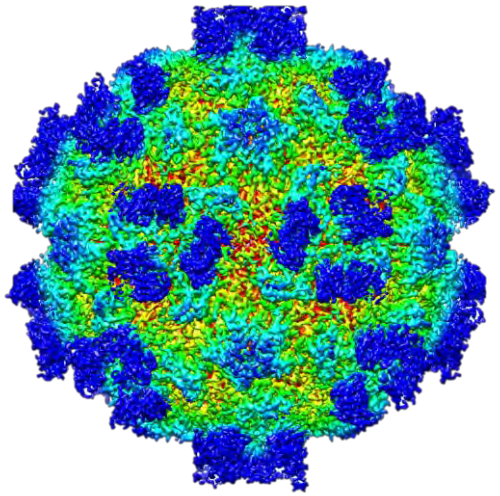
Type 2

Type 3

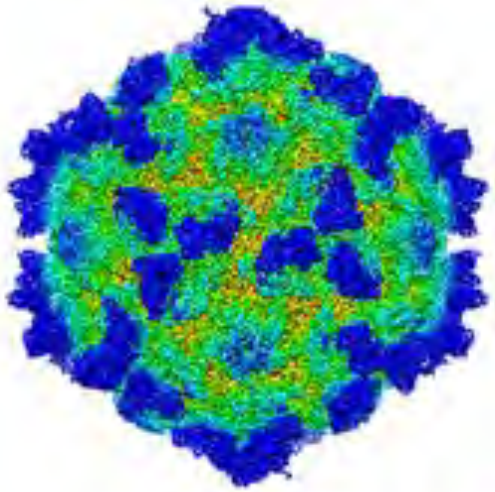
Pan-polio mAb

	Type 1			Type 2			Type 3	
	Mahoney	Sabin 1		MEF1	Sabin 2		Saukett	Sabin 3
doma mAbs								
6D11							1,158,500	364,900
6D2							7,200	14,400
7A1							444,800	91,200
7A2							1,459,600	459,800
8A12	364,900	182,500						
1E4	91,200	45,600		200			17,800	57,500
8F9				1,158,500	364,900			
2H5	900	300		200	200			
12F8	1,158,500	57,500		229,900	57,500		100	200
2F11	289,631	1,131						
3C10							289,631	1,158,524
2F7	289,631	289,631		72,408	36,204			
6B8	100	50		18,102	12,800		100	400
2F11-2							144,815	144,815
8E1							144,815	144,815
1E10							144,815	144,815
6A1	2,263	1,600		72,408	144,815		144,815	144,815
2B3				71			144,815	4,525
6G7							144,815	144,815
10D1	144,815	144,815		3,200	1,600			
7B1				144,815	102,400		18,102	18,102
8H4	72,408	72,408		72,408	72,408			
7D3							72,408	72,408
9F10	12,800	72,408		1,131	400			
8F2							72,408	72,408
9H2	72,408	72,408		72,408	72,408		1,600	36,204
10D2				12,800	1,600		18,102	36,204
3G9		400		283	3,200			6,400
8D3							72,408	72,408
7C9				9,051	3,200		18,102	72,408
6B5							36,204	72,408
7E5	72,408	72,408		72,408	72,408			
6B4							72,400	72,408

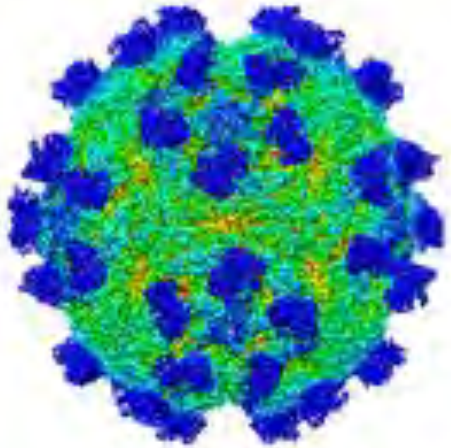
sIPV D antigen ELISA Universal Reagents



IPV1-2D6

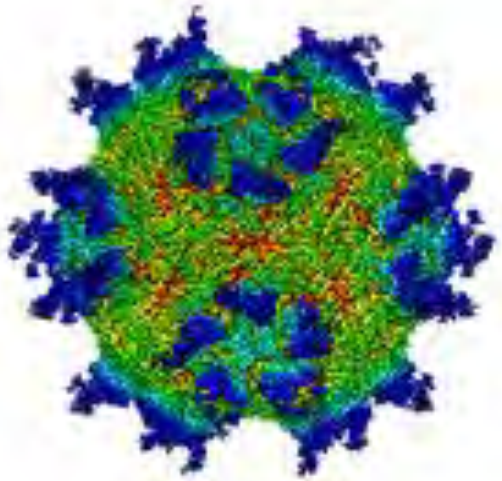


IPV2-1B8

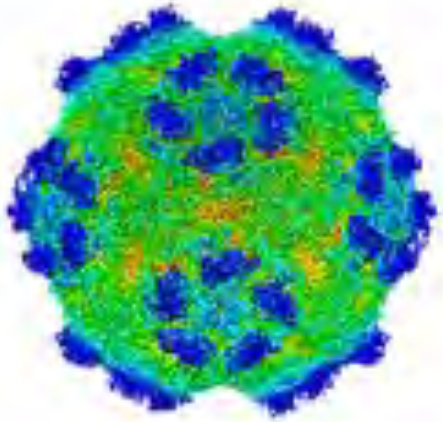


SIPV3-FAb 6B5

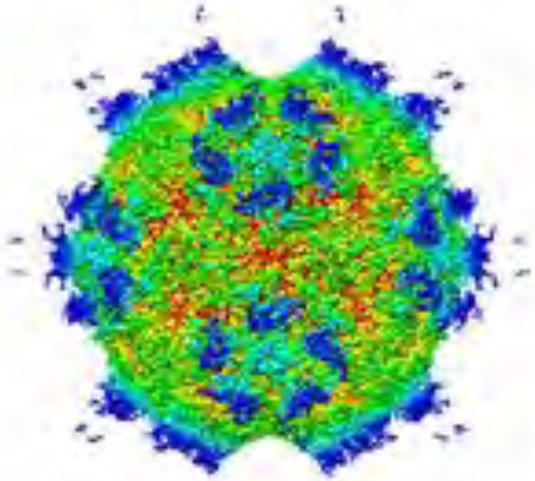
type 1, 2 & 3
specific huMabs
For capture



SIPV1-9H2



SIPV2-9H2



SIPV3-9H2

pan-cross reactive
huMab
For detection

3.6 A^0

PATH

nature communications

Article

https://doi.org/10.1038/s41467-023-41052-9

A human monoclonal antibody binds within the poliovirus receptor-binding site to neutralize all three serotypes

Received: 19 August 2022

Accepted: 17 August 2023

Published online: 10 October 2023

Check for updates

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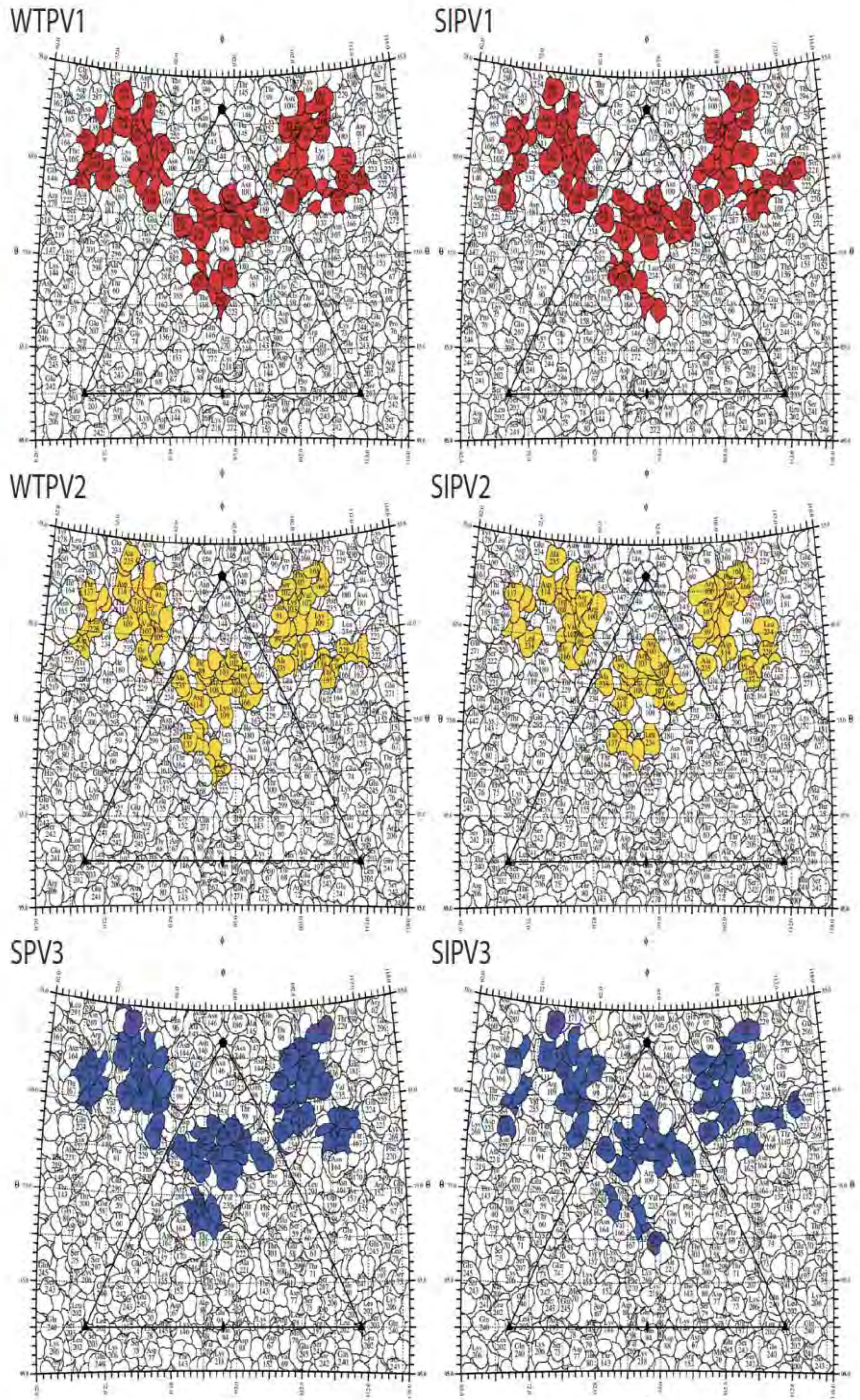
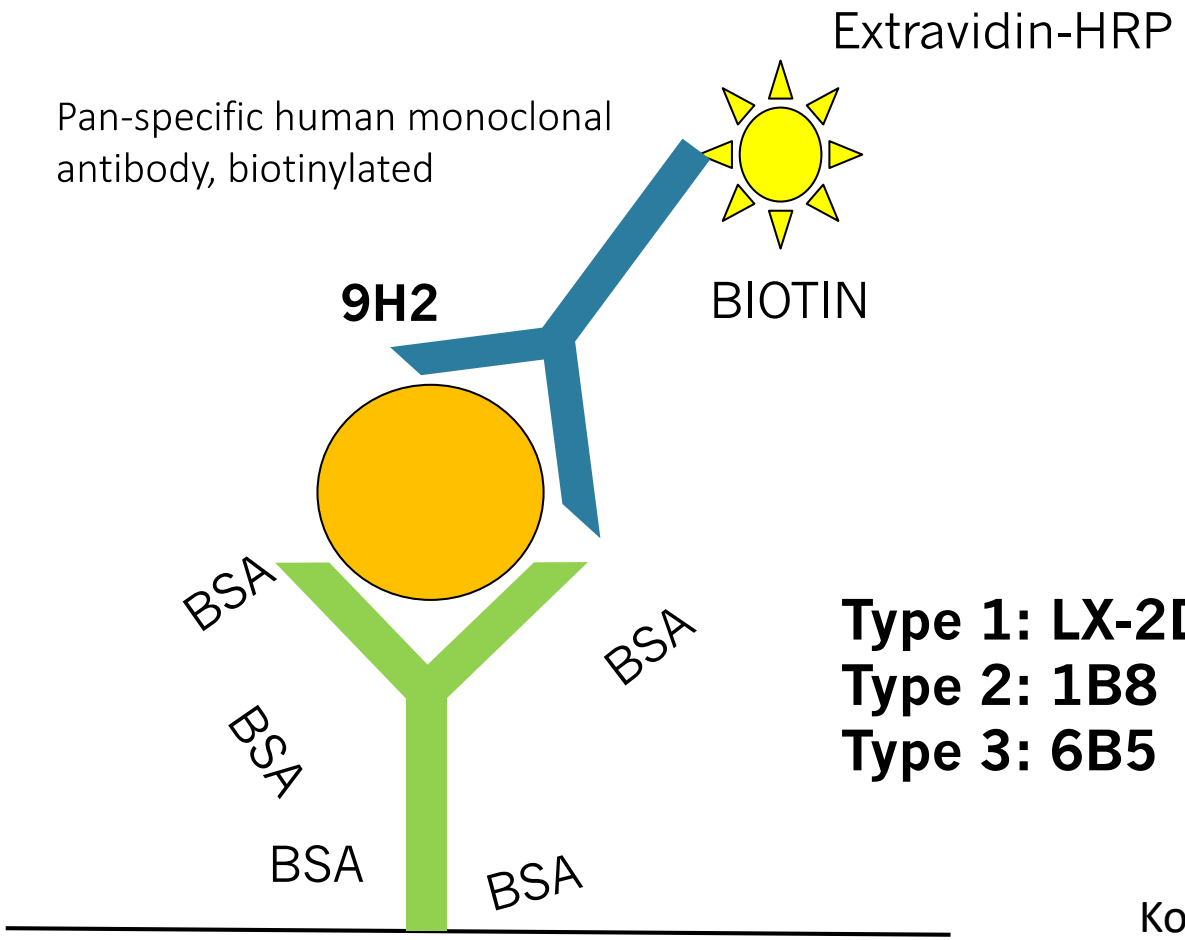


Universal ELISA protocol to measure potency of both conventional and Sabin IPV



	Mahoney	Sabin 1	MEF1	Sabin 2	Saukett	Sabin 3
LX_2D6	204,800	289,630				
1B8			23,170	46,341		
6B5					72,408	36,204
9H2	289,631	144,815	1,158,524	3,276,800	1,600	36,204

Type 1: LX-2D6
Type 2: 1B8
Type 3: 6B5

Serotype-specific human monoclonal antibody

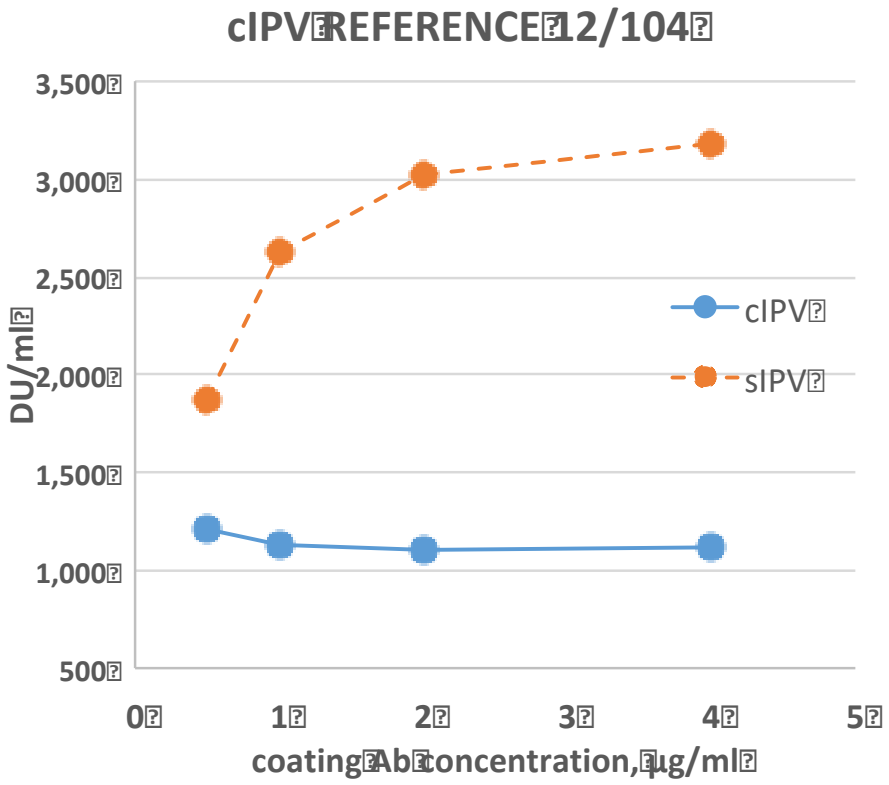
Kouiavskaia et al., Universal ELISA for quantification of D-antigen in inactivated poliovirus vaccines. Journal of Virological Methods, Vol. 276, February 2020, 113785

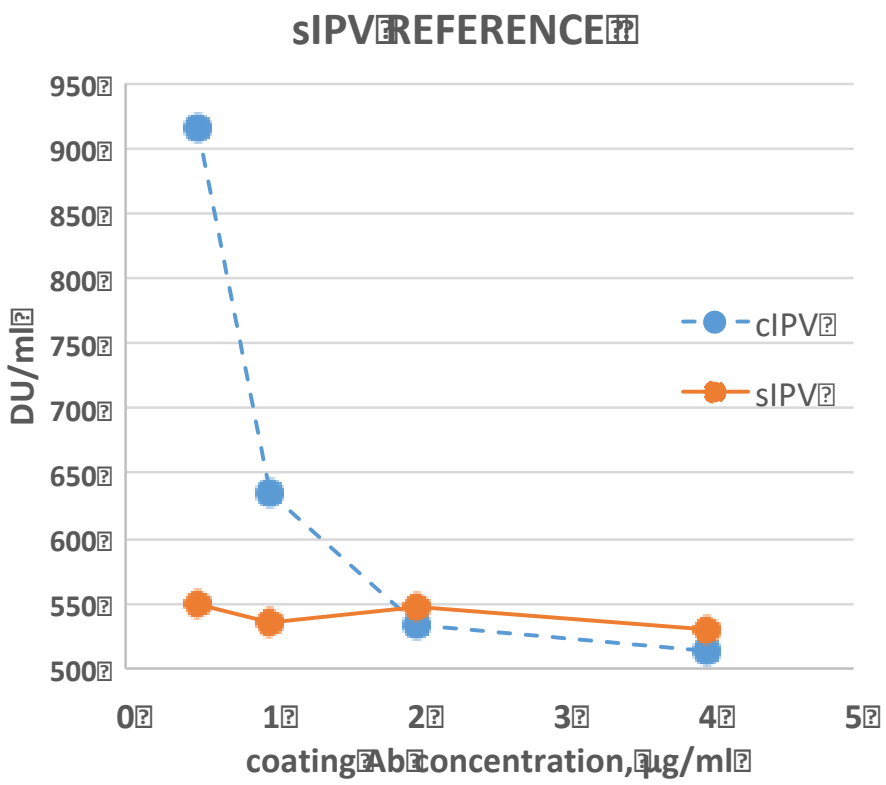
These reagents react equally well with Salk and Sabin strains and can be used to test potency of both cIPV and sIPV

However, each type of vaccine must be used with respective reference

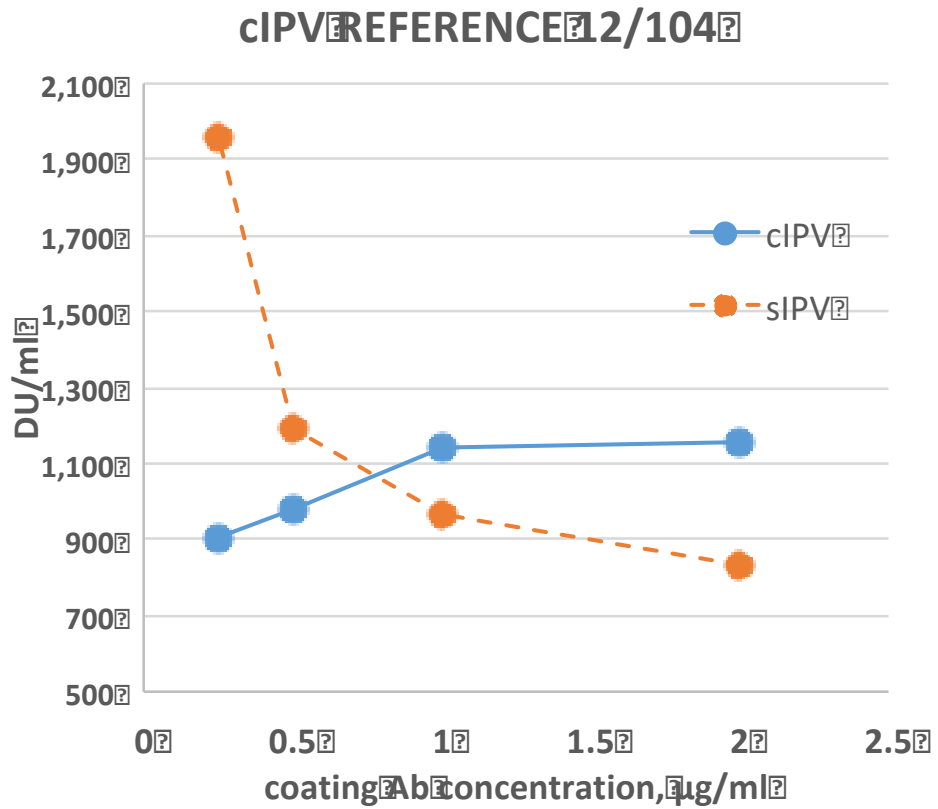
Is this a big deal?

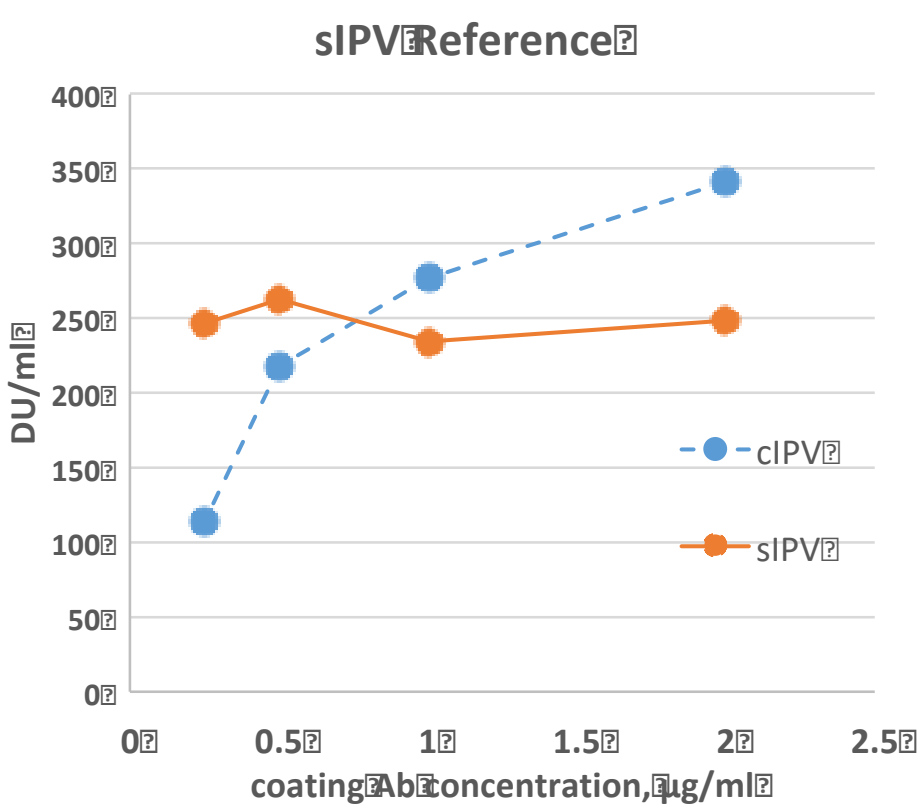
Cross-calibration of type 1 IPV



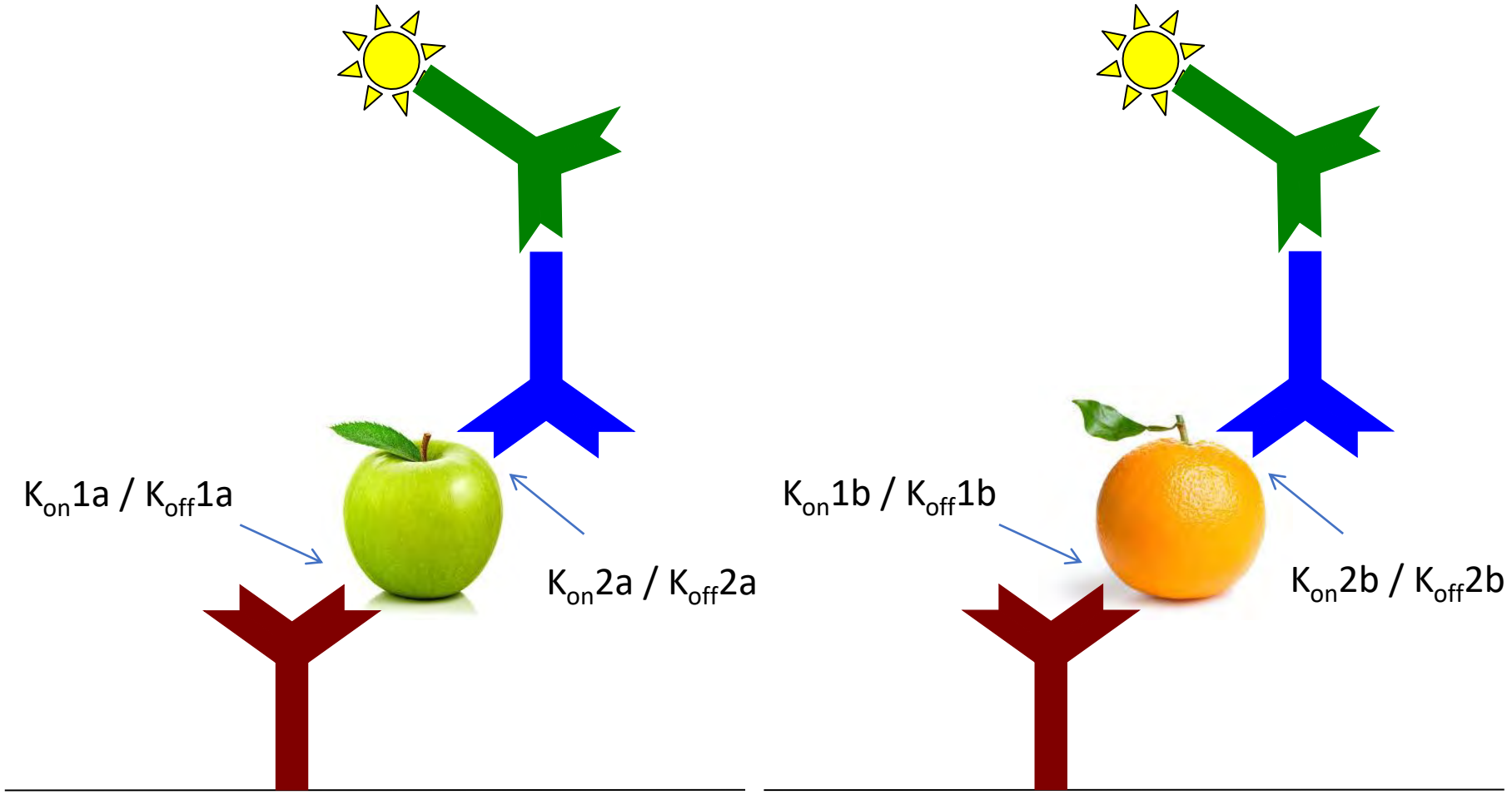


Cross-calibration of type 3 IPV





Why only homologous references must be used?



cIPV

sIPV

Conclusions

- A new sIPV International Standard 17/160 was prepared, validates and approved by ECBS
- A new Sabin IPV potency unit was established (sDU)
  - 17/160 reference material was arbitrarily assigned potency of 100 sDU
- Universal potency reagents based on human monoclonal antibodies were prepared and validated.
  - They can be used to test potency of both Salk and Sabin IPV
  - Only homologous International Standard must be used in conjunction with these reagents
  - The reagents are available from NIBSC
- D-antigen ELISA protocol using these reagents was optimized and validated
- Manufacturers are free to chose whether to use universal reagents or to prepare their own
  - New IPV manufacturers could benefit from adopting fully validated reagents and the test protocol
  - Formulation of IPV-containing products is different (e.g. adjuvants, combination vaccines). Therefore the protocol for testing final products must be validated by each manufacturer.

Acknowledgements

- **Diana Kouiavskaia**, *CBER/FDA* — ELISA protocol development
- **Scott Dessain**, *Lankenau Institute* — mAb discovery
- **Ramdev Puligedda**, *Lankenau Institute* — mAb discovery
- **Andrew Charnesky**, *PennState University* — Cryo-EM structure
- **Susan Hafenstein**, *PennState University* — Cryo-EM structure
- **Javier Martin**, *NIBSC* — Collaborative studies
- **Alison Tedcastle**, *NIBSC* — Collaborative studies
- **Kutub Mahmood**, *PATH* — Support and coordination

Thank you!

Medicines & Healthcare products Regulatory Agency

Standardization of D-antigen ELISA potency test for IPV

Alison Tedcastle

1st November 2023

Background

- The potency of IPV is measured *in vitro* using a validated ELISA test with a suitable reference and is expressed as D-Antigen units.

- Reference standards are essential to establish potency assays, calibrate internal references, compare batches of vaccines to ensure consistency of production, compare vaccines from different manufacturers and more.

- Since the development of conventional IPV products, reference standards have been available and has allowed manufacturers to share common target human doses for type 1, 2 and 3 poliovirus serotypes.
  - 40, 8 and 32 D-Antigen units for types 1, 2 and 3 poliovirus respectively per human dose.

- The current cIPV IS is 12/104 and is used internationally to calibrate internal reference reagents.
  - Other reference reagents (e.g Biological Reference Preparation – BRP) are also commonly used in these *in vitro* potency assays.

- They have been shown to be suitable for the determination of antigenic content and/or the immunogenicity of IPVs by *in vitro* and/or *in vivo* assays.

Current situation

- The current IS (12/104) is running low and a proposal for the 4th International Standard for IPV has been submitted to ECBS.
- 513 vials left of the current IPV IS - 12/104
- Two candidates have been proposed for the 4th IPV IS and were tested in conjunction with 12/104.
- A new collaborative study will be completed to identify the best candidate.
- Proposal has been endorsed by ECBS.

Proposal (title)	4th WHO International Standard for Inactivated Polio Vaccine		
Proposer (name of Institution)	MHRA	**Principal contact**	Mark Hassall
Rationale	Replacement of 3rd International Standard for inactivated Polio Vaccine (IPV) based on wild type strains (replacement for 12/104). We have a stock of 513 vials of the 3rd IS left, which will be depleted by 2026.		
Anticipated uses and users	This standard is for use with IPV derived from wild type polio virus strains and is suitable for determining the antigenic content by in vitro assays. It is intended for Clinical and public health laboratories, Vaccine manufacturers and national control laboratories (NCL) for calibration of secondary reference standards as well as harmonising and controlling assay performance.		
Source/type of materials	There are 2 possible candidate materials already which were tested in conjunction with the 3rd IS. These are prepared from commercially available lots of IPV donated by European manufacturers		
Outline of proposed collaborative study	A collaborative study incorporating both candidate materials with up to 10 participants running bioassays for D-antigen content will be set up to identify the best candidate and to calibrate to the 4th IS.		
Issues raised by the proposal			
Action required	ECBS to endorse proposal		
Proposer's project reference		**Date proposed:**	January 2023
CONSIDERATIONS FOR ASSIGNMENT OF PRIORITIES (TRS932)			
Approval status of medicine or in vitro diagnostic method	IPV is the vaccine of choice in the world with increasing usage across the globe considering the WHO Global Polio Eradication Initiative (GPEI). Multiple IPV products have been licensed in the world and prequalified by the WHO.		
Number of products or methods	IPV is used for routine vaccination and can be administered singly or as part of a multiple vaccine programme.		
Public health importance	Provision of a replacement IS will provide continued support to the standardization of the potency of IPVs globally and aid NCL's in the control of these. This will ensure the safety of the vaccine for use in disease control and prevention.		
Global importance	Most of the world is free of Polio, but there is a continued need for vaccines in the drive to eradicate it fully. Therefore, there is a need to be able to access suitable reference materials to assess the quality of vaccines and ensure there is enough available to meet the current and future needs.		
Global need from regulatory & scientific considerations	Provision of the IS will support the standardization of the potency of IPV and aid manufacturers and NCLs in the control of IPVs globally, and ultimately contribute to the global polio eradication programme.		
ECBS outcome	[BLANK]		

Sabin IPV

- In October 2019, the GCC concluded that wild poliovirus type 3 had been eradicated worldwide. This follows the declaration of eradication of wild poliovirus type 2 in 2015.
  - Historic achievement towards polio eradication and global health
- Detection of wild poliovirus type 1 is now limited to Pakistan and Afghanistan
- The focus is to now permanently interrupt all poliovirus transmission in endemic countries and stop circulating vaccine-derived poliovirus (cVDPV) transmission.
- cVDPVs are a rare result of live attenuated oral polio vaccine (OPV) strains evolving to virulent strains causing a number of poliomyelitis outbreaks across the world. The post eradication era ultimately requires cessation of all OPV use to prevent cVDPV outbreaks.
- The switch from trivalent OPV to bivalent OPV and eventually the removal of OPV entirely from routine vaccination regimens requires the global introduction of IPV.

- The higher containment level now required for type 2 poliovirus and ultimately all poliovirus has led to the increased production of IPV using Sabin strains – a safer alternative to the usual wild-type strains.

- The increase in demand for sIPV has led to new manufacturers starting production which in turn has created a need for harmonisation of reagents and test methods.

- The available evidence suggests that there may be significant differences in the antigenic composition of various IPV products developed independently, particularly when comparing sIPV to cIPV.

- However, during the introduction of sIPV vaccine manufacture there were no International standards or reference reagents for sIPV and there are no defined requirements in terms of specific D-Antigen units / human dose.
  - Manufacturer specific references were not calibrated against a common reference causing issues when comparing different products.

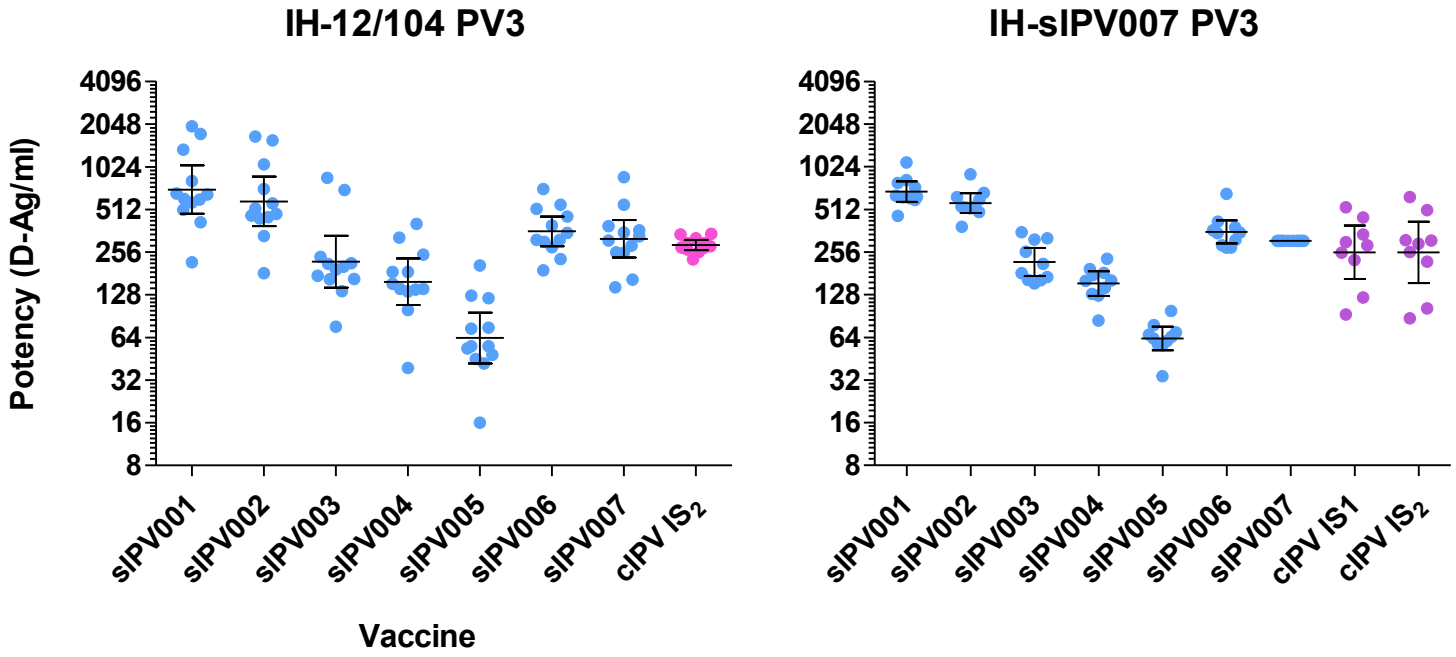
- In 2016, NIBSC conducted a study to identify whether the current cIPV reference reagent would be suitable to analyse sIPV products.
  - Conclusions were that the highest level of consistencies were observed when a homologous reference was used in addition to a common method.

In-house method

Virus	Sample	All labs		
		GM	%GCV	N
Type 1	15001	49	71	9
	15002	73	53.8	10
	15003	142	51.4	10
	15004	191	80.7	10
	15005	17	76.7	11
	15006	461	95	10
	15007	423	54.6	10
	08/143	357	6.4	11
Type 2	15001	513	33	9
	15002	509	38.6	8
	15003	29	21.2	8
	15004	71	14.8	8
	15005	28	58.2	8
	15006	160	47.1	9
	15007	165	38.9	9
	08/143	81	12.7	11
Type 3	15001	687	50.2	8
	15002	435	35.9	8
	15003	202	45.9	7
	15004	121	61.4	9
	15005	55	47.5	8
	15006	308	31.2	9
	15007	283	30.3	8
	08/143	276	8.7	11

NIBSC method

Virus	Sample	All labs		
		GM	%GCV	N
Type 1	15001	53	23.2	9
	15002	49	24.7	9
	15003	125	12.2	9
	15004	142	8.8	9
	15005	10	3.8	6
	15006	378	11.8	9
	15007	367	13.4	9
	08/143	352	8	9
Type 2	15001	487	10.3	9
	15002	486	9.5	9
	15003	29	6.5	9
	15004	74	20.1	8
	15005	27	23.3	9
	15006	207	27.7	9
	15007	207	7.5	9
	08/143	79	13.9	9
Type 3	15001	706	9.1	7
	15002	581	17.7	7
	15003	197	8	8
	15004	164	12.2	7
	15005	59	12.5	9
	15006	244	23.4	9
	15007	303	8.7	9
	08/143	291	10.8	9



World Health Organization

WHO/BS/2018.2338
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 29 October to 2 November 2018

Report on the WHO collaborative study to establish the 1st International Standard for Sabin inactivated polio vaccine (sIPV)

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- Two sIPV candidates were proposed
- Both sIPV samples and cIPV references were tested alongside 12/104 and newly proposed sIPV candidates
- Study concluded that there was generally good agreement between laboratories when using the common method and 12/104 as the reference but not when using 12/104 and in-house methods.
- Improvement was seen when using the sIPV products as a reference with either the in-house or common method.
- The study revealed differences in the reactivity of antibodies used in the various in-house methods with cIPV and sIPV products when using a heterotypic reference.

- Candidate <u>17/160</u> was chosen as it showed better overall results in terms of assay validity, intra and inter laboratory variability and thermostability profiles.
- It was recommended that a new antigen unit was assigned.
  - Sabin D-Antigen (SDU), specific for sIPV products.

		In-house method				NIBSC method		
	Reference	12/104	17002	17004	Reference	12/104	17002	17004
Type 1	17001	91.3	74.7	60.6		34.4	40.2	44.5
	17002	73.7		23.2		55.4	45.2	
	17003	81.3	28.1	17.5		22.0	12.5	50.4
	17004	74.0	23.2			26.9		45.2
	17005	67.7	11.1	19.4		52.9	43.1	12.0
	17006	75.9	8.3	16.1		40.0	33.5	25.0
	17007	65.2	18.7	6.7		22.6	11.0	46.2
Type 2	17001	24.7	12.7	7.9		36.2	53.1	26.1
	17002	21.2		9.2		15.8		19.7
	17003	21.6	19.9	19.9		6.7	19.2	19.8
	17004	26.3	8.2			29.2	19.7	
	17005	25.0	17.0	28.0		17.7	15.5	18.7
	17006	39.2	50.7	59.8		25.0	27.0	28.6
	17007	28.4	7.8	5.0		27.4	19.0	6.1
Type 3	17001	34.2	19.8	7.1		30.1	36.5	18.4
	17002	32.6		13.0		18.2		27.7
	17003	40.2	49.2	39.4		14.3	15.7	27.9
	17004	26.2	13.0			13.9	27.5	
	17005	33.4	10.3	13.0		19.7	21.1	31.0
	17006	35.2	7.6	19.6		21.9	22.1	25.7
	17007	26.6	16.0	6.5		16.2	31.2	8.5

Calculating potency and validity

The assigned potency for 12/104 Type 1

Standard		
Id.	12/104	
Ass. pot.	277 Du/ml	
Doses	(1)	(2)
1/20	1.7511	1.7796
1/40	1.2842	1.2968
1/80	0.8658	0.8843
1/160	0.5410	0.5684

Sample 1		
Id.	17/160	
Ass. pot.	100 Du/ml	
Doses	(1)	(2)
1/20	1.7497	1.7331
1/40	1.2430	1.2384
1/80	0.8077	0.8029
1/160	0.5194	0.5081

Sample 2		
Id.	Monitor	
Ass. pot.	? Du/ml	
Doses	(1)	(2)
1/20	1.9709	1.9717
1/40	1.3919	1.5339
1/80	0.9097	1.0920
1/160	0.5505	0.7499

Sample 3		
Id.	Sample 1	
Ass. pot.	? Du/ml	
Doses	(1)	(2)
1/40	2.3211	2.3589
1/80	1.8708	1.9146
1/160	1.3846	1.4361
1/320	0.9208	0.9850

Model: Sigmoid curves
Design: Completely randomised
Transformation: y' = logit(y)
Variance: Observed residuals

Common slope(factor) = 0.937186 (0.899756 to 0.974616)
Correlation | r |: 0.995581 (Weighted), 0.996978 (Unweighted)
Asymptotes: 0.0902133 and 3.16130

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	3	0.616642	0.205547	186.524	0.000 (***)
Regression	1	5.60752	5.60752	1696.18	0.000 (***)
Non-parallelism	3	0.00185215	0.000617384	0.560245	0.905
Non-linearity	8	0.000632399	7.90498E-05	0.191290	1.000
Standard	2	0.000139522	6.97611E-05	0.0422031	0.979
Sample 1	2	0.000139392	6.96959E-05	0.0421637	0.979
Sample 2	2	0.000237029	0.000118515	0.0716974	0.965
Sample 3	2	0.000116455	5.82277E-05	0.0352258	0.983
Treatments	15	6.22665	0.415110	1883.46	0.000 (***)
Residual error	16	0.0528955	0.00330597		
Total	31	6.27954	0.202566		

The statistical analysis shows no significant deviation from linearity or parallelism

All standards and samples show no significant deviation from linearity

Sample 1			
Id.	17/160		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	233.581	257.162	283.060
Rel. to Ass.	233.6%	257.2%	283.1%
Rel. to Est.	90.8%	100.0%	110.1%

Sample 2			
Id.	Monitor		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	326.015	357.911	393.218
Rel. to Ass.	?	?	?
Rel. to Est.	91.1%	100.0%	109.9%

Sample 3			
Id.	Sample 1		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	1190.00	1307.20	1439.34
Rel. to Ass.	?	?	?
Rel. to Est.	91.0%	100.0%	110.1%

Monitor is within range for PV type 1;

354.4-511.8 D-Antigen/ml

Calculating potency and validity

The assigned potency for 17/160 Type 1

Sample 1		
Id.	12/104	
Ass. pot.	277 Du/ml	
Doses	(1)	(2)
1/20	1.7597	1.7352
1/40	1.2386	1.2065
1/80	0.8170	0.8673
1/160	0.5025	0.5323

Standard		
Id.	17/160	
Ass. pot.	100 Du/ml	
Doses	(1)	(2)
1/20	1.5741	1.6903
1/40	1.1273	1.2144
1/80	0.7424	0.8137
1/160	0.4809	0.5141

Sample 2		
Id.	Monitor	
Ass. pot.	? Du/ml	
Doses	(1)	(2)
1/20	~~1.8260~~	~~1.8796~~
1/40	1.5934	1.5319
1/80	1.0318	1.0317
1/160	0.7049	0.7050

Sample 3		
Id.	Sample 1	
Ass. pot.	? Du/ml	
Doses	(1)	(2)
1/40	2.3009	2.2246
1/80	1.7165	1.7349
1/160	1.2394	1.2601
1/320	0.8579	0.8602

Model: Sigmoid curves
Design: Completely randomised
Transformation: y' = logit(y)
Variance: Observed residuals

Common slope(factor) = 0.781213 (0.759365 to 0.803062)
Correlation | r |: 0.997460 (Weighted), 0.998010 (Unweighted)
Asymptotes: 0.00256217 and 3.88944

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	3	0.834436	0.278145	586.256	0.000 (***)
Regression	1	4.92321	4.92321	3458.94	0.000 (***)
Non-parallelism	3	0.00274297	0.000914323	1.92715	0.588
Non-linearity	7	0.00526372	0.000751960	3.69817	0.814
Sample 1	2	0.00121333	0.000606666	0.852461	0.653
Standard	2	0.000168261	8.41306E-05	0.118217	0.943
Sample 2	1	0.00311892	0.00311892	2.19129	0.139
Sample 3	2	0.000763206	0.000381603	0.536212	0.765
Treatments	14	5.76566	0.411833	4050.83	0.000 (***)
Residual error	15	0.0213499	0.00142333		
Total	29	5.78701	0.199552		

The statistical analysis shows no significant deviation from linearity or parallelism

All standards and samples show no significant deviation from linearity

Sample 1			
Id.	12/104		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	105.547	112.511	119.958
Rel. to Ass.	38.1%	40.6%	43.3%
Rel. to Est.	93.8%	100.0%	106.6%

Sample 2			
Id.	Monitor		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	160.547	171.831	183.910
Rel. to Ass.	?	?	?
Rel. to Est.	93.4%	100.0%	107.0%

Sample 3			
Id.	Sample 1		
(Du/ml)	Lower limit	Estimate	Upper limit
Potency	427.700	455.461	485.686
Rel. to Ass.	?	?	?
Rel. to Est.	93.9%	100.0%	106.6%

No current reference reagents for sIPV to use as internal monitors. End users will need to validate their own reagents against the IS to ensure assays are valid.

Calculating potency and validity

Reference validation – 17/130

- A monitor needs to be included in the D-Antigen potency test for batch release of IPV.
- The D-Antigen content of the monitor sample must lie within a specific range for the test to be valid.
- To establish a new monitor, the aim is to analyse between 5 and 10 assays to determine the D-Antigen content of the reagent.
  - All validity criteria must be met as per SOP.

	Assays									GM	SD	3 x SD	Range	
Type	22.08.18	23.08.18	30.08.18	11.05.21	13.5.21	18.5.21	29.09.21	30.09.21	30.10.21					
1	26.9	27.6	31	16.7	23.4	24.9	22.8	21.3	26.4	24.2	4.1	12.4	11.8	36.6
2	412.4	458.3	427.2		396.6	387.1	431.9	446.8	335	410.2	39.2	117.7	292.5	527.8
3	225.9	244.8	171.6		156.2	176.8	173.7	141.5	156.6	178.0	35.9	107.7	70.4	285.7

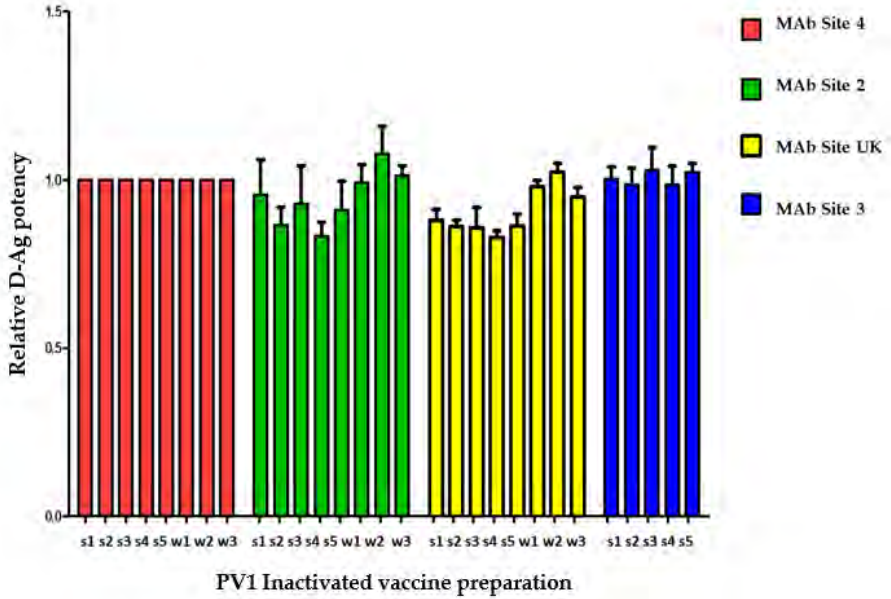
- This is a preliminary range and the table will be continually updated to include all new tests.

- The current D-Antigen ELISA method used to calculate vaccine potency requires a suitable reference and employs either polyclonal or monoclonal antibodies.
  - These all have slightly different specificities and can lead to disagreements between laboratories which makes comparisons difficult.
  - It is recognized that IPV derived from attenuated strains or adjuvanted IPV may require different D-antigen content to induce adequate immune responses in humans.

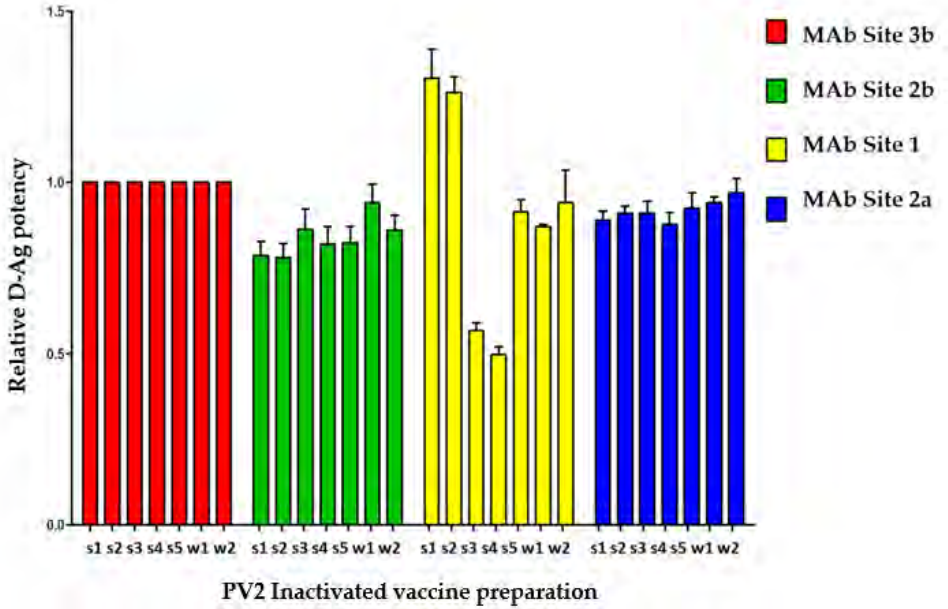
Appendix 1.
Details of Assay Methods used by Participants

Lab.	Coating antibody	Detection antibody	Conjugated antibody	No. of replicas/ dilutions	Substrate
1	Bovine polyclonal serum	Rabbit polyclonal serum	Goat anti-rabbit IgG-HPRO	2	ABTS
2	Bovine polyclonal serum	Mouse MAb	Sheep anti-mouse IgG-HRPO	2/5	TMB
3	Bovine polyclonal serum	Mouse MAb	Sheep anti-mouse IgG-HRPO	2/7	TMB
4	Rabbit PSerl	Mouse MAb	Sheep anti-mouse serum-HRPO	2 / 7	TMB
5	Mouse MAb	Rabbit polyclonal serum	Anti-rabbit IgG-HRPO	3/5	OPD
6	Rabbit polyclonal serum - IgG fraction	-	Rabbit polyclonal anti-polio serum-HRPO	2/5	OPD
8	Manufacturer antibody	Manufacturer antibody	Goat anti-rabbit IgG-HRPO	2/5	PNPP
9	Rabbit polyclonal serum	-	Polyclonal anti-IPV serum- HRPO	3/4	TMB
10	Rabbit polyclonal serum	-	Rabbit polyclonal anti-polio serum-HRPO	2/5	TMB
11	Bovine polyclonal serum	Rabbit polyclonal serum	Goat anti-rabbit serum-HRPO	3	ABTS
12	Bovine polyclonal serum	Rabbit polyclonal serum	Anti-rabbit IgG-HRPO	2/6	ABTS
13	Bovine polyclonal serum	Mouse MAb	Anti-mouse IgG-HRPO	Duplicate	TMB
14	Bovine polyclonal serum	-	Sheep anti-mouse IgG-HRPO	2/8	TMB
15	Bovine polyclonal serum	Rabbit polyclonal antibodies	Goat anti-rabbit IgG-HPRO	3/6	ABTS
16	Mouse MAb	Rabbit polyclonal antibodies	Anti Rabbit IgG-HRPO	3/5	OPD
17	Biacore Technology (see method below)*				
18	Rabbit polyclonal serum - IgG fraction	Rabbit biotinilated polyclonal serum	Streptavidin-peroxidase	2/7-8	TMB
19	Sheep/Rabbit polyclonal serum	Mouse MAb	Anti-mouse Ig-HPRO	4/4	OPD

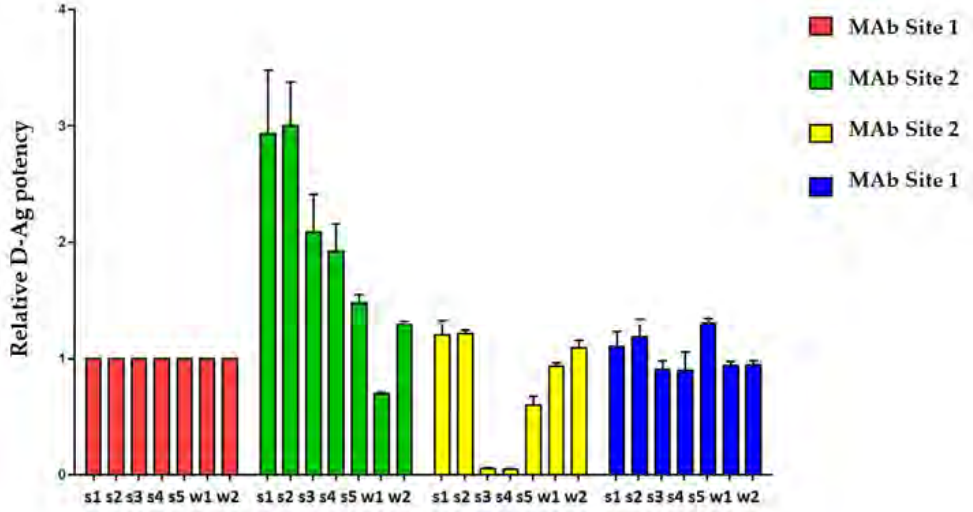
* The D-Ag content of study samples were measured in a Biacore T100 (GE Healthcare), equipped with an anti-polio biosensor. Goat anti-mouse IgG Fc-specific were covalently immobilized on the dextran layer of a CM3 sensorchip by primary amine coupling. MAbs were bound to the sensor at a flow rate of 10 µl/min during 120 s, followed by IPV at the same flow rate. The sensor chip was regenerated with 10 mM glycine-HCl, pH 1.5. Assay data were analysed by a four-parameter logistic curve fitting using the Biacore T100 evaluation software. Antigenicity was calculated relative to the international reference PU91-01



PV1 Inactivated vaccine preparation



PV2 Inactivated vaccine preparation



PV3 Inactivated Vaccine Preparation

World Health Organization

WHO/BS/2022.2432
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 24 to 28 October 2022

Report on the WHO collaborative study to establish Universal Reagents for the D-Antigen potency testing of Inactivated Polio Vaccines

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Aim: To evaluate the suitability of a set universal reagents (Monoclonal antibodies) and associated method for the potency testing of IPVs.

Source Material

- Vaccine samples were kindly provided by licensed vaccine manufacturers and were a combination of cIPV and sIPV products.
  - These were tested using 12/104 and 17/160 as the references.

CBER method

- The universal reagents are the purified poliovirus type 1, 2 and 3 specific and cross-reactive recombinant human monoclonal antibodies – these were provided by PATH.

  Generated from human B cell hybridomas and the heavy and light chains cloned into CHO cells.

NIBSC method

- The poliovirus specific sheep polyclonal and mouse monoclonal antibodies are the stocks currently available in the NIBSC products catalogue and have been previously validated – these were used as a control.

Source Material

Formulation

- Human monoclonal antibodies were filled and freeze-dried using a Bausch and Strobel AFV5090 machine with a 4-day cycle
- The human type specific monoclonal antibodies were diluted to the desired concentration in PBS before filling commenced.
- The human pan specific antibody was diluted in 1% BSA in PBS before filling commenced.

Presentation

- Freeze-dried material in 2.5 ml glass ampoules
- Material to be reconstituted in 500 ul sterile water before use
- 4 candidates:
  - Human monoclonal antibody 2D6 – Type 1 (NIBSC 20/250)
  - Human monoclonal antibody 1B8 – Type 2 (NIBSC 20/252)
  - Human monoclonal antibody 6B5 – Type 3 (NIBSC 20/254)
  - Human pan specific monoclonal antibody 9H2 (NIBSC 20/256)

Source Material

Table 1. Product Summary of the universal reagents.

NIBSC Code (study code)	20/250 (2D6)	20/252 (1B8)	20/254 (6B5)	20/256 (9H2)
Presentation	2.5 ml glass ampoule	2.5 ml glass ampoule	2.5 ml glass ampoule	2.5 ml glass ampoule
No. of containers	3127	2919	3192	4249
Mean fill mass	0.52g	0.52g	0.52g	0.52g
CV fill mass	0.19%	0.34%	0.19%	0.44%
Mean dry weight	0.005g	0.005g	0.05g	0.01g
CV of dry weight	4.47%	6.30%	1.15%	1.57%
Mean residual moisture	1.26%	1.27%	0.43%	1.00%
CV residual moisture	16.40%	19.40%	21.43%	12.77%
Mean oxygen headspace	0.36%	0.50%	0.59%	0.36%
CV of oxygen headspace	30.60%	40.20%	17.92%	24.64%
Date of fill / lyophilisation	December 2019	December 2019	December 2019	December 2019
Storage temperature	-20ºC	-20ºC	-20ºC	-20ºC
Microbial contamination	None detected	None detected	None detected	None detected

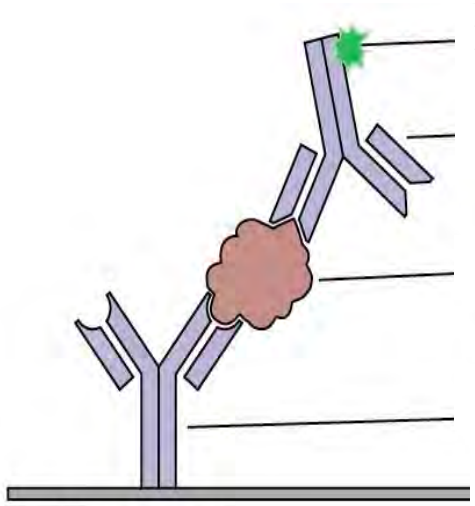
HuMAb and CBER method

Antibody	Specificity	Name of antibody	Working concentration, µg/ml	Amount of antibody provided, mg/vial	Concentration after reconstitution in 0.5 ml water
Coating	Type 1	LX-2D6	1.5	0.5	1 mg/ml
	Type 2	1B8	1	0.25	0.5 mg/ml
	Type 3	6B5	1.5	0.5	1 mg/ml
Detection (biotinylated)	Types 1, 2, and 3	9H2	1	0.1	0.2 mg/ml

HuMAb and CBER method

Step #	Step	Reagent	Concentration	Volume per well, µl	Incubation time	Incubation temperature
1	Coating	Coating Ab, in Coating buffer	See table in 3.1	60	Overnight	4° C
2	Wash, 3X	Washing buffer		300	n/a	RT
3	Blocking	Blocking buffer		100	45-60 min	25° C
4	Wash, 3X	Washing buffer		300	n/a	RT
5	Antigen (IPV; sIPV)	in dilution buffer		50	Overnight	4° C
6	Wash, 3X	Washing buffer		300	n/a	RT
7	Detection	Detection antibody, biotinylated, in dilution buffer	See table in 3.1	50	1h 30min	25° C
8	Wash, 3X	Washing buffer		300	n/a	RT
9	Avidin-HRP	in dilution buffer	1:1000	50	40 min	25° C
10	Wash, 3X	Washing buffer		300	n/a	RT
11	TMB substrate	TMB		100	15 min	RT, shaking
12	STOP	STOP solution		100	20-30 sec	RT, shaking
13	Reading: 450 nm				Read within 15 mins	

HuMAb and CBER method



Biotinylated

Cross Reactive Detection Antibody

Viral Antigen

Type specific Capture antibody

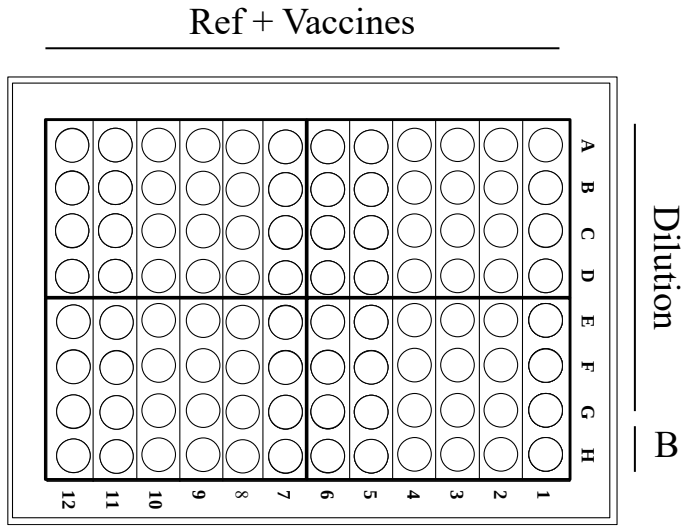
Solid support

Capture HuMAbs;

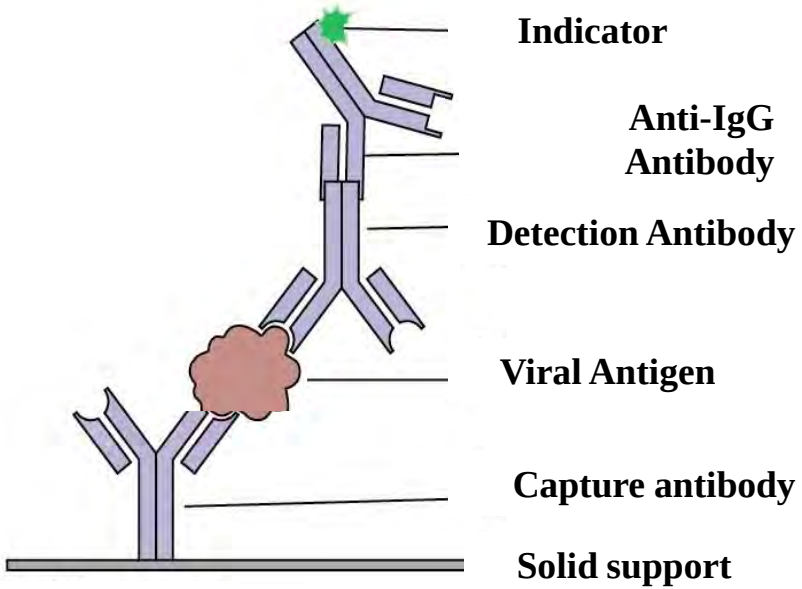
Type 1	2D6 – 20/250
Type 2	1B8 – 20/252
Type 3	6B5 – 20/254

Detection HuMAb;

Cross reactive	9H2 – 20/256



NIBSC reagents method

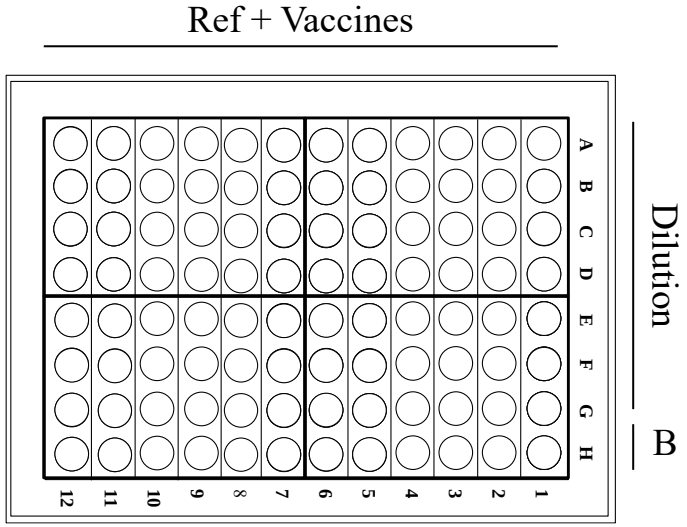


Capture mMAbs;

Type 1	13/118
Type 2	13/120
Type 3	13/122

Detection mMAb;

Type 1	234
Type 2	1050
Type 3	520



Outline of Collaborative Study

19 global laboratories were invited to participate in the study

15 laboratories returned data on time

- These were from nine different countries and three different continents

Participants were requested to;

- Determine the D-Antigen content of a panel of 3 coded trivalent cIPV samples (21001-21003) and 3 coded trivalent sIPV samples (21004-21006) using standards 12/104 and 17/160. This was to be completed using the CBER method and the NIBSC method as the control.

- Perform three independent assays per serotype per method.

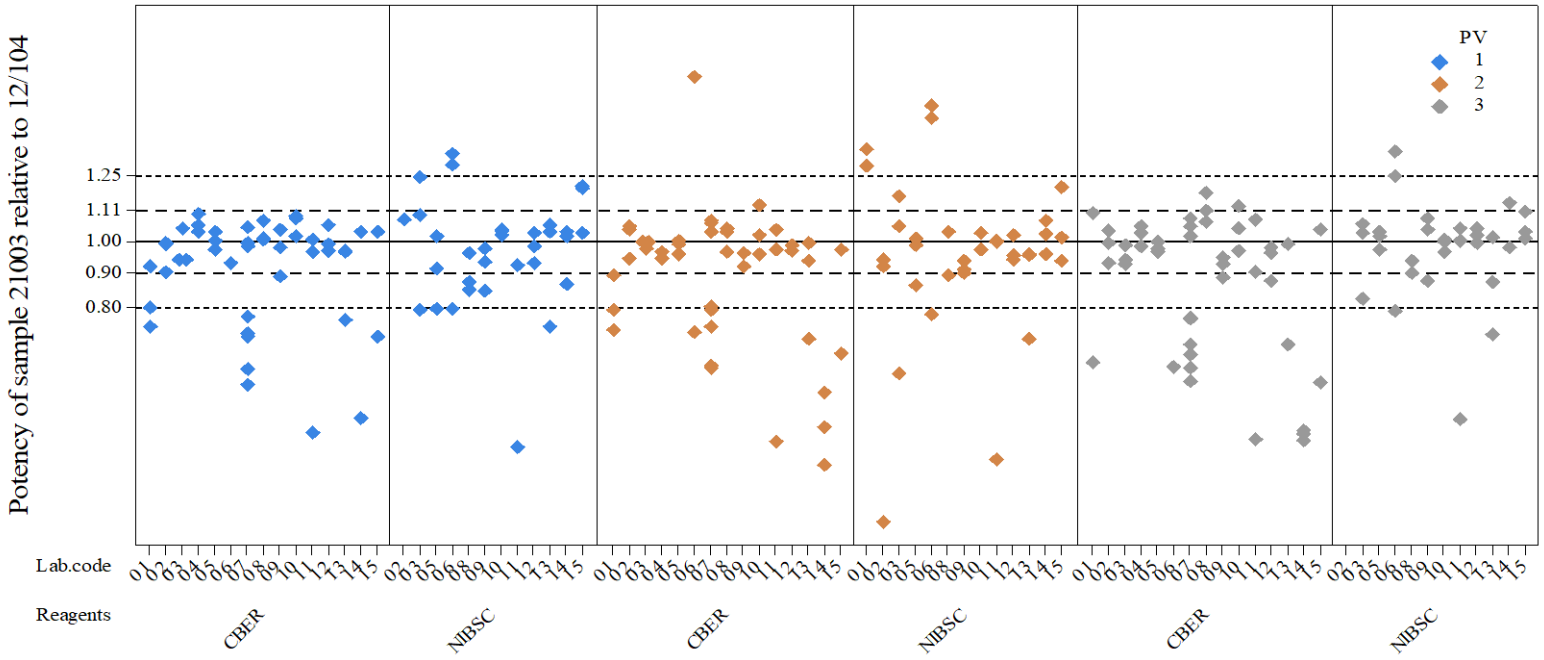
- Use both 12/104 and 17/160 to calculate the D-Antigen content of the 6 coded samples

- Test all study samples at the same time for each of the three independent determinations.

Collaborative Study Results

- There was less than 2% of exclusions due to non-parallelism

- Sample 21003 was a coded 12/104 sample therefore could be used to assess intra-assay variability
  - Majority of laboratories had low intra-assay variability (within 0.80-1.25) with some higher individual cases



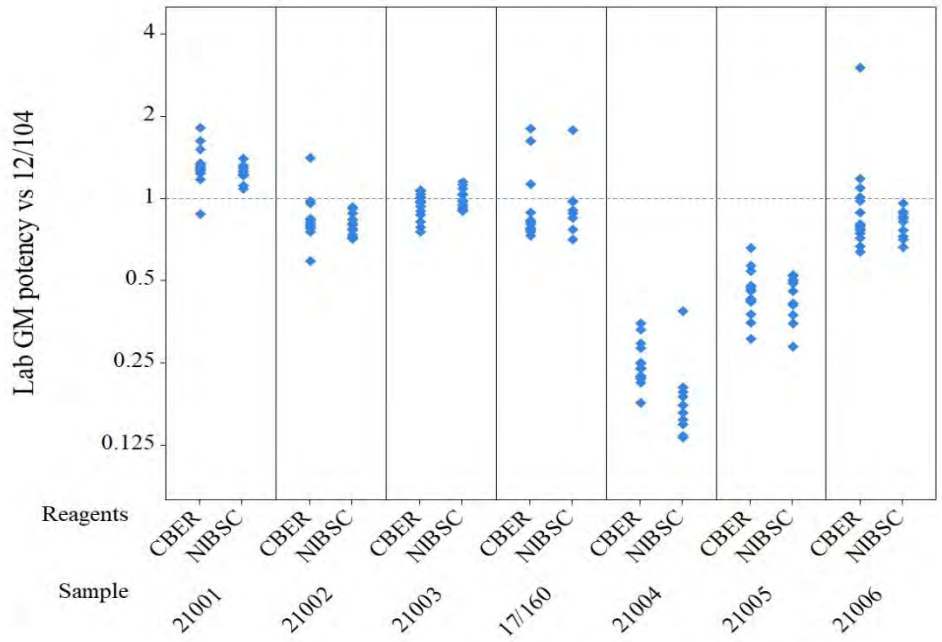
Collaborative Study Results

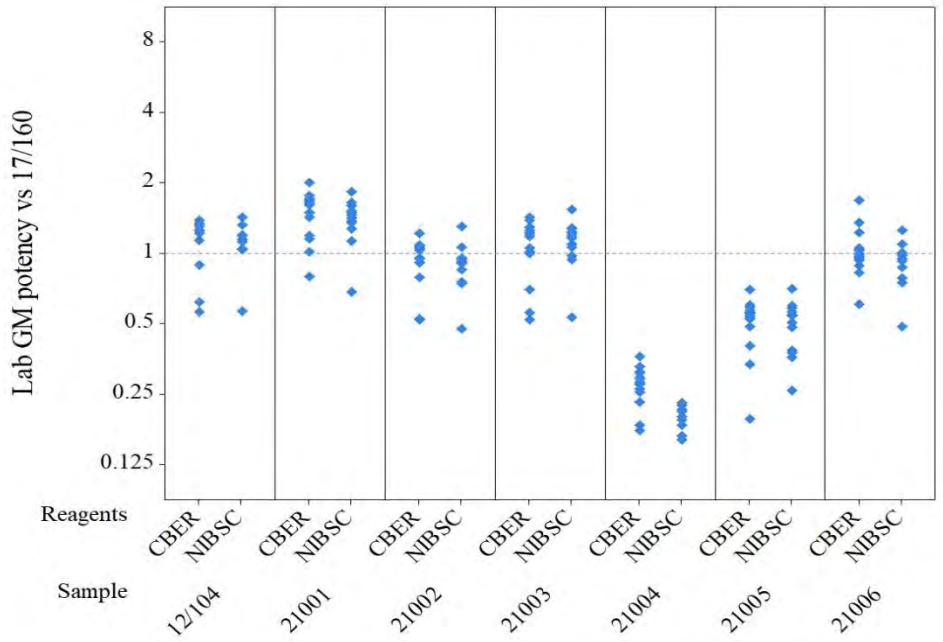
Relative potencies

In general, the inter-laboratory variability was slightly higher for 17/160 compared to 12/104.

PV type 1

There was highly significant differences observed between reagents for sample 21004 using both references



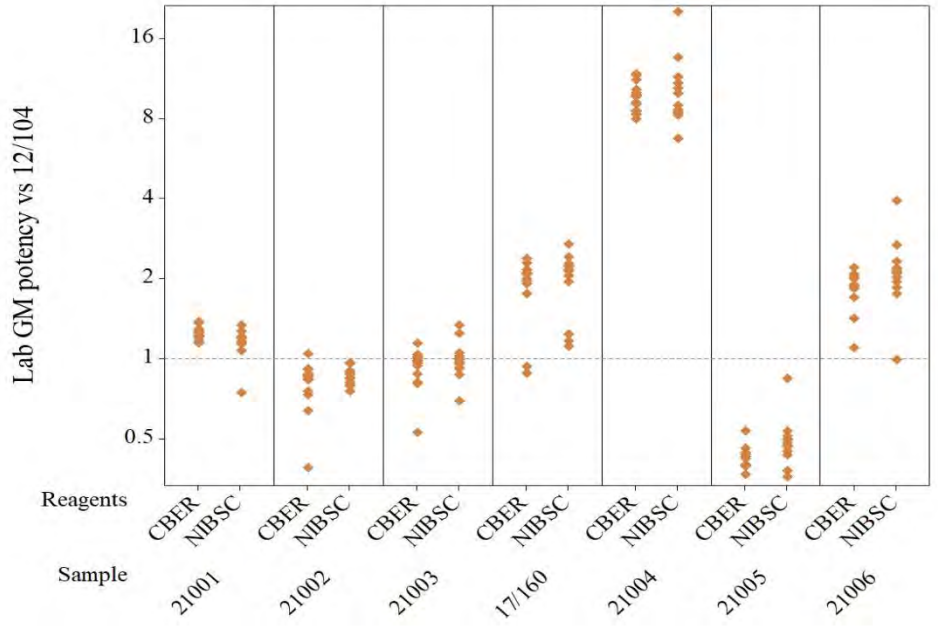


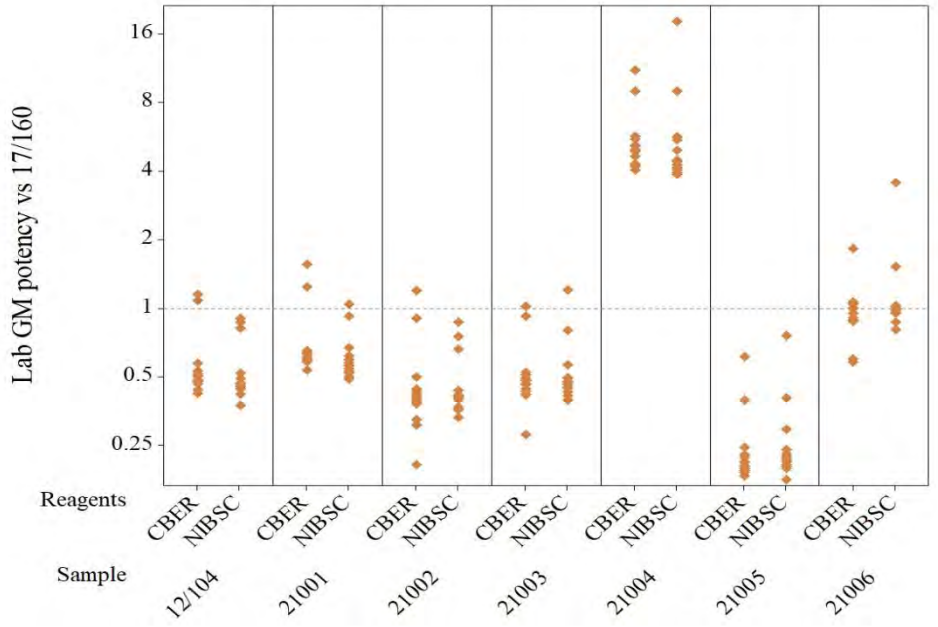
Collaborative Study Results

PV type 2

There were significant differences for samples 21005 and 21006 when using 12/104 as the reference only

- This highlights the need for homologous references

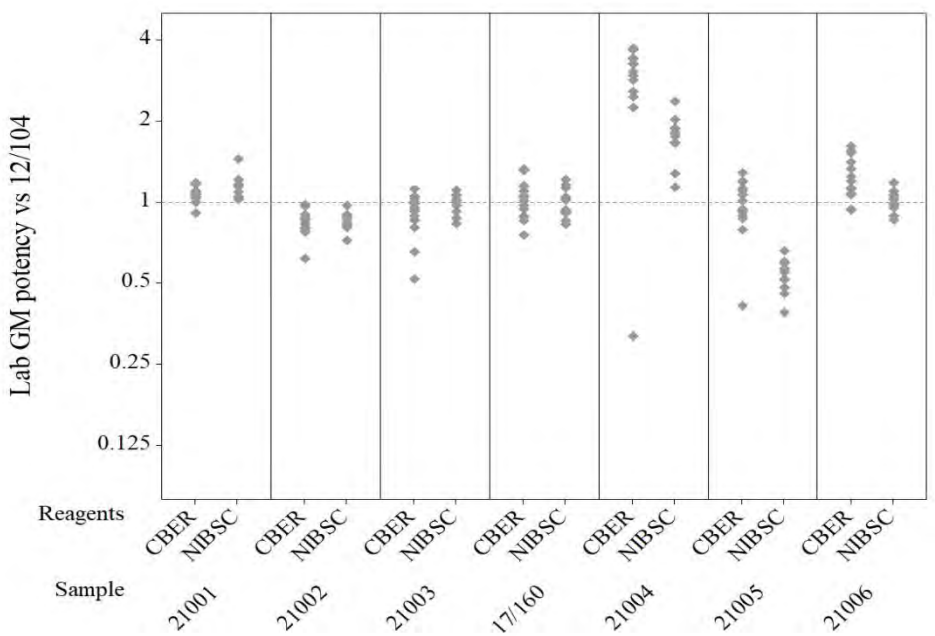


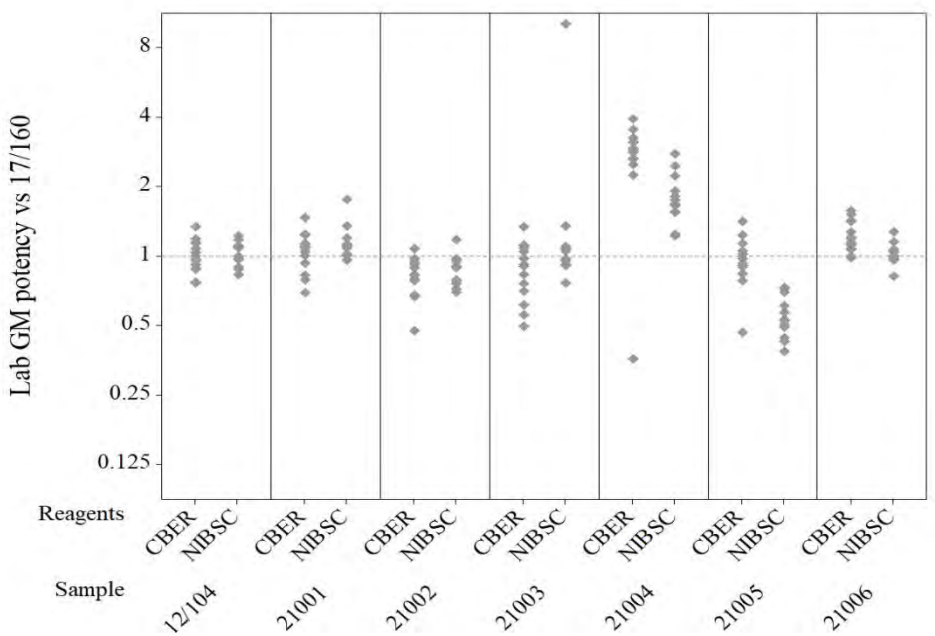


Collaborative Study Results

PV type 3

There were highly significant differences in relative potencies for samples 21004, 21005 and 21006 when using both references





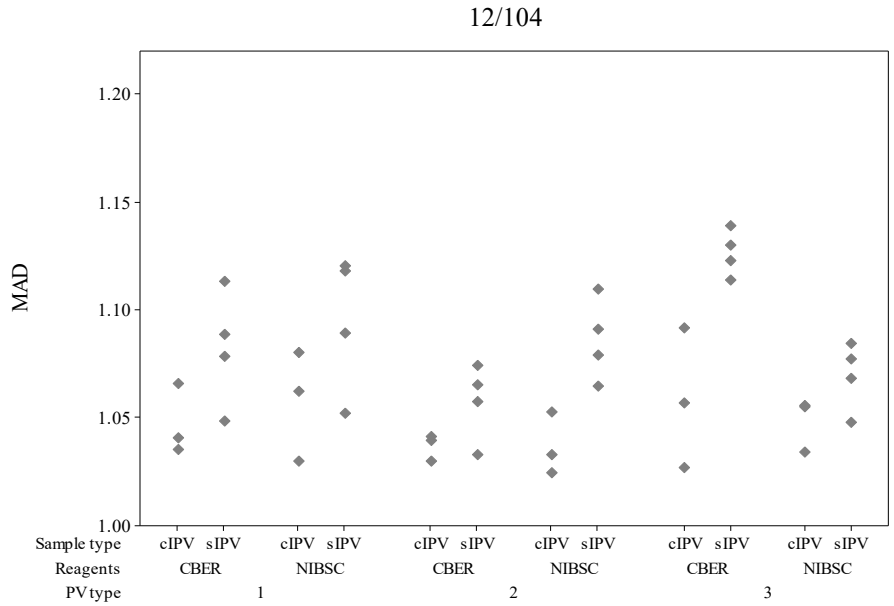
Collaborative Study Results

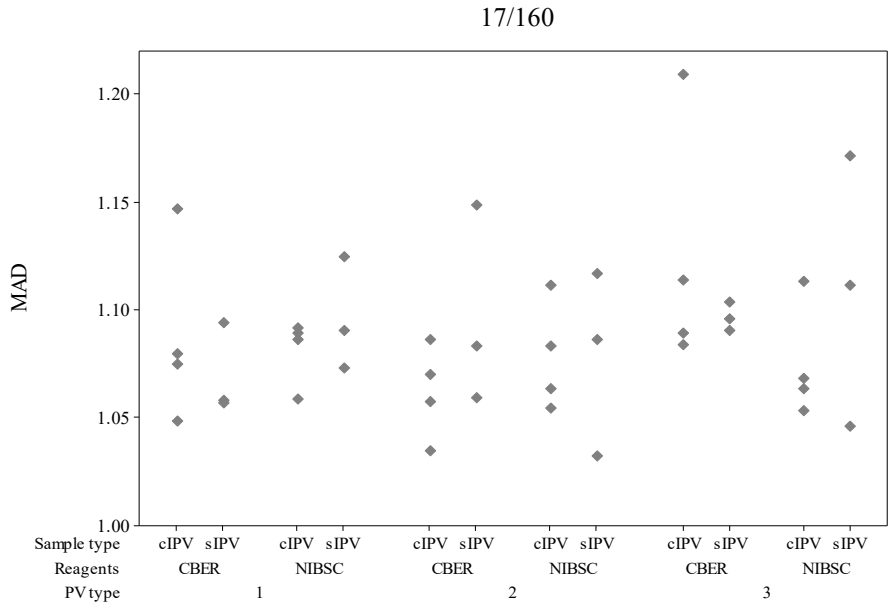
- There was low inter-assay variability for both sets of reagents
  - Slightly lower for the HuMAbs.

- There was higher inter-laboratory variability for sIPV samples compared to cIPV.
  - This was dependent on sample type and PV type not reagents used.

PV Type	Reagents	Median	LQ	UQ
1	CBER	5.8%	3.2%	10.5%
1	NIBSC	7.5%	4.2%	16.1%
2	CBER	5.0%	3.6%	9.9%
2	NIBSC	6.0%	3.4%	20.2%
3	CBER	6.7%	4.3%	11.2%
3	NIBSC	7.5%	3.9%	18.9%

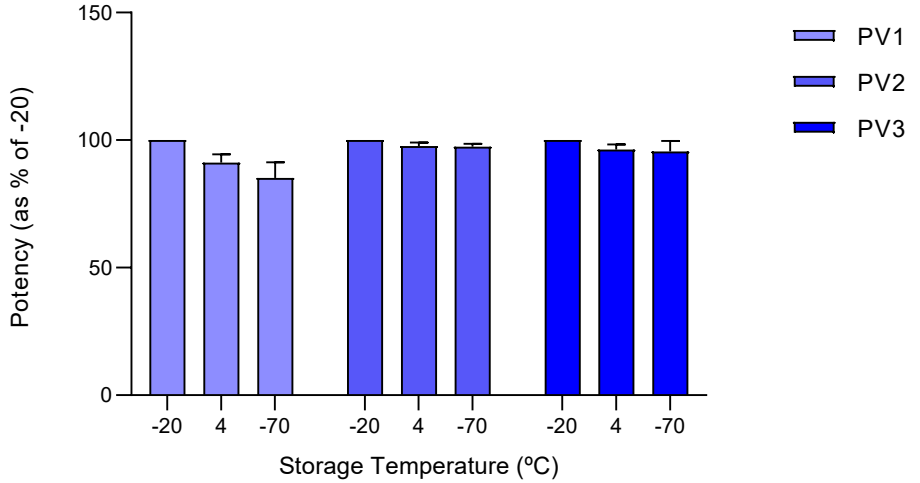
Table 2. Summary of inter-assay GC values.



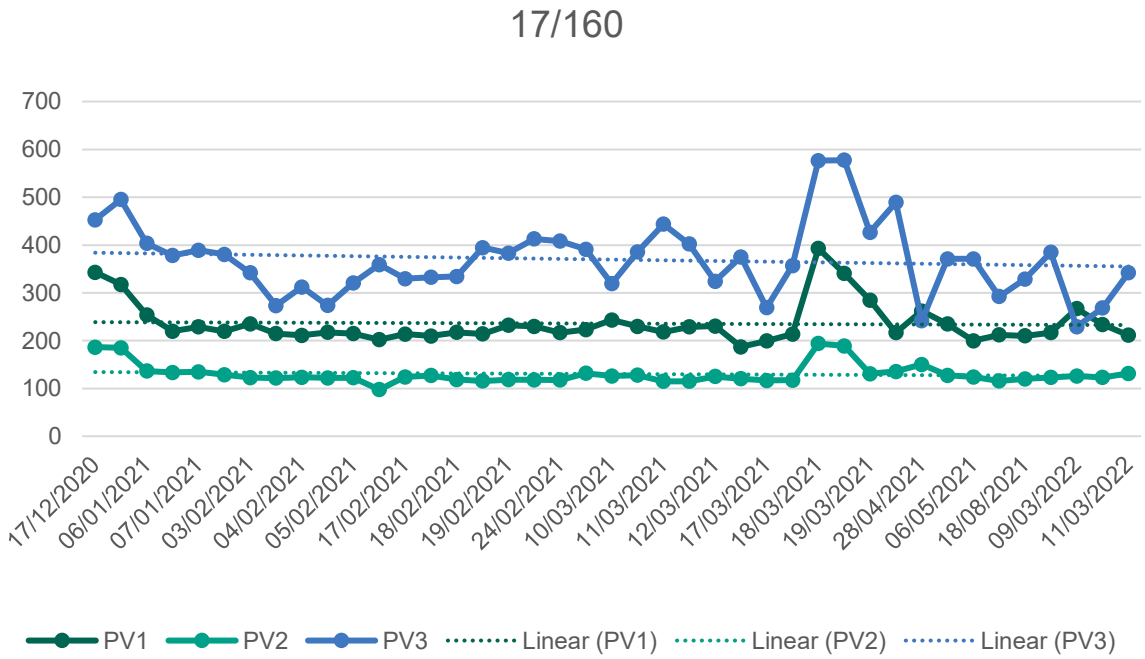


Stability - HuMAbs

- Completed at NIBSC. Based on data for 12-month storage at -20^{0}C, -70^{0}C and +4^{0}C, there was a slight drop in potency for PV1 but this was not significant. There was no loss of potency for PV2 and 3 using 17/160.

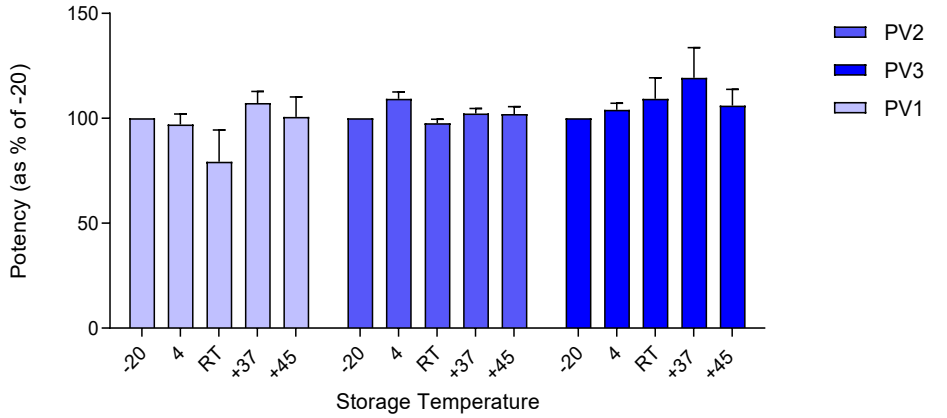


- Real time stability at the intended storage temperature (-20^{0}C) revealed no real loss in potency over time for all four HuMAbs.



Stability testing - HuMAbs

- Accelerated degradation studies after 6 weeks showed no loss in potency at any of the higher temperatures suggesting good stable products. There was a slight drop at room temperature for PV1 but not significant.

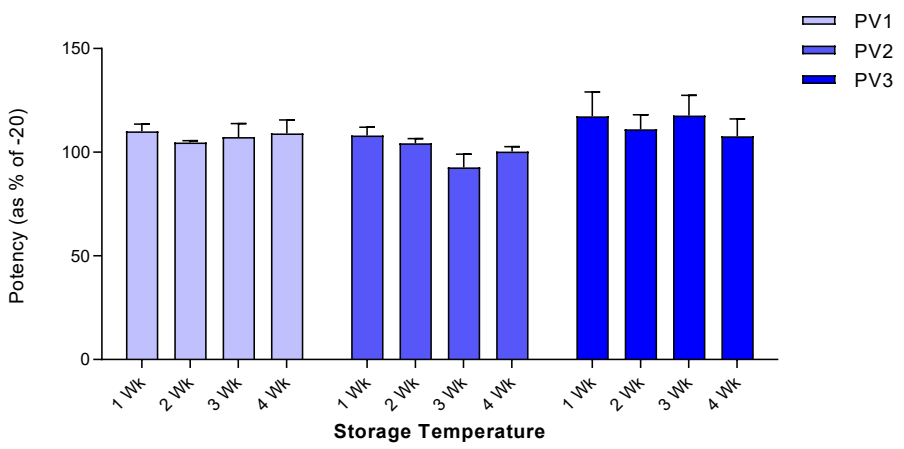


Accelerated degradation. Potencies after 6 weeks of storage at temperatures; -20°C, RT, +37°C and +45°C (as a % of -20°C) .

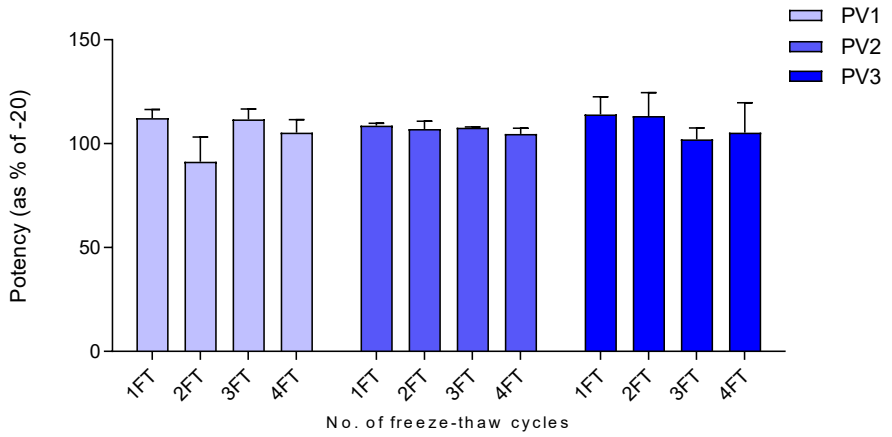
Stability testing - HuMAbs

- There was no significant loss of activity for any of the reconstituted materials that had been stored for 4 weeks at 4˚C.

- There was good maintenance of stability for all antibodies up to four rounds of freeze/thawing.



Reconstitution. Potencies after reconstitution and held at 4°C for up to 4 weeks.



Freeze-thaw. Potencies after multiple rounds of freeze-thawing after reconstitution, from -20°C to room temperature.

Summary and Proposal for ECBS

- The universal reagents showed low intra-assay and inter-assay variability.
- The products were shown to have a good level of stability at varying conditions.
- The potency values obtained with the CBER method for cIPV samples and sIPV IS 17/160 were comparable to the NIBSC method.
  - The significant differences observed particularly with the sIPV products highlights the need for a harmonized method and suggests there may be a need for specific references.

It is concluded that the human monoclonal antibodies along with the associated CBER method are suitable for the evaluation of D-antigen content of IPV products and are recommended for use.

They are provided by NIBSC at the following concentrations:

20/250 - 0.5 mg/ampoule

20/252 - 0.25 mg/ampoule

20/254 - 0.5 mg/ampoule

20/256 - 0.1 mg/ampoule

Acknowledgements

Elaine Pegg
Peter Rigsby
Javier Martin
CBRM

Kutub Mahmood

sIPV HuMAb study group

Diana Kouiavskaia
Konstantin Chumakov

Health Canada Santé Canada

Canada

Scientific and regulatory considerations for management of reference standards

Tong Wu, Ph.D.
Vaccine Quality Division 3, Health Canada
Oct 31 – Nov 2, 2023, Jakarta, Indonesia

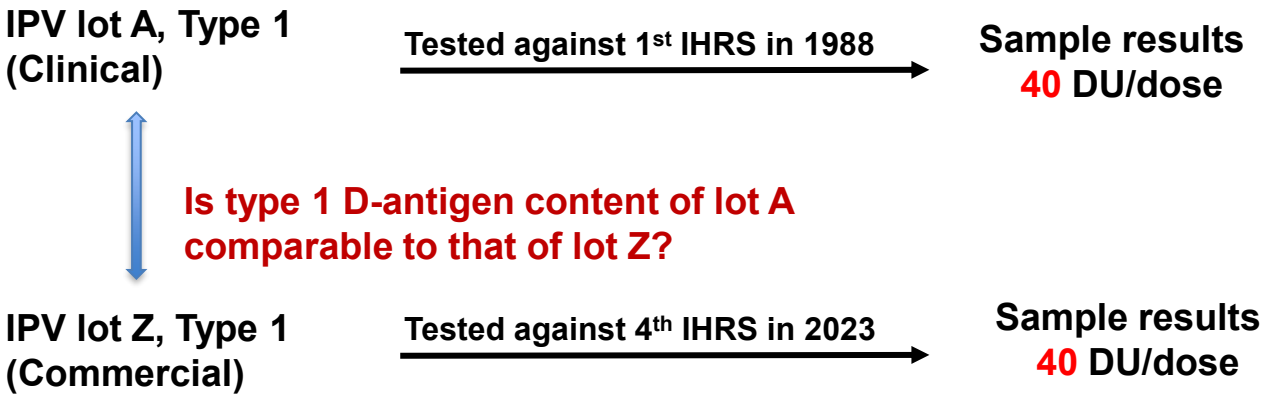
YOUR HEALTH AND SAFETY... OUR PRIORITY.

Reference standards play a critical role in vaccine quality control

- Reference standards (RS) are used to calculate relative test results by comparing the absolute readout of a test sample to that of a RS (e.g., ELISA, immunogenicity assays).
- During vaccine development, the 1st in-house RS (IHRS) should be
  - calibrated against an international standard (IS) if available.
  - assigned an arbitrary units if an IS is not available.
- The aim of IHRS management program is to ensure that all IHRS replacements are comparable to the **1st IHRS**.
  - A key strategy to ensure consistent and clinically effective commercial lots throughout the lifecycle of a vaccine.
  - The 1st IHRS may not be a clinical lot →Ensure clinical performance of commercial lots through setting appropriate specification.

Characteristics of potency reference standards

Potency RS almost always uses arbitrary unit (e.g., D-antigen unit for IPV) → The numerical potency value of the sample has no meaning other than in relation to the RS.



Numerical potency values cannot be compared independent of the reference standards used in assays.

Components of an in-house reference standard (IHRS) program

- Criteria for candidate material as an IHRS replacement:
  - Similar dose-response curves when compared to test samples (e.g., a lot representative of commercial lots).
  - Composition and storage conditions maximize IHRS stability (e.g., addition of stabilizer).
- Qualification of an IHRS replacement
  - Assign a value to the candidate against the current IHRS based on a large data set (test runs) → minimize measurement uncertainty.
  - Confirm equivalence of candidate against the current IHRS in potency assays using common samples → based on statistical analysis (e.g., use of Two one-sided t-test (TOST)).
  - Verify against International Standards (IS) if available.
- Stability monitoring of IHRS

Challenge #1: detect potential drift due to successive bridging

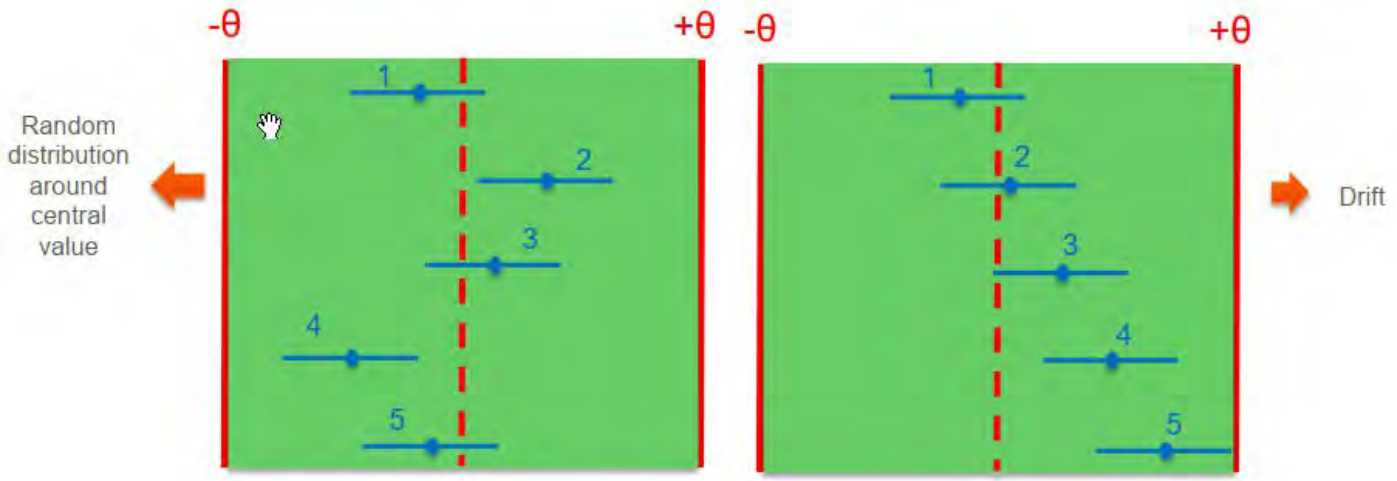
- Is the current equivalence assessment sufficient?
  - The equivalence assessment routinely performed by vaccine manufacturers compares new IHRS candidate against previous IHRS.
  - Measurement uncertainty associated with each qualification of an IHRS replacement can accumulate over several successive bridging.
    - Propagation of error
  - Current practice ensures the candidate IHRS (X^{th} IHRS) is equivalent to the previous IHRS but does not ensure that the X^{th} IHRS is comparable to the **1^{st} IHRS**.

Challenge #1: detect potential drift due to successive bridging (cont.)

- How to improve?
  - Examining the outcomes of all equivalence assessments for all IHRS replacements may allow the detection of a drift in relation to the 1st IHRS.

TOST results for all RS replacements (Simulated data)

(Courtesy of GSK, November 2015 at BRDD)



However, it is challenging to estimate the drift between the IHRS candidate and the 1st IHRS, as it could be impractical to test them side-by-side.

Challenge #2: stability monitoring of reference standards

- Vaccine IHRS are typically complex biologics that may undergo conformational changes or degrade even under optimal storage conditions.
- There are many challenges to implement an effective stability monitoring program for IHRS, due to:
  - Use of arbitrary unit.
  - Lack of suitable measurements for trending purposes.
- It is often assumed that an IHRS retains its assigned potency throughout its use.

The greatest challenge in IHRS management is the lack of effective tools to detect drift

- Potential drift of IHRS and its replacements is due to:
  - Measurement uncertainty during calibration of IHRS replacements (challenge #1).
  - Conformational changes and/or degradation of IHRS during storage (challenge #2).
- Limitations of current monitoring strategies:

Current strategy	Limitation
Trending of positive controls (control chart)	**Positive controls often have similar stability characteristics as IHRS and are measured against IHRS (and its replacements).**
Use of orthogonal methods	**Those methods often require the same IHRS or are highly variable.**
Stability prediction based on Arrhenius equation (WHO TRS 932, Annex 2)	**Arrhenius equation is only applicable to a first-order reaction rate. However, the decay rates of many vaccines at different temperatures don't follow a first-order kinetics.**
Trending of assay readouts, such as ED_{50}, GMT.	**The results are often too variable to be useful.**

International Standard (IS) is a useful tool

- An IS is a useful tool. However,
  - IS for potency assay often uses arbitrary rather than absolute unit → Measurement uncertainty is not applicable to 1st IS.
  - IS replacements are calibrated against previous IS → Measurement uncertainty is applicable to IS replacements (2^{nd}, 3^{rd}…).
  - IS and its replacements may be supplied by different manufacturers → difficult to verify based on product-specific characteristics (e.g., "specific activity" of IPV).
- WHO TRS 932, Annex 2 (Recommendations for the preparation, characterization and establishment of international and other biological reference standards) states:
  - Once a replacement standard has been established, **the units defined by the previous standard formally cease to exist**.
  - In practice, every effort is made to assign a value to the new reference preparation that will preserve as closely as possible the value of the IU over time (continuity of the unit).

Summary of IS and its replacement for wIPV D-antigen ELISA

DU definition	A vaccine preparation that produced a precipitin line at the distance of 25 mm from the centre was arbitrarily assigned a value of 600 D-antigen units using a particular antibody at a particular concentration.				
	Year adopted by ECBS	Comment	D-antigen content (DU/mL)		
			Type 1	Type 2	Type 3
IS1	1962	For *in vivo* assay	N/A	N/A	N/A
PU78-02 (RIVM)			400	40	160
IS2 (91/574)*	1994	Calibrated against PU78-02	430	95	285
IS3	2013	Calibrated against Eur. Ph. BRP2**	277	65	248

***A separate aliquot, from the same bulk sued to prepare IS2, was established by the European Pharmacopoeia Commission as the Biological Reference Preparation batch 1 (BRP1).**

**** Calibrated against BRP1 and with assigned potencies of 320, 67 and 282 D-antigen units per mL for types 1, 2 and 3, respectively.**

WHO recommendations concerning the 1st IS for Sabin inactivated poliomyelitis vaccine (sIPV)

- The First WHO International Standard for Sabin inactivated poliomyelitis vaccine (17/160) was established by the WHO Expert Committee on Biological Standardization in 2018.
- A unitage of 100 SDU/mL was assigned to each of the three poliovirus serotypes. The value 100 is an arbitrary unit.
- Manufacturers of existing sIPV products, including those already licensed and those in late-stage development, that use potency values expressed in DU measured against their internal standards can continue to use these values with the approval of the NRA. It is recommended that these manufacturers also determine the potency of their sIPV products in SDU using the sIPV International Standard, and establish the correlation between “SDU” and “DU”. This can serve as a useful quality characteristic to ensure product comparability.

International Standard (IS) – additional considerations

- Amendment to Annex 3 of WHO TRS, No. 993 recommends:
  - There are still gaps in the scientific knowledge required for the further standardization of IPV products. Some differences have been noted in the antigenic profile of different IPV products, highlighting the importance of product-specific assessment of future IPV products, particularly sIPV products, against current international standards.
- When investigating inconsistent results between IHRS and IS replacements:
  - Considering that IS may decay and IS replacements have measurement uncertainty.
  - The resolution must ensure the comparability between the IHRS replacements and the 1st IHRS → important for maintaining quality comparability between commercial and clinical lots.

Product-specific characteristics

D-antigen units

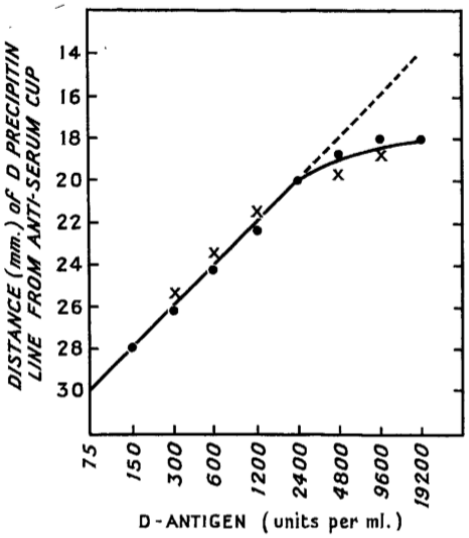


Fig. 1—Relationship between poliovirus type-1 D-antigen concentration and position of precipitin line.

600 units of D-antigen produce precipitin line at 24 mm

Potency of IPV is determined relative to reference vaccine

1 D-antigen unit ≈
$6*10^6$ infectious units
10^9 particles
~15 nanogram

From: Beale and Ungar, *Lancet* 280 (7260): 805-808, 1962

From Kostya

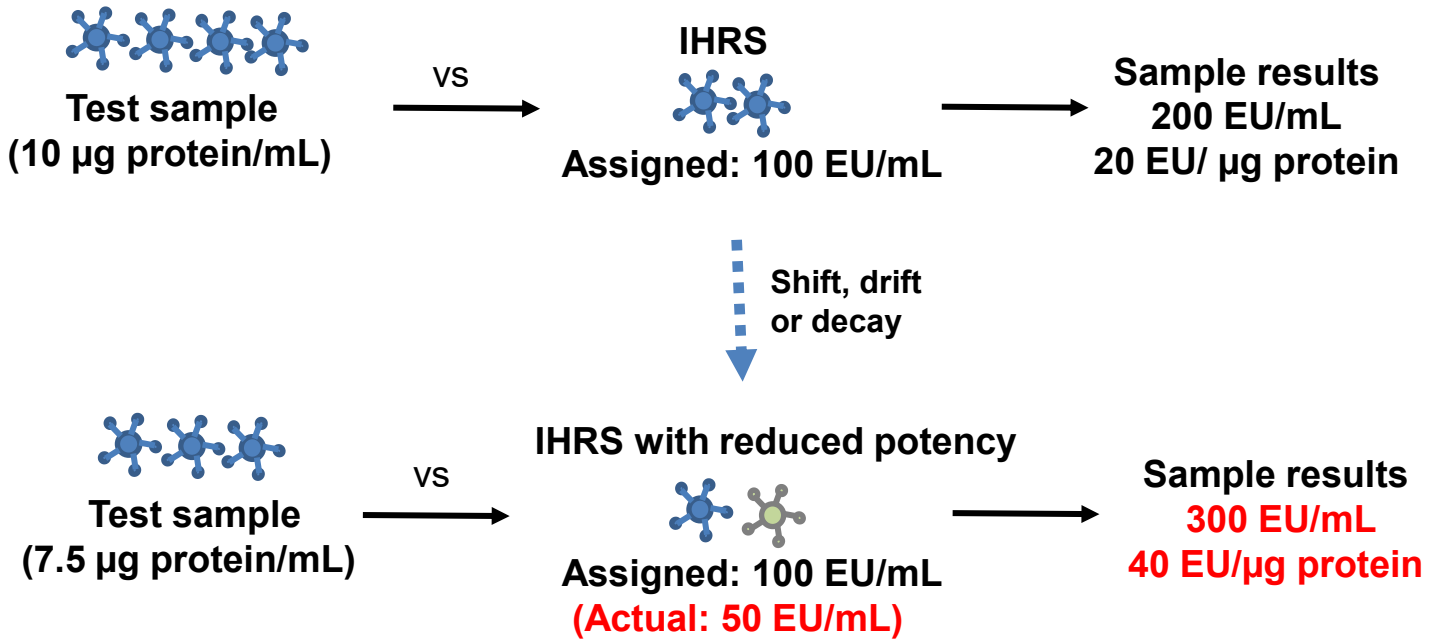
How to improve the monitoring of IHRS?

Link potency to other quality attributes for the same antigen

- Multiple assays are usually performed to verify critical quality attributes of an antigen (e.g., ELISA, total protein and purity are usually performed for protein-based antigens).
- Vaccine manufacturing process has inherent variability:
  - The antigen concentration (by ELISA or protein) at DS manufacturing stage is more variable → no impact on final product quality.
- "Specific activity" [e.g., antigen (by ELISA) to total protein (by Kjeldahl) ratio] is an intrinsic quality attribute of a protein-based antigen*.
  - "Specific activity" of an antigen is expected to remain relatively stable over time.
  - Antigen content by ELISA is susceptible to issues with relative measures, including potential drift of IHRS replacements.
  - Total protein content can be measured accurately and precisely (e.g., by Kjeldahl method) over time.
  - Trending of "specific activity" of vaccine lots → indirect monitoring of IHRS and their replacements.

***Many vaccines contain multiple antigens → impractical to monitor "specific activity" at DP manufacturing stage.**

Impact of a decayed IHRS on testing results



Vaccine lots tested against decayed IHRS:

- Higher "specific activity".
- Higher relative potency results for the same sample

In conclusion, trending of "specific activity" (mean and range over time) of a purified antigen (DS) can improve monitoring of IHRS.

Impact of a decayed IHRS on product quality

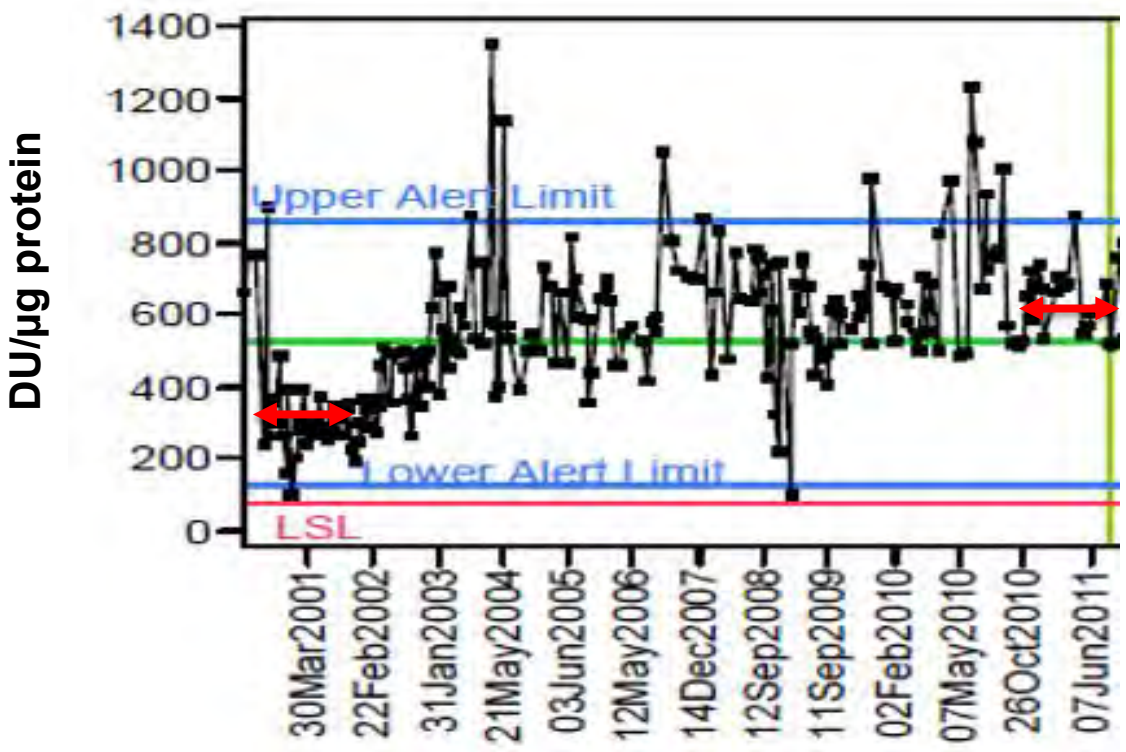
- When a drug product is formulated based on protein content (e.g. by Kjeldahl):
  - Assigned potency results of DP lots by ELISA → trend upward.

In reality: No impact on DP potency!

- When a drug product is formulated based on antigen content (by ELISA):
  - Assigned potency results of DP by ELISA → no trend.

In reality: Reduced antigen in DP lots!

Example: Trending of “specific activity” of type 1 IPV detects potency decay of IHRS



DU: D-antigen unit
Protein content: based on total nitrogen
Conclusion: An approximately 50% reduction in IHRS potency over 10 years.

Proposed approach to improve stability monitoring of a potency assay IHRS

- Link the arbitrary unit of a potency assay IHRS with another relevant vaccine quality attribute that can be measured accurately and precisely (e.g., protein content).
- Monitor “specific activity” of a purified antigen → indirectly monitor the potency assay IHRS and its replacements.
- Select an appropriate assay to measure the relevant quality (e.g., protein content):
  - The assay does not use the same IHRS for calculation (independent of IHRS).
  - The assay performance is consistent over a long period time (conformational or structural changes to antigen protein do not impact the test results).
  - For example, Kjeldahl method (based on total organic nitrogen) is more suitable for protein content determination than HPLC method (relative value using the same IHRS) for monitoring of “specific activity”.

The proposed approach to improve stability monitoring of potency RS (cont.)

- As part of demonstrating manufacturing consistency during product development and early commercial manufacturing stage:
  - Establish the range and the mean value of “specific activity” (e.g., potency to protein ratio) based on a sufficient number of lots.
- Trend “specific activity” data during routine commercial manufacturing, as part of IHRS monitoring.
- Re-assess the impact on “specific activity” when introducing manufacturing changes.

Trending of "specific activity" of an antigen – applications and limitations

- Trending of "specific activity" of an antigen during commercial manufacturing, where the potency is measured against an IHRS has the potential to detect drift of the IHRS.
  - Comparison of "specific activity" between the 1st IHRS and its xth replacement has the potential to detect drift.
- "Specific activity" (mean and range) is antigen specific and product specific.
  - It is not practical to trend "specific activity" in DP that contains multiple antigens.
  - It is not possible to trend "specific activity" of International Standard and its replacements, as the candidate materials are supplied by different manufacturers.
  - "Specific activity" of an IHRS lot remains unchanged through its use → assigned potency value and protein content remain the same.

Conclusions

- The composition and storage conditions of IHRS may be different from the vaccine product.
  - IHRS and test samples should have similar dose response curves.
  - It is important to preserve the integrity of IHRS and reduce the need for frequent replacements that may lead to drift.
- Effective IHRS management → ensure IHRS replacements are comparable to 1st IHRS.
  - The assigned value of an IHRS replacement is based on a large data set.
  - Equivalence assessment should examine equivalence assessments of all previous IHRS → improve the detection of a drift in relation to the 1st IHRS.
  - Periodically test IHRS against IS if available.
  - Establish, monitor, and trend the range and the mean value of a "specific activity" for each antigen during routine commercial manufacturing and the qualification of an IHRS replacement → improve the detection of a drift.

Reference

- Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO Expert Committee on Biological Standardization: fifty-fifth report. Geneva: World Health Organization; 2006: Annex 2 (WHO Technical Report Series, No. 932; https://www.who.int/publications/m/item/annex2-trs932, accessed 23 October 2023).
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- WHO manual for the establishment of national and other secondary standards for vaccines. Geneva: World Health Organization; 2011 (Document WHO/IVB/11.03; https://www.who.int/publications/i/item/WHO-IVB-11.03, accessed 30 March 2022).

Medicines & Healthcare products Regulatory Agency

History of in vivo (rat) potency assay for QC of IPV

WHO workshop on implementation of international standards for
The quality control of polio vaccines including OPV and IPV
31 October- 2 November 2023
Jakarta, Indonesia

Javier Martin

IPV potency assays

- When IPV was first developed potency was not assessed.
- Instead, each dose of IPV was designed to be the equivalent of a specific volume of harvest fluid from PV-infected primary monkey kidney tissue cells. Consequently, IPV products showed variable immunogenicity in humans.
- In response to the Cutter incident in which vaccine recipients were paralyzed by the use of incompletely inactivated IPV, filtration steps were introduced to remove aggregates which lowered the antigencity immunogenicity immunogenicity of the vaccine so potency assays for were required.
- Initially, in vivo assays were used as the official batch release for IPV while the in vitro assays were principally used for in-process monitoring.
- This situation has since changed and due to ethical considerations for the use of vertebrate animals for experimental and other scientific purposes, it is possible to waive the in vivo assay and assess the potency solely by in vitro assays, should certain conditions be met.

In vivo rat potency assays for IPV

- A range of in vivo assays for IPV have been historically developed in monkeys, chicks, guinea pigs, mice and rats.
- Although guidelines for in vivo potency assays exist in Pharmacopeial documents, a common assay design is not used by manufacturers and control laboratories.
- However, the rat assay is long regarded as the most suitable in vivo potency assay for IPV as rats were found to give the highest titers, a good linear dose response in the IgG class and to better resemble the antibody response in humans.
- We aim at supporting this area by establishing standardize methods that can be used by different organizations with a special focus on validating current IPV IS preparations for in vivo assays.

International Symposium on Reassessment of Inactivated Poliomyelitis Vaccine, Bilthoven 1980,
Develop. biol. Standard. 47, pp. 119-128 (S. Karger, Basel 1981)

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POTENCY TESTING OF KILLED POLIO VACCINE IN RATS

G. van Steenis, A.L. van Wezel and V.M. Sekhuis

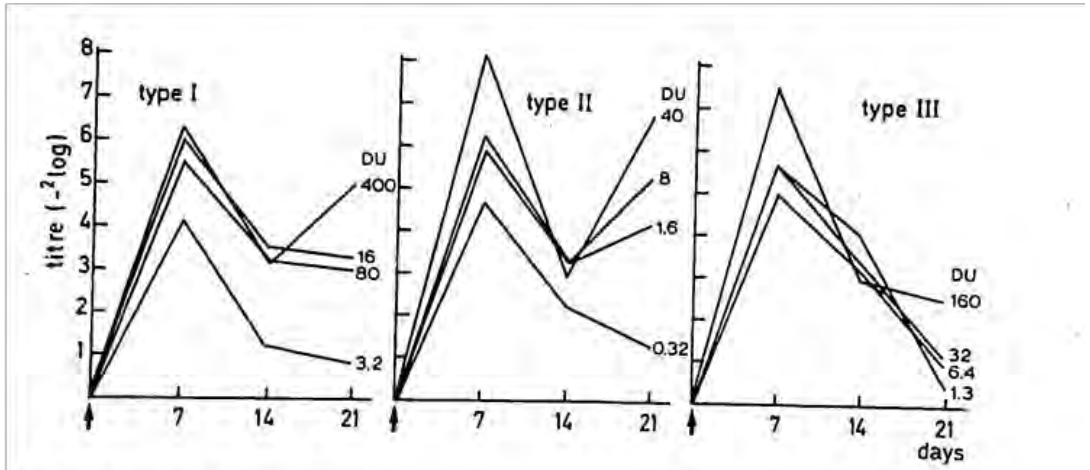

Fig. 1. Antibody development in guinea pigs inoculated with trivalent polio vaccines of different D-antigen content. Each titre represents the geometric mean of 6 animals.

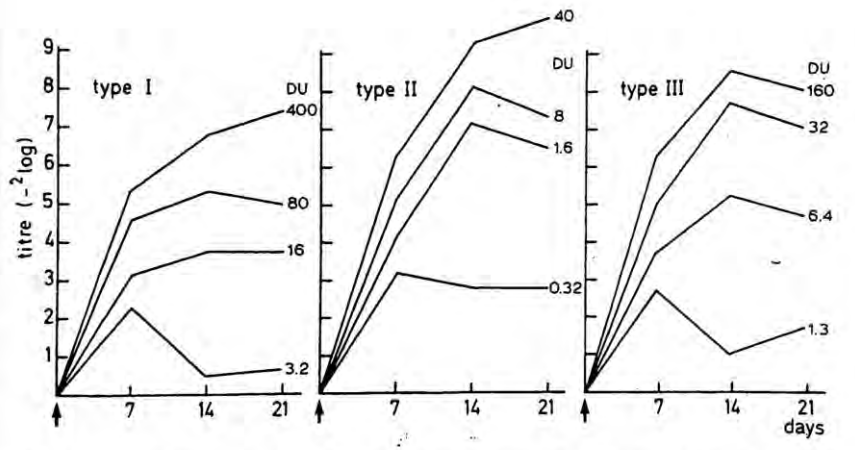

Fig. 2. Antibody development in rats inoculated with trivalent polio vaccines of different D-antigen content. Each titre represents the geometric mean of 6 animals.

Antibody responds in guinea pigs

Antibody responds in rats

Biologicals (1995) **23**, 301–311

A WHO Collaborative Study of Immunogenicity Assays of Inactivated Poliovirus Vaccines

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Abstract. Ten laboratories studied the immunogenicity of six trivalent inactivated poliovirus vaccines in a WHO Collaborative Study. The antigenic content of all six vaccines had previously been estimated in an earlier study (Wood *et al.*, 1995). In collaboration with the European Pharmacopoeia Commission an additional preparation, European Pharmacopoeia Biological Reference Preparation Batch 1, was also included. Laboratories were requested to use established immunogenicity assays and six used the European Pharmocoepia guinea pig/chick test while three used a rat potency test. One laboratory contributed data from both methods. Apart from one laboratory, within laboratory variation was low (less than five-fold). However, very large (greater than 100-fold) variation was seen between laboratories for ED50 results in the guinea pig/chick test. Different decisions on pass/fail outcome would have occurred for some of the samples tested. Between laboratory variation was much lower (less than five-fold) in the rat test. Expression of results as potencies relative to a standard reduced between laboratory variation for both methods, substantially so for the guinea pig/chick test. The correlation between *in vivo* and *in vitro* results was generally good with the exception of the type 3 component of one preparation. This showed that the relationship between immunogenicity and antigenicity was not necessarily predictable. There is an urgent need to revise the European Pharmocoepia immunogenicity test but it is premature, on the basis of this study, to recommend either the rat test or *in vitro* tests as replacements. Two candidate reference materials were both found suitable for *in vivo* assay of inactivated poliovirus vaccine.

In vivo potency assays for IPV

- Critical to assess correlation between *in vitro* and *in vivo* IPV potency
- D-Ag values might provide indication of virus/protein quantity in IPV samples but do not tell us much about the immunogenicity of vaccine preparations
- *In vivo* laboratory potency assays are very useful for early vaccine development stages (for both IPV and VLP preparations), assessment of the effect of mutations that arise during virus cell growth, vaccine dose finding determination, and batch release quality control assays
- Following establishment of consistency of vaccine production, *in vivo* assays can be waived in favour of *in vitro* potency assays providing full correlation between *in vivo* and *in vitro* has been established and has been approved by the NRA

In vivo potency assays for IPV – WHO TRS for IPV

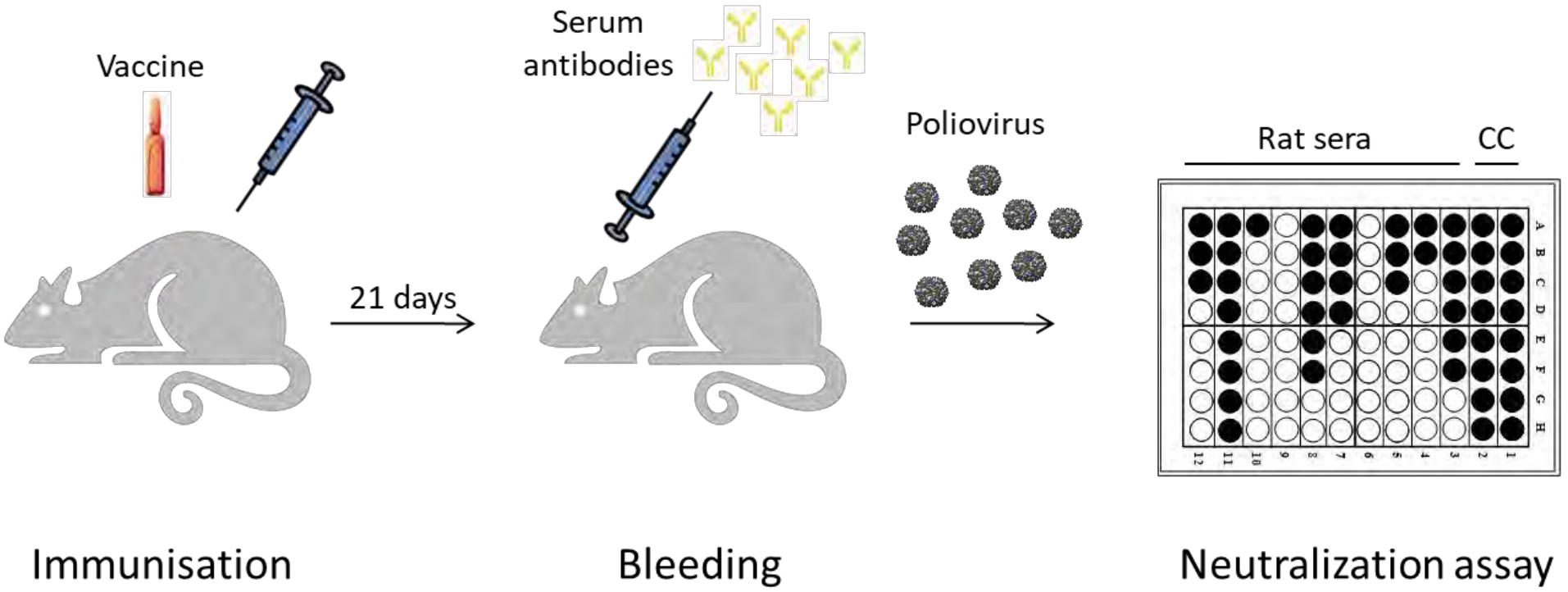
Appendix 2

In vivo potency assay of IPV

Tests for evaluating the potency of IPVs include an in vivo assay for immune response. An appropriate WHO International Standard should be used to validate the assay. However, because of the diversity in the reactivity of different vaccines it is unlikely that an International Standard will be suitable for the standardization of in vivo assays of vaccines from all manufacturers. If this is shown to be the case, manufacturers should establish a product-specific reference preparation which is traceable to a lot of vaccine shown to be efficacious in clinical trials. The NRA should approve the reference preparation used and should agree with the potency limits applied. The performance of this reference vaccine should be monitored by trend analysis using relevant test parameters and it should be replaced when necessary.

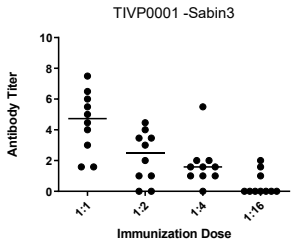
In recent investigations the in vivo potency assay in rats has been standardized (*1*) and shown to have advantages over previously described in vivo tests for IPV (*2*).

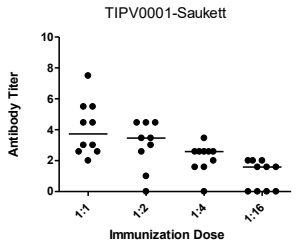
In vivo potency: Rat Assay

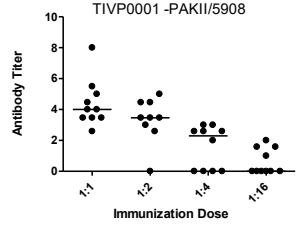


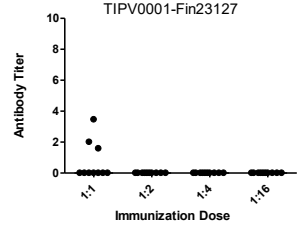
In vivo potency: Rat Assay

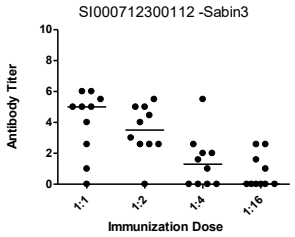
Example using wild and Sabin IPV preparations and different challenge viruses

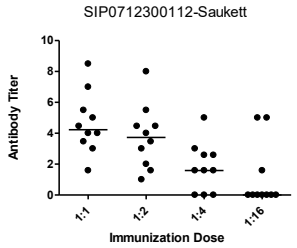


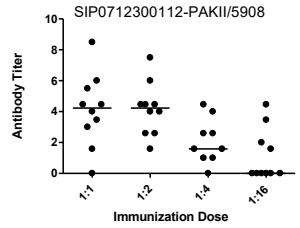


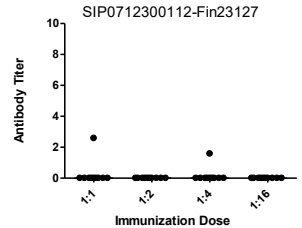


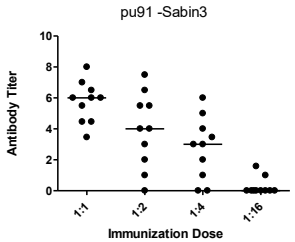


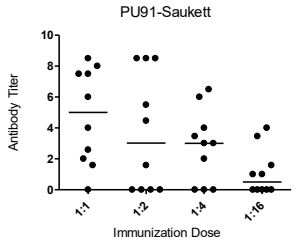


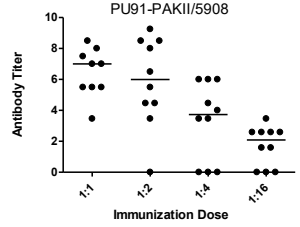


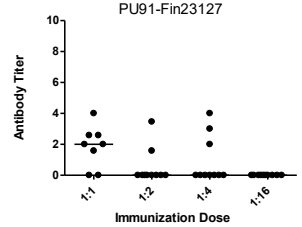


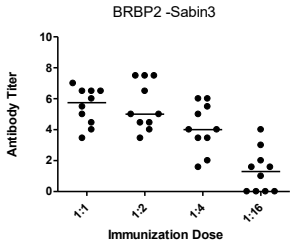


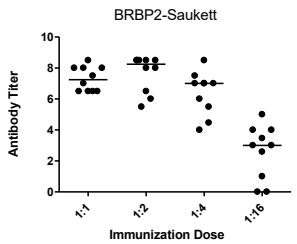


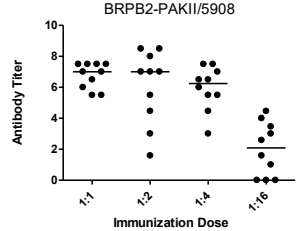


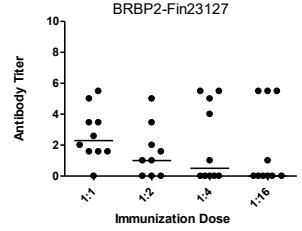




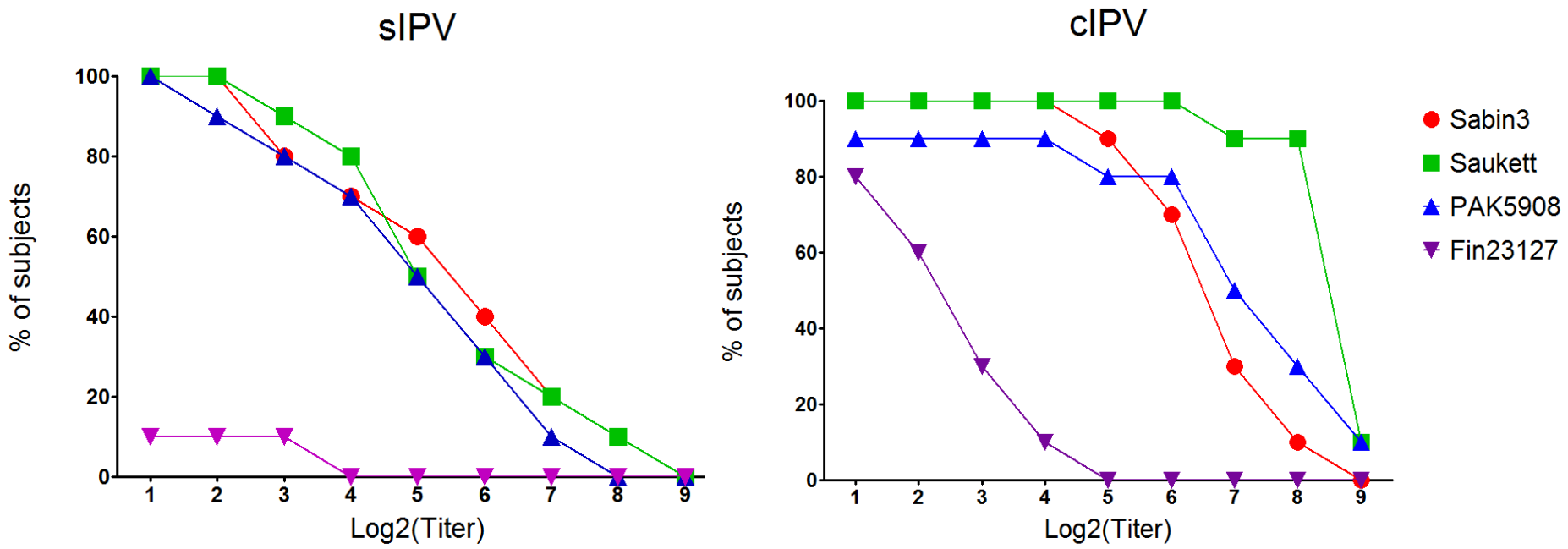






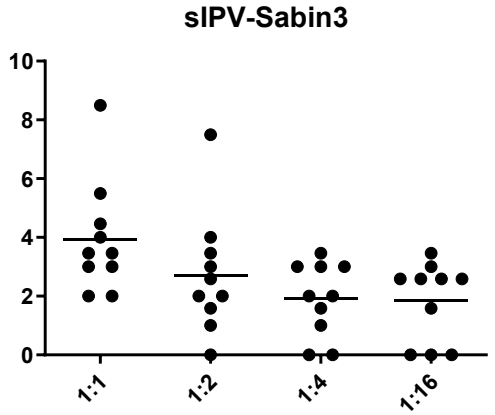


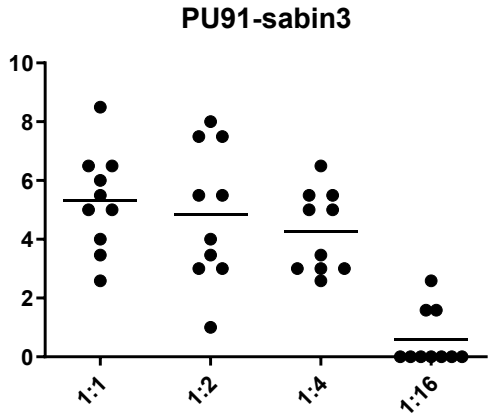
Immune response against PV3 strains

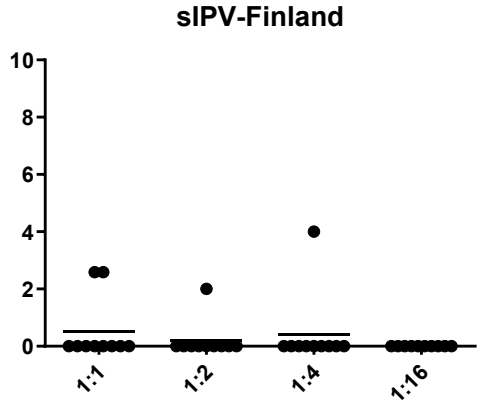


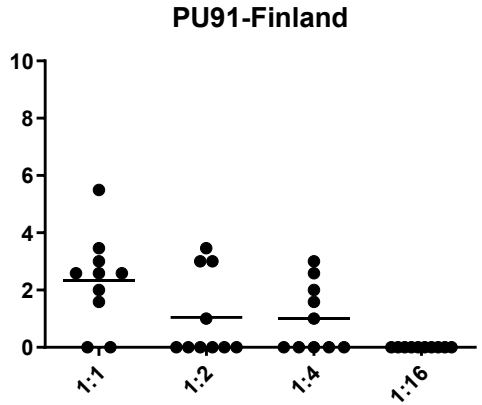
Reverse cumulative distribution curves of virus neutralizing titers against different PV3 strains

In vivo potency: Rat Assay – Effect of adjuvant

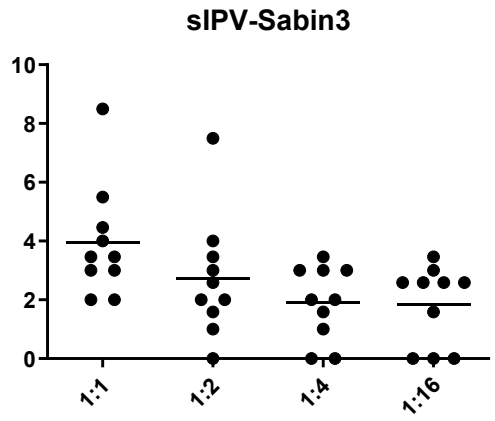


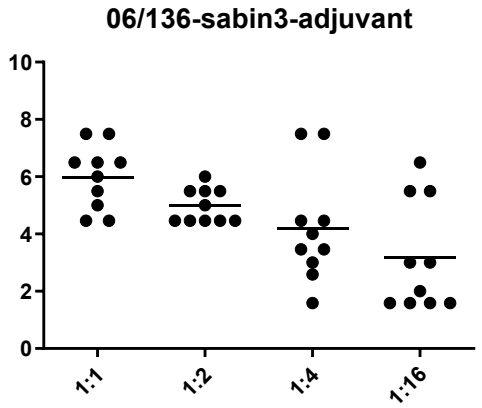


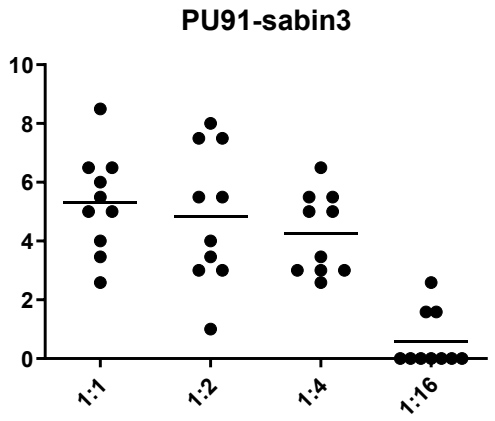


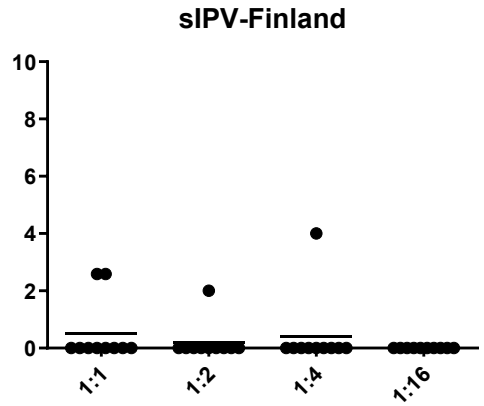


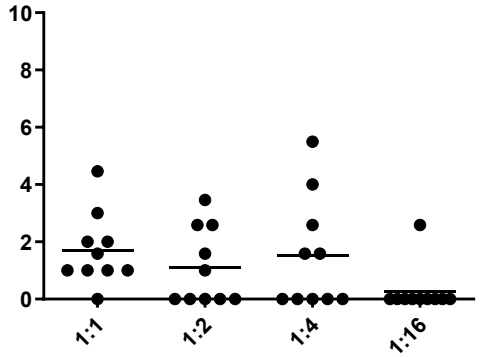
In vivo potency: Rat Assay – Effect of adjuvant

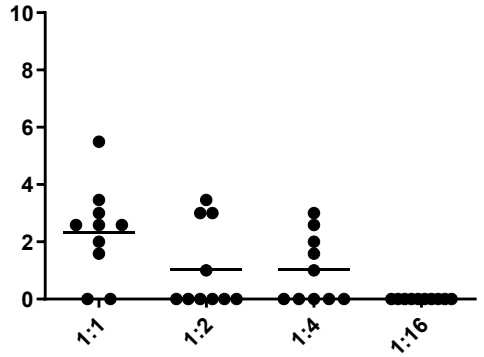












WHO Technical Report Series No. 993, 2015

Annex 3

Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

Replacement of Annex 2 of WHO Technical Report Series, No. 910

C.2.1 Assessment of the immune response; C.2.4 Endpoint and analysis; Part B. Non-clinical evaluation

For the evaluation of IPV derived from attenuated strains (Sabin strains and strains derived by recombinant DNA technology), serum neutralizing antibody titres against both Sabin and wild-type poliovirus should be determined in order to ensure that the conclusions of clinical studies are applicable to both types of strains. In view of the antigenic differences between the wild-type poliovirus strains, it may be useful to assess the neutralizing antibody titres using both recent wild-type isolates and the conventional strains in a subset of study subjects, if relevant.

The presence of neutralizing antibody against polioviruses is considered a reliable correlate of protection against poliomyelitis. However, immunity induced by one serotype does not provide protection against the other two serotypes.

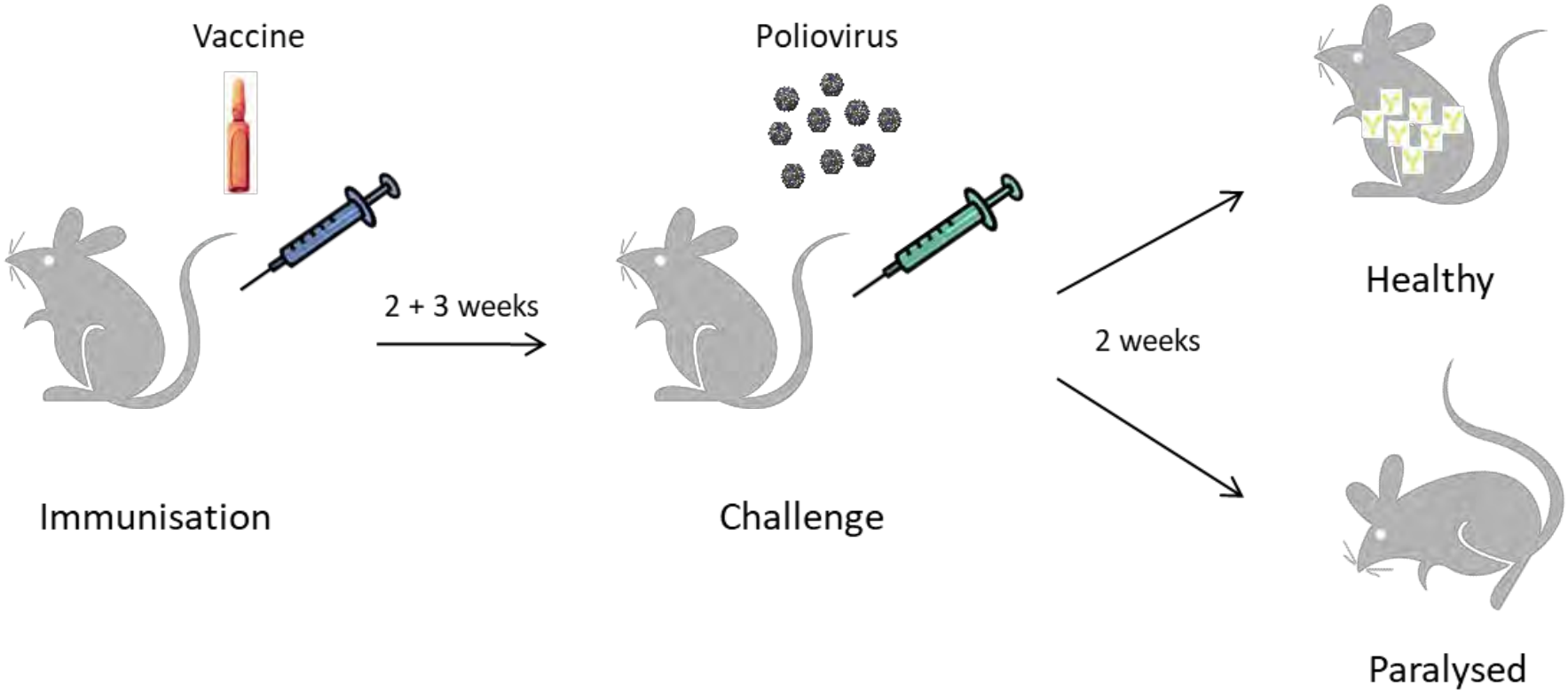
In vivo potency: Tgm immunization/challenge test – WHO TRS for IPV

B.4 Evaluation of immunogenicity in animal models

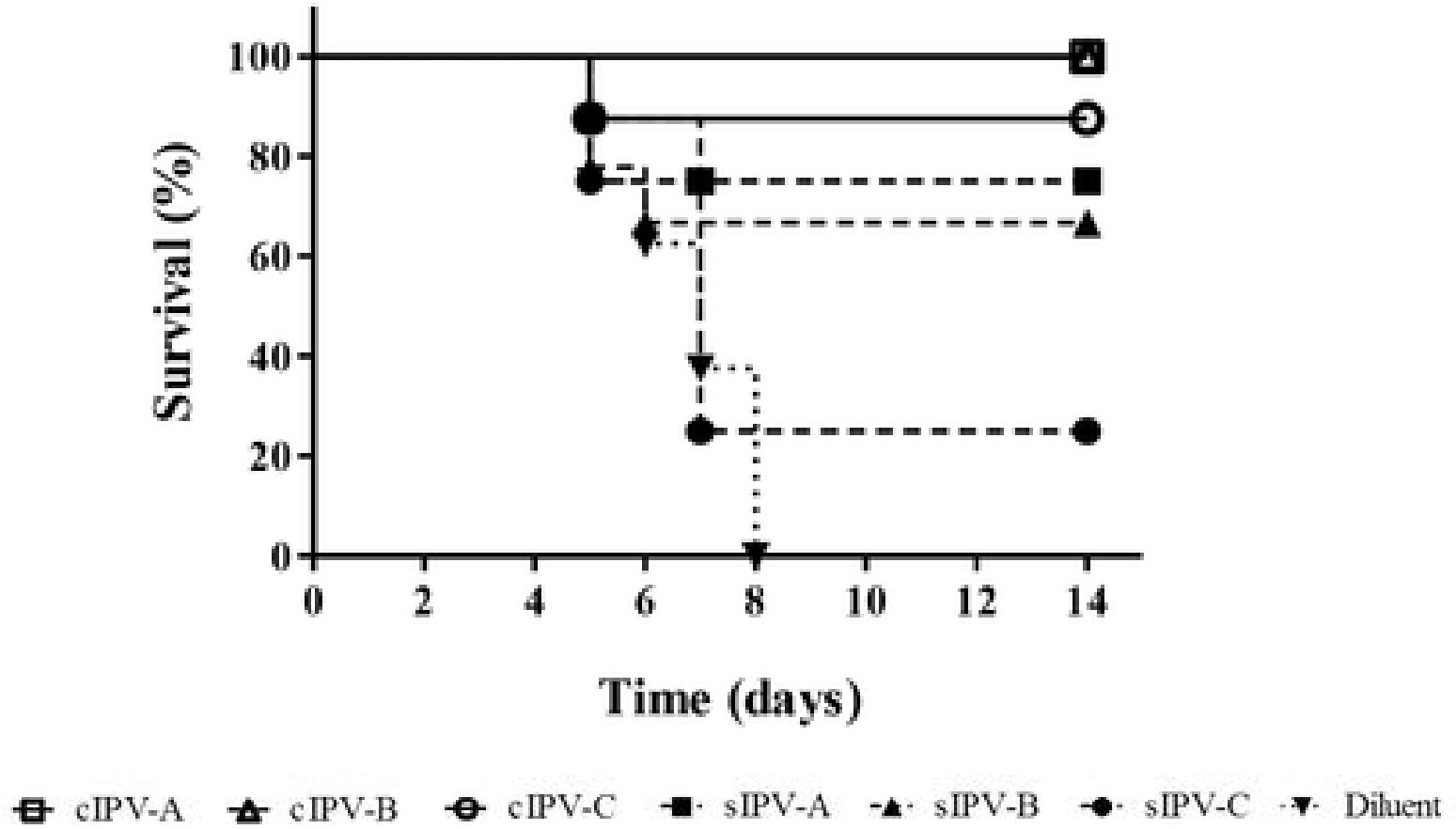
Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in suitable animal models (for example, rats). Proof-of-concept nonclinical studies should include the comparison of immunogenicity between a candidate IPV and a licensed IPV based on type-specific serum neutralizing antibody titres against both Sabin and wild-type strains. These studies may also assist in the selection of D-antigen content to be tested in the dose-finding studies in humans. However, it is important to note that immunogenicity data in animals do not reliably predict the antigen content that might be appropriate for inclusion as a single human dose in the final vaccine formulation. An assay using transgenic mice may be performed to compare the immune response and protection against virulent challenge induced by a candidate IPV to that induced by a licensed IPV (*31*, *32*). In vivo tests are also important tools to be used as characterization tests to demonstrate comparable manufacturing processes when major changes are introduced.

When an adjuvant is included in the formulation, manufacturers should provide a rationale and immunogenicity data to support the use of an adjuvant in the vaccine (*62*).

In vivo potency: Tgm immunization/challenge test



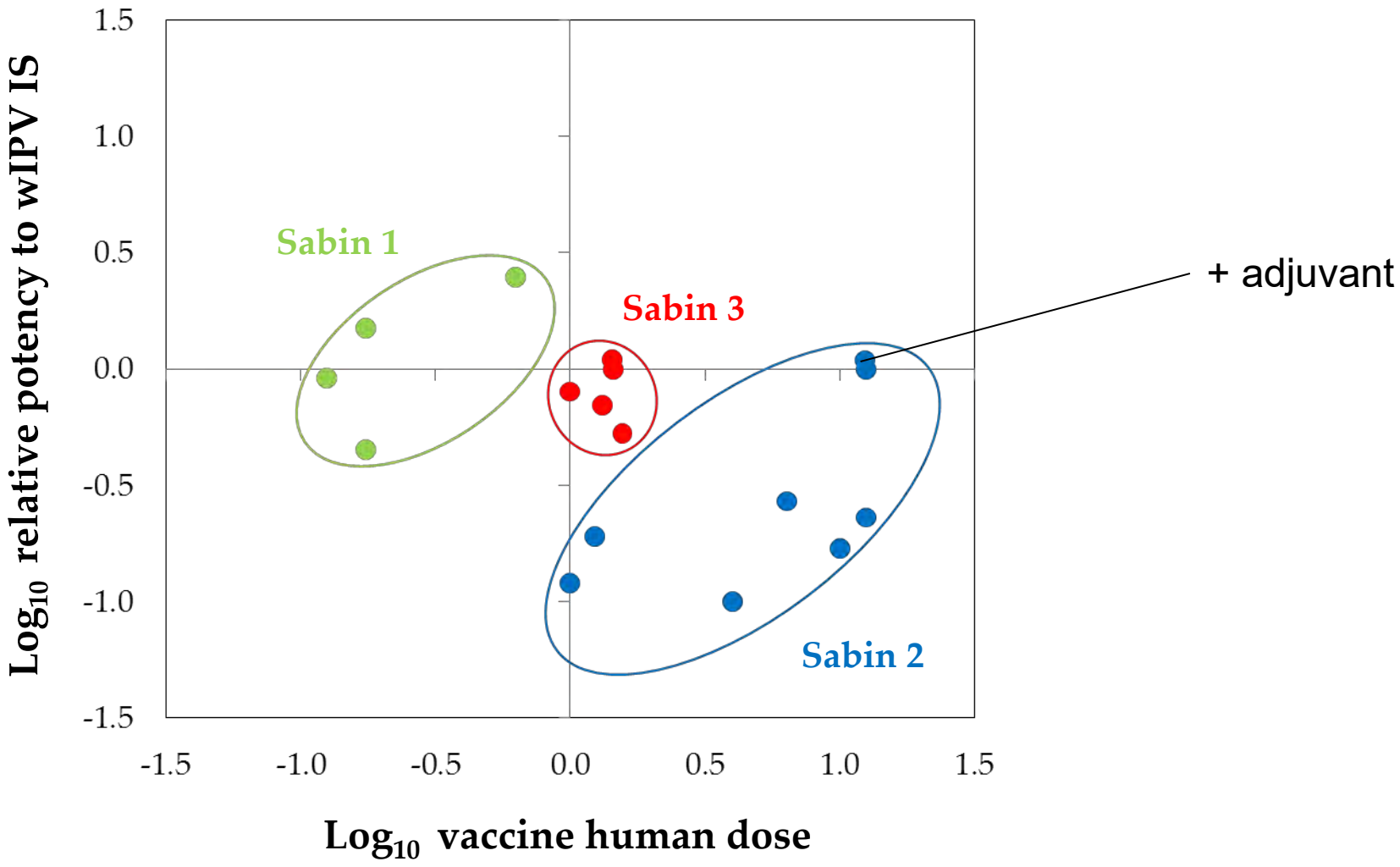
Protection by different vaccines against paralysis induced by highly evolved iVDPV2 isolate in TgM



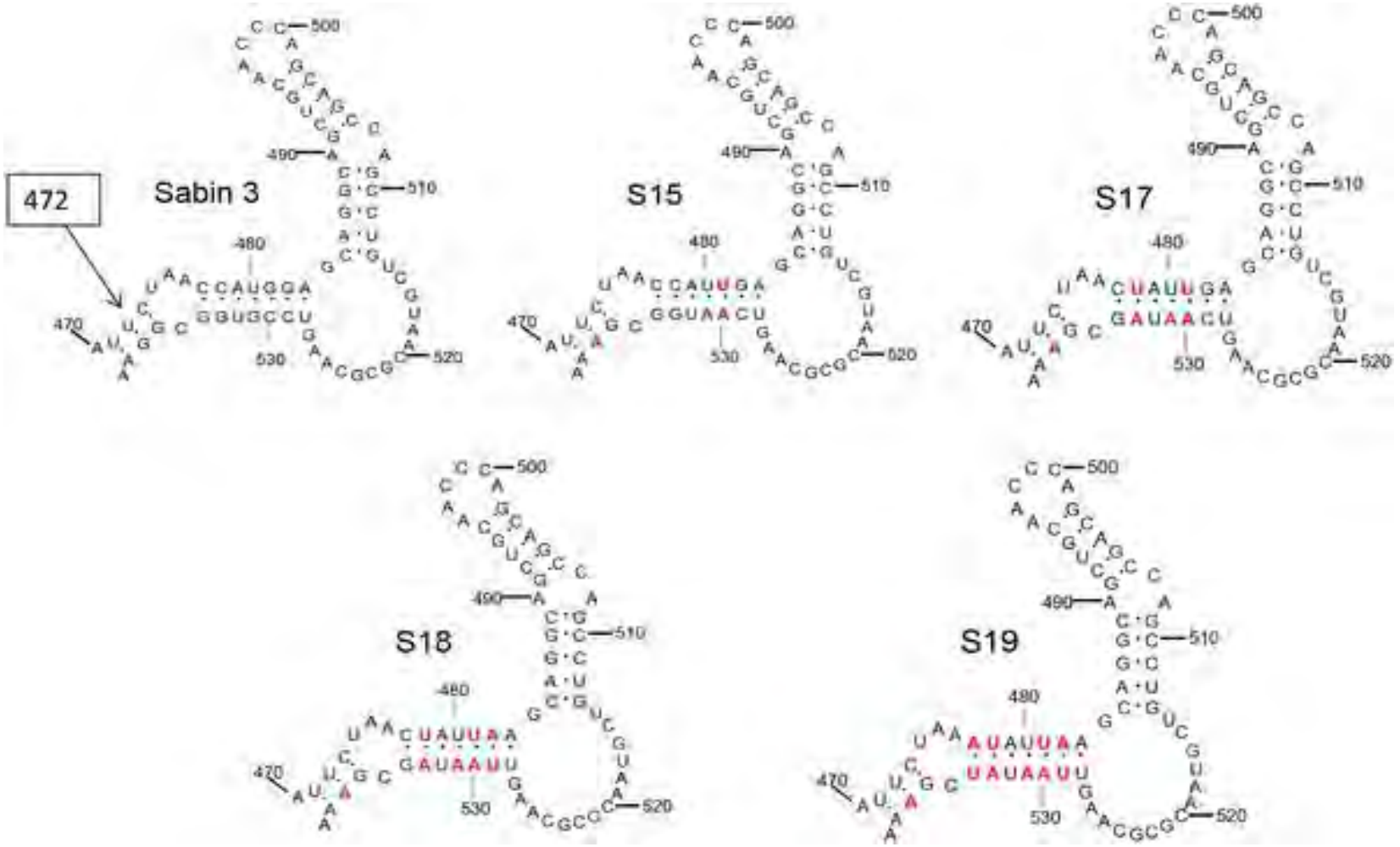
Acknowledgments

Alison Tedcastle	FDA, USA
Elaine Pegg	Lankenau Institute, USA
Gill Cooper	WHO, Switzerland
Laura Crawt	Vaccine manufacturers
Peter Rigsby	NRAs
Helen Fox	BMGF, USA
Sarah Carlyle	PATH, USA
Andrew Macadam	Collaborating laboratories
Thomas Wilton	
Dimitra Klapsa	
Shaun Baker	
Graham Crossland	
Lisa Johnson	

In vivo potency of sIPV

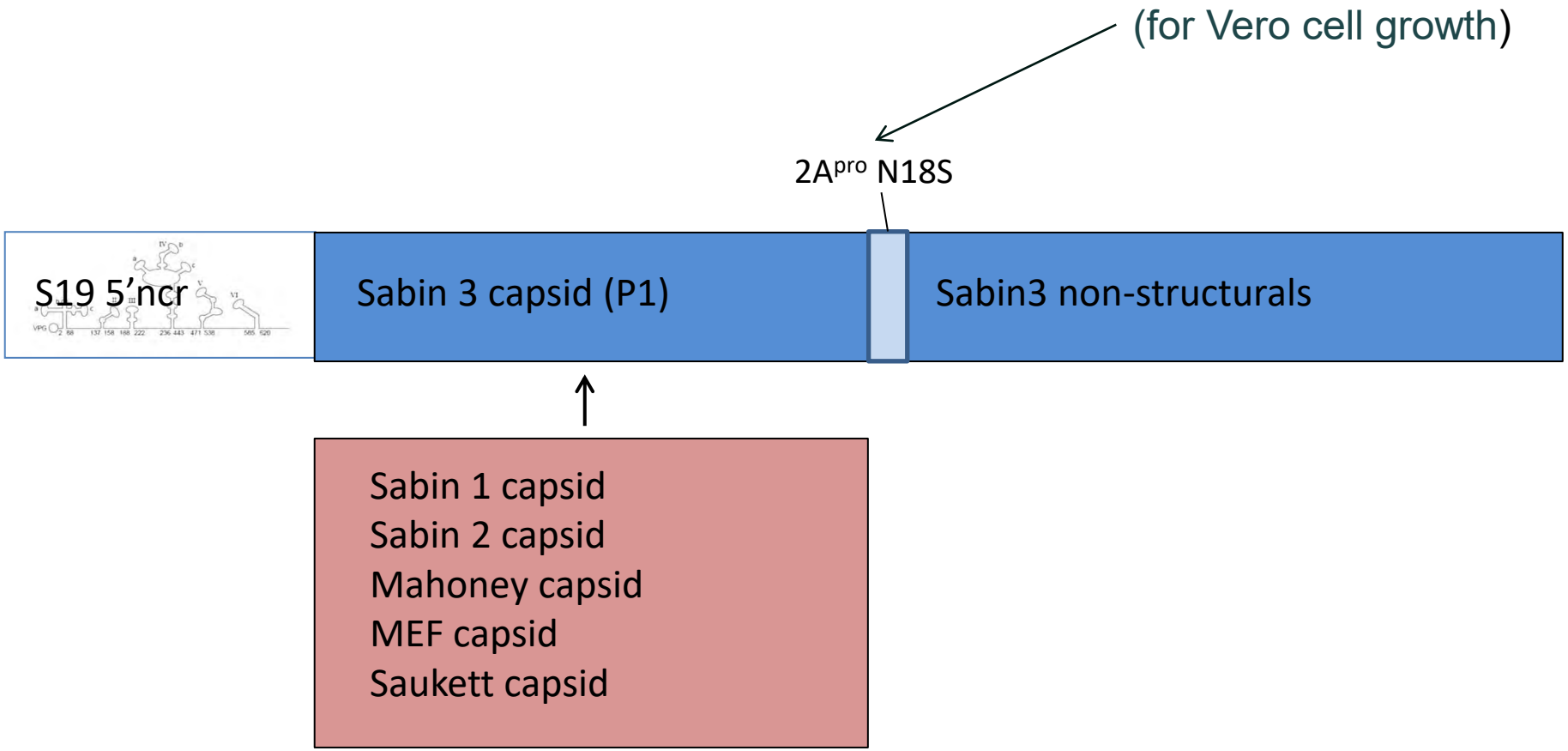


Genetic manipulation of domain V of 5’NTR – S19 PV strains



Base paired stem loop structures in domain V of the 5’ noncoding region of type 3 poliovirus.

S19-poliovirus strains



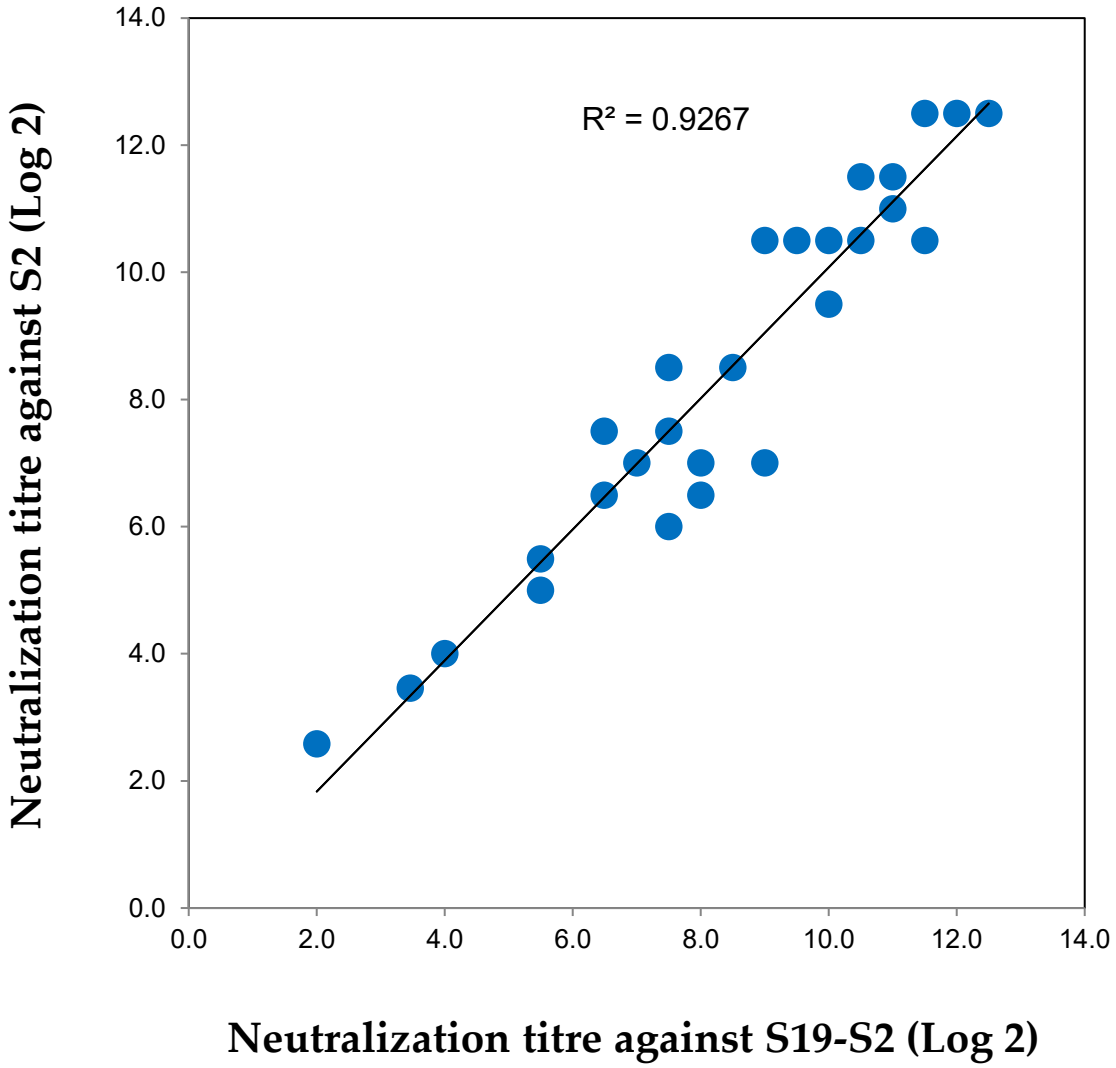
S19-poliovirus strains

- Very low infectivity
  - > 1 million-fold increase in PD_{50} in transgenic mouse spinal cord
  - Oral ID_{50} $>10^{12}$ $CCID_{50}$ in non-human primates
- High yields in cell culture
- Genetically stable
  - no reversion after >20 passages under selective conditions
- Wild-type and VDPV capsid sequences can be used
- Unaltered antigenicity and immunogenicity
- Can be used as seeds for IPV production – assessment in progress
- Can be used as challenge strains for serology assays requiring lower containment – validation in progress (human sera + rat IPV sera)

Use of S19-poliovirus strains

Virus Stocks	IPV Production	IPV QC	IgG QC	Serology Assays	Cell Sensitivity
S19S1_N18S	√				
S19S2_N18S	√				
S19S3_N18S	√				
S19S1		√		√	?
S19S2		√		√	?
S19S3		√		√	?
S19Mah_N18S	√				
S19MEF1_N18S	√				
S19Skt_N18S	√				
S19Mah		√	√		
S19MEF1		√			
S19Skt		√			

Immune response in humans – S2 vs S19-S2



Differences not statistically significant

	p-value
Log Data + Paired t-test	0.813
Wilcoxon Signed Rank Test	0.136

Medicines & Healthcare products Regulatory Agency

In-vivo potency testing for Sabin Inactivated Poliovirus Vaccines

Alison Tedcastle

1st November 2023

Background / Rationale

- Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be evaluated in a suitable animal model i.e. Rats.

- This should include comparison of immunogenicity between the candidate and a licenced IPV based on serotype specific serum neutralising antibody titres against an appropriate challenge virus.

- Since there is limited clinical experience with sIPV, it is also recommended to assess the neutralising antibody titres induced by the candidate against heterologous poliovirus strains that differ in antigenicity.
    - Wild type poliovirus strains used for cIPV manufacture or their S19 equivalents.

- Important to bear in mind the neutralising antibody titre will likely be higher against the homologous production strains.
    - Vital to understand the D-Antigen content selected for further studies is sufficient to induce protective immunity against heterologous strains.

Background / Rationale

- The *in vivo* potency assay is used to assess the immunogenicity of IPV alongside the D-Antigen *in vitro* potency test.
- A range of *in vivo* tests have been developed including monkey, chicks, guinea pigs, mice and rats and all are based on the assessment of neutralising antibodies titres.
- Due to sub-optimal testing conditions and lack of sensitivity, the rat potency test has become the test of choice
    - Produce highest titres
    - Give a good dose related titre response
    - Consistent across different poliovirus strains
    - Antibody patterns of rats and humans were found to be similar

- Historically it has been shown that rat assays reflect well the immunogenicity in humans so they could be used to further characterize and standardize sIPV.
- Results can be used for dose finding, monitor consistency, assess effects of mutations and test different virus challenge strains.

European Pharmacopoeia

- The capacity of the vaccine to induce an immune response (produce neutralising antibodies) is determined *in vivo* by the rat potency test.
    - *Suitable test involves intramuscular injection into the hind limb of not fewer than 3 dilutions of the vaccine to be examined and a reference vaccine*
    - *Using for each dilution a group of 10 specific pathogen-free rats of a suitable strain*
    - *A weight range of 175-250 g has been found to be suitable.*
    - *An inoculum of 0.5 ml per rat is used.*
    - *Bleed the animals after 20-22 days.*
    - *Validity range TCID50*
    - *Results of rat assays on all final bulks should be included in all data generated for demonstration of consistency of production.*

European Pharmacopoeia

- Guidelines on waiving of the *in vivo* assay, the following conditions should be met before performance of the validation study;
  - Full validation of the D-Antigen assay
  - Establishment of the acceptance criteria for the D-Antigen assay based on a number of consecutive lots.
  - Establishment of production consistency on recent final bulks using the currently approved *in vivo* assay; the final bulks should correspond to the final lots used to establish the acceptance criteria for the D-Antigen assay and represent all three poliovirus types.

- The Validation study should be performed on a final bulk/lot that is representative of the current production method and 2 sub-potent batches (e.g. prepared by heat-inactivation and should have the expected titres of about half of the final bulk).
  - The batches are assayed by the currently approved *in vivo* assay and by the D-Antigen assay.
  - Waiving of the *in vivo* assay is accepted once the final bulk/lot complies with the *in vivo* and *in vitro* assays and the sub-potent batches fail to comply.

- This validation should be carried out for each product and should be repeated wherever there is a significant change to the manufacturing process.

Methodology – NIBSC SOP

The *in vivo* potency test involves immunising rats with a reference and test vaccine/s. A vaccine is satisfactory if the potency is not significantly less than that of the reference preparation. This testing is undertaken in line with European Pharmacopoeia (Ph Eur) potency test for IPV.

Inoculation of Rats

- Dilutions of test vaccine/s and reference are prepared in diluent
    - Neat, ½, ¼ and 1/16
        - ‘Neat’ is established as a relevant concentration i.e. one human dose
- Each group of rats are inoculated with 2 × 0.25 ml of diluted material into each hind leg.
- Following 20–22 days post-inoculation rats are bled out by cardiac puncture.
- Blood samples are allowed to clot at +4°C for 1–2 days.
- Sera is removed in a sterile manner and stored at −20°C or −80°C until required for testing.

Titration of sera; for each serotype

- Each rat sera should be added at a starting concentration of ½ to the top row of a 96 well tissue culture plate in duplicate.
- Serial twofold dilutions are made down the plate.
- Positive and negative control rat sera should also be included in the assay.

Challenge virus strains

- The challenge virus is diluted to 100 $TCID_{50}$ per well
- At the same time, 1ml of virus is mixed with 1ml of diluent to perform the back-titration of the challenge virus.

Addition of virus challenge

- The appropriate challenge virus is then added to all serum samples at a 1:1 ratio and incubated overnight at 4°C alongside the 1 + 1 virus challenge.
- The plates are then moved to 35°C and incubated for three hours.

Back Titration of Virus Challenge

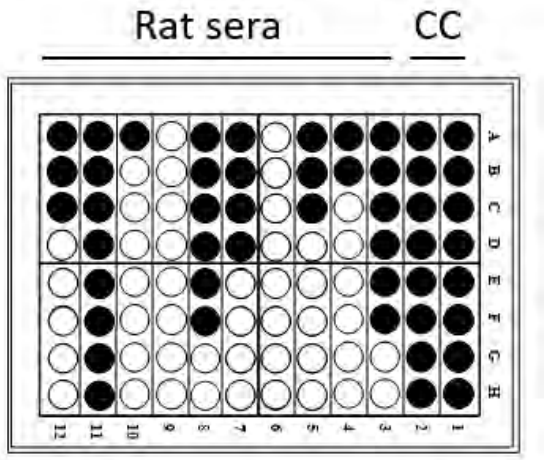
- After incubation, prepare Neat, 10^{-1} , 10^{-2} and 10^{-3} of the 1 + 1 challenge virus mix.
- Add these dilutions to a new tissue culture plate.

Addition of HEp2-C Cells

- Approximately 1–2 × 10^5/ml of HEp-2-C cells are prepared.
- Cells are added to the plates and incubated at 35°C for 5–7 days. Ensure at least two rows or columns of cell controls are included in each test.

Staining

- Plates are then stained with Naphthalene black for at least 1 hour or overnight.
- Subsequently washed with water and read for CPE.
- End point titres are recorded.



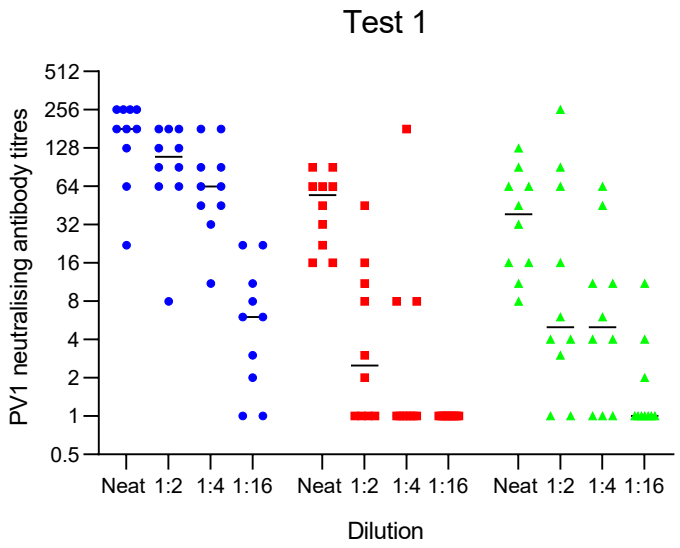
Neutralization assay

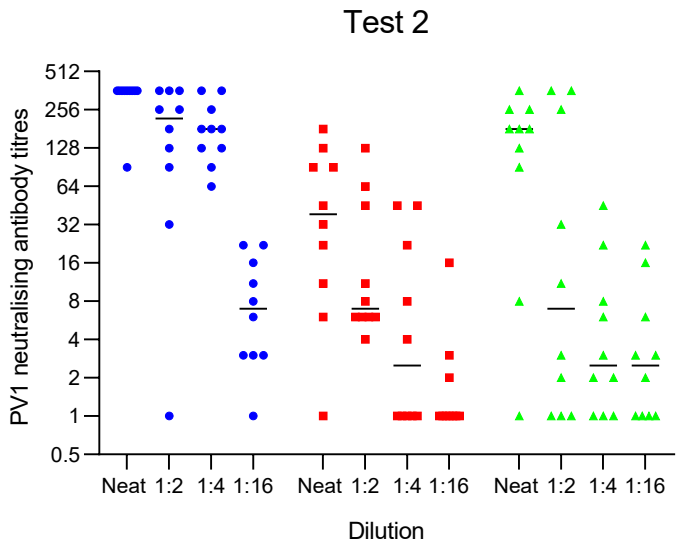
NIBSC Tests

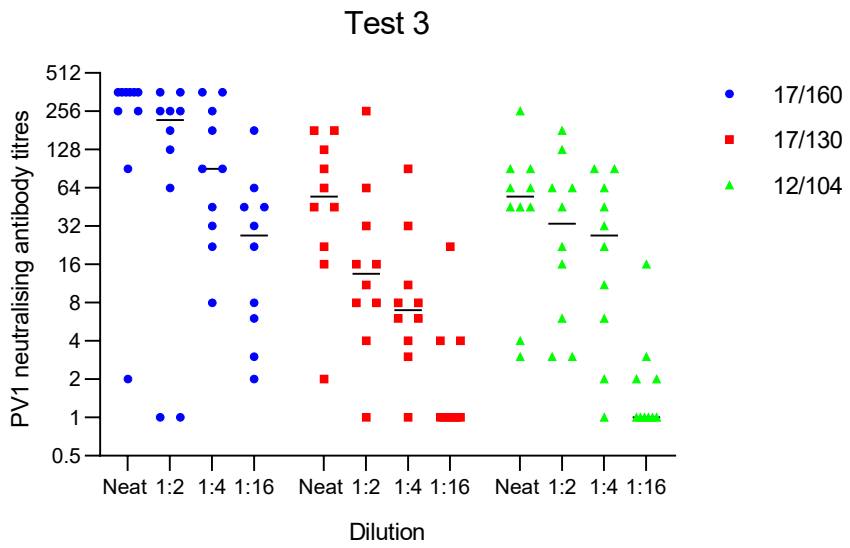
- Performed 3 independent rat tests
  - 17/160, 12/104 and 17/130
- Tested 10 rats at the following dilution; Neat, ½, ¼ and 1/16
- Challenged with monovalent virus strain
- Calculated end point titres
- Note; for a valid antibody assay, the titre of each challenge virus must be shown to be within the range 10-1000 $TCID_{50}$ and the neutralising antibody tire of control serum must be within 2 twofold dilutions of the GM titre of the serum.

NIBSC Results

PV type 1

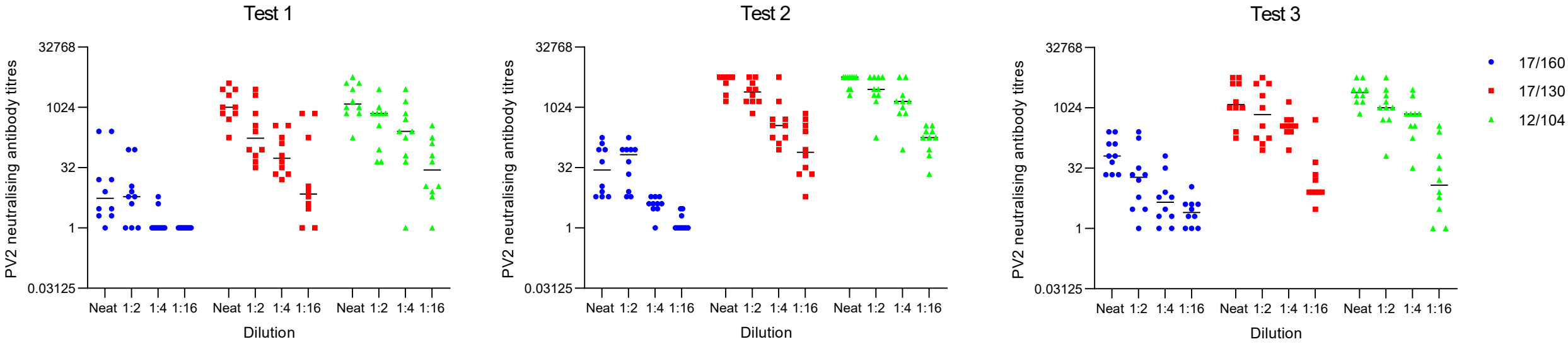






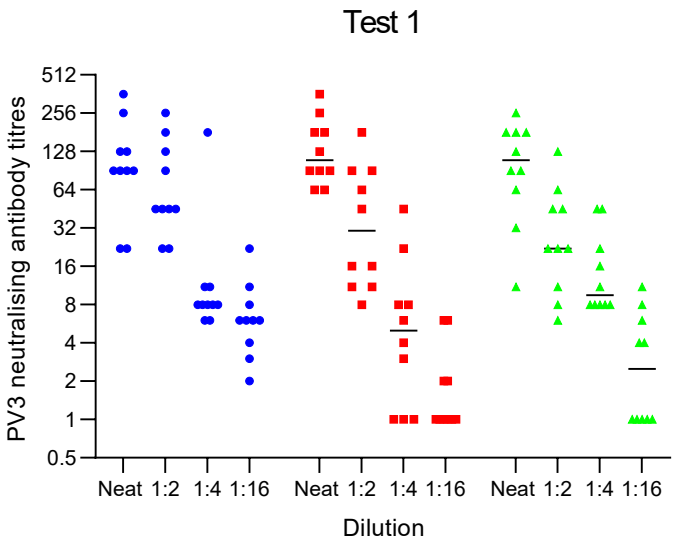
NIBSC Results

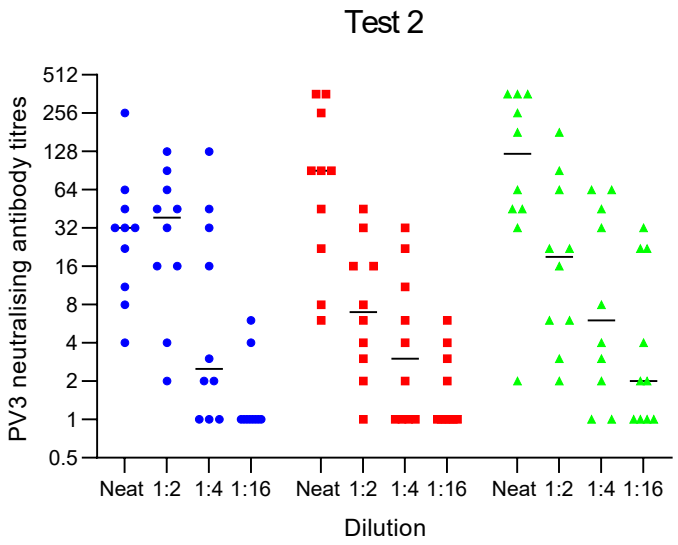
PV type 2

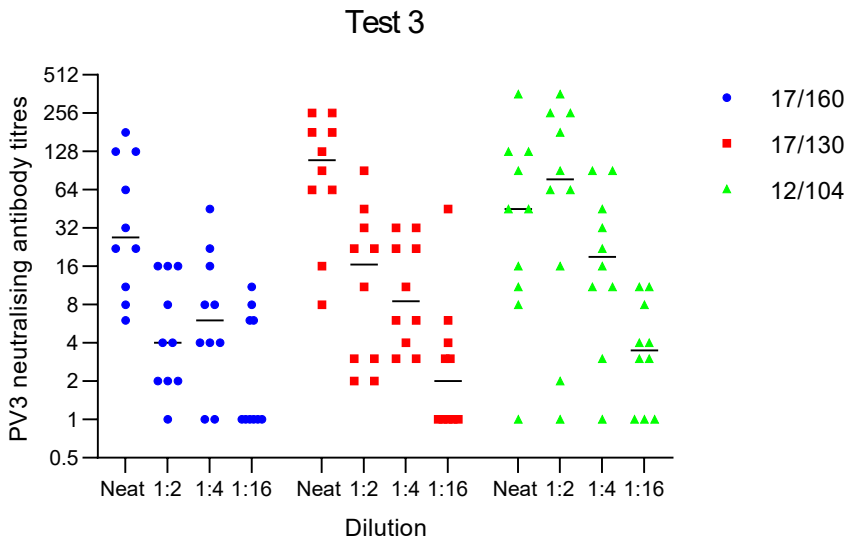


NIBSC Results

PV type 3







NIBSC Results

Establishing a cut off

Test	Dilution of 17/160	End point titres										GM	Mid-point GM	Cut off	Response
1	**Neat**	181	256	22	256	256	64	181	181	256	128	146.6	26.99476181	40.97702464	9/10
	1:2	90	181	64	64	128	8	90	181	128	181	87.3			9/10
	1:4	64	45	32	181	45	181	64	90	90	11	61.5			9/10
	1:16	11	3	22	6	6	8	2	1	22	1	5.0			0/10
2	**Neat**	362	362	362	362	362	362	362	90	362	362	315.0	44.80011551		10/10
	1:2	362	256	362	362	256	1	90	128	181	32	107.6			8/10
	1:4	181	181	90	64	181	256	362	362	128	128	168.8			10/10
	1:16	3	6	22	1	3	8	11	22	16	3	6.4			0/10
3	**Neat**	362	256	362	256	2	90	362	362	362	362	174.7	56.89349704		9/10
	1:2	1	256	362	256	128	1	256	64	181	362	71.0			8/10
	1:4	90	32	181	8	22	362	256	362	45	90	81.2			7/10
	1:16	45	8	181	3	22	6	45	64	2	32	18.5			4/10

Calculating relative potency and validity

Response

Standard	
Id.	17/160
Ass. pot.	1 IU/ml
Doses	(1)
1/1	9/10
1/2	9/10
1/4	9/10
1/16	0/10

Sample 1	
Id.	17/130
Ass. pot.	1 IU/ml
Doses	(1)
1/1	6/10
1/2	1/10
1/4	1/10
1/16	0/10

Sample 2	
Id.	12/104
Ass. pot.	1 IU/ml
Doses	(1)
1/1	5/10
1/2	3/10
1/4	2/10
1/16	0/10

Model: r/n=(phi(x)) where x=c.+b*ln(dose)
Design: Completely randomised
Weight function: w=n/(m*(1-m))
Theoretical variance: 1

Common slope(factor): b = 1.08285 (0.739585 to 1.42612)
Correlation | r |: 0.889661 (Weighted)

Source of variation	Degrees of freedom	Sum of squares	Mean square	Chi-square	Probability
Preparations	2	7.40612	3.70306	7.40612	0.025 (*)
Regression	1	26.9235	26.9235	26.9235	0.000 (***)
Non-parallelism	2	1.10382	0.551911	1.10382	0.576
Non-linearity	6	7.93956	1.32326	7.93956	0.243
Standard	2	5.98621	2.99310	5.98621	0.050
Sample 1	2	1.39090	0.695452	1.39090	0.499
Sample 2	2	0.562442	0.281221	0.562442	0.755
Treatments	11	43.3730	3.94300	43.3730	0.000 (***)
Theoretical variance			1.00000		
Total	11	43.3730	3.94300		

The statistical analysis shows no significant deviation from linearity or parallelism

Standard			
Id.	17/160		
(IU/ml)	Lower limit	Estimate	Upper limit
Potency	1.00000	1.00000	1.00000
Rel. to Ass.	100.0%	100.0%	100.0%
Rel. to Est.	100.0%	100.0%	100.0%
IU/ED50	0.0959439	0.169982	0.270482
Rel. to Ass.	369.7%	588.3%	1042.3%
Rel. to Est.	62.8%	100.0%	177.2%

Sample 1			
Id.	17/130		
(IU/ml)	Lower limit	Estimate	Upper limit
Potency	0.0658904	0.170778	0.332879
Rel. to Ass.	6.6%	17.1%	33.3%
Rel. to Est.	38.6%	100.0%	194.9%
IU/ED50	0.617501	0.995339	1.91605
Rel. to Ass.	52.2%	100.5%	161.9%
Rel. to Est.	51.9%	100.0%	161.2%

Sample 2			
Id.	12/104		
(IU/ml)	Lower limit	Estimate	Upper limit
Potency	0.0886826	0.214267	0.409702
Rel. to Ass.	8.9%	21.4%	41.0%
Rel. to Est.	41.4%	100.0%	191.2%
IU/ED50	0.502971	0.793318	1.42006
Rel. to Ass.	70.4%	126.1%	198.8%
Rel. to Est.	55.9%	100.0%	157.7%

Relative potency

Confidence limits are not less than 25% or more than 400% of the estimated potency

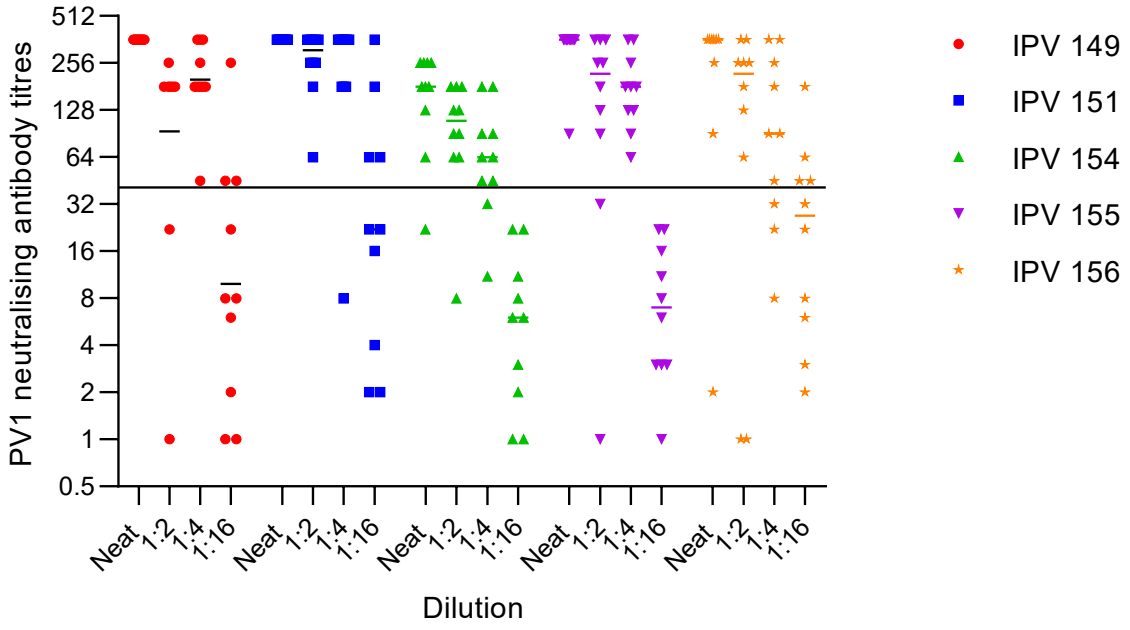
For both the vaccine to be examined and the reference vaccine, the ED50 lies between the smallest and the largest doses given to the animals;

For this test, between 1 and 0.0625

Trend data

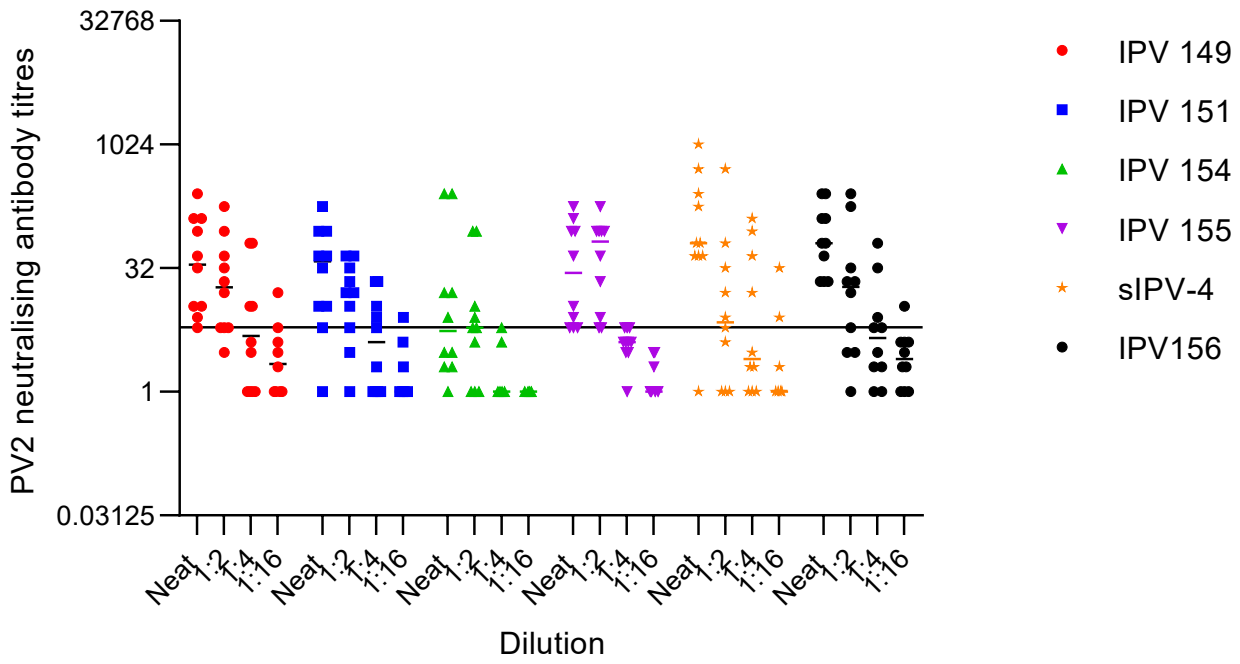
Data from all tests completed using 17/160 were analysed together to assess consistency between tests

PV type 1 - 17/160 trend with Sabin monovalent challenge



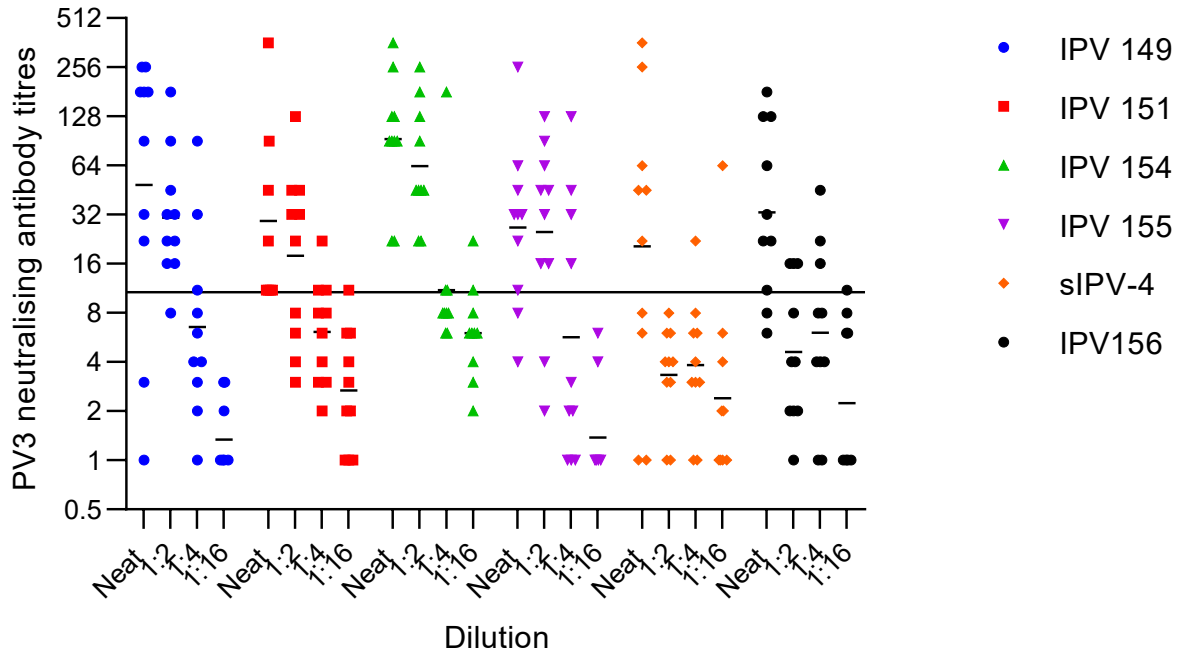
Test	Dilution	Response	Validity	Relative potency
151	Neat	10/10	Yes - All criteria met	1.40955
	1:2	8/10		
	1:4	10/10		
	1:16	3/10		
154	Neat	9/10	Yes - All criteria met	0.606949
	1:2	9/10		
	1:4	9/10		
	1:16	0/10		
155	Neat	10/10	Yes - All criteria met	0.698002
	1:2	8/10		
	1:4	10/10		
	1:16	0/10		
156	Neat	9/10	Yes - All criteria met	0.681401
	1:2	8/10		
	1:4	7/10		
	1:16	4/10		

PV type 2 - 17/160 trend with Sabin monovalent challenge



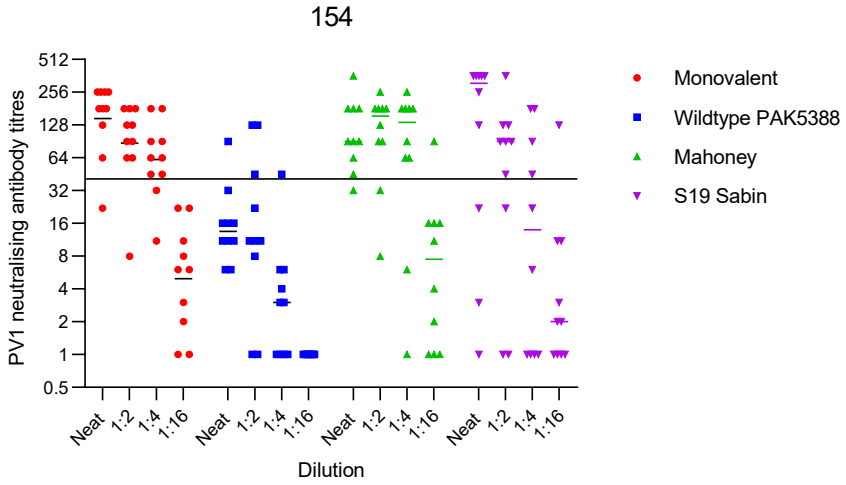
Test	Dilution	Response	Validity	Relative potency
151	Neat	8/10	Yes - All criteria met	1.11071
	1:2	7/10		
	1:4	4/10		
	1:16	1/10		
154	Neat	5/10	Yes - All criteria met	0.393593
	1:2	4/10		
	1:4	0/10		
	1:16	0/10		
155	Neat	7/10	Yes - All criteria met	0.686744
	1:2	8/10		
	1:4	0/10		
	1:16	0/10		
sIPV-4	Neat	9/10	Yes - All criteria met	1.06097
	1:2	4/10		
	1:4	4/10		
	1:16	2/10		
156	Neat	9/10	Yes - All criteria met	1.14606
	1:2	3/10		
	1:4	2/10		
	1:16	0/10		

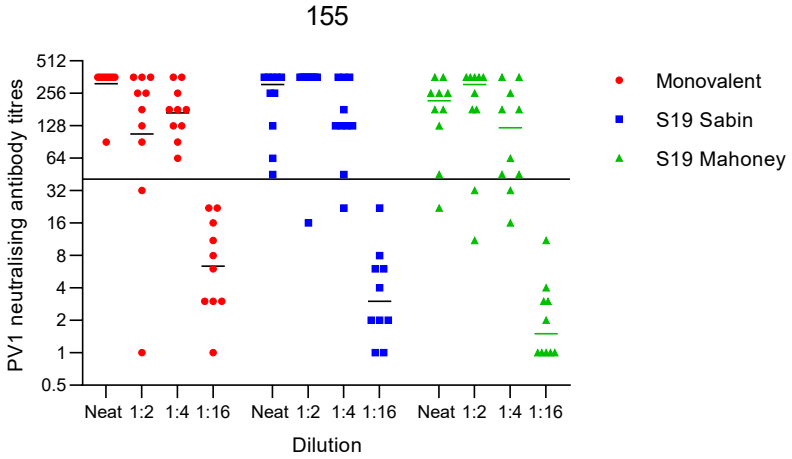
PV type 3 - 17/160 trend with Sabin monovalent challenge

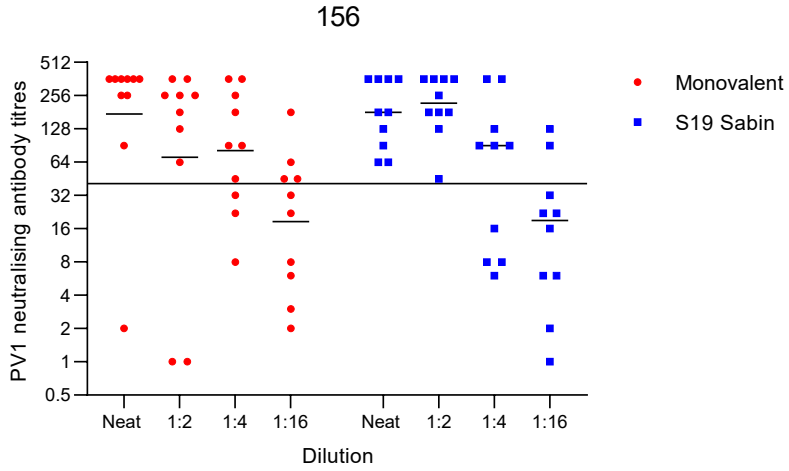


Test	Dilution	Response	Validity	Relative potency
151	Neat	10/10	Yes - All criteria met	1.39389
	1:2	7/10		
	1:4	5/10		
	1:16	1/10		
154	Neat	10/10	Yes - All criteria met	1.76465
	1:2	10/10		
	1:4	3/10		
	1:16	2/10		
155	Neat	8/10	Yes - All criteria met	0.99869
	1:2	8/10		
	1:4	4/10		
	1:16	0/10		
sIPV-4	Neat	7/10	Yes - All criteria met	0.458219
	1:2	1/10		
	1:4	2/10		
	1:16	1/10		
156	Neat	8/10	Yes - All criteria met	0.663967
	1:2	3/10		
	1:4	3/10		
	1:16	1/10		

Different challenge viruses – PV type 1





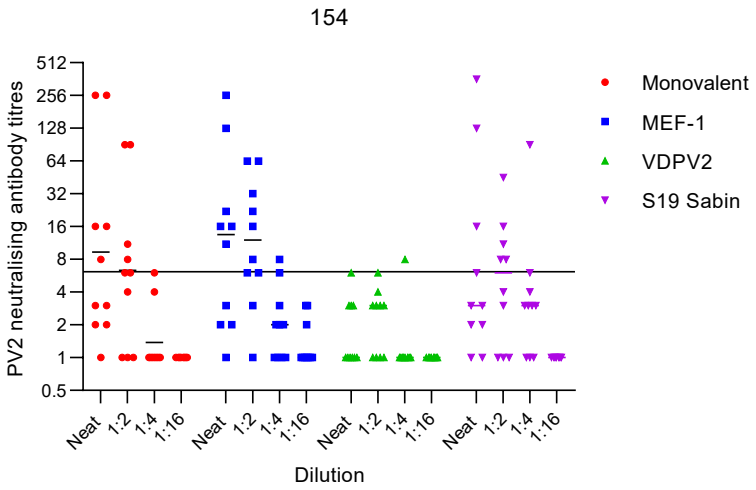


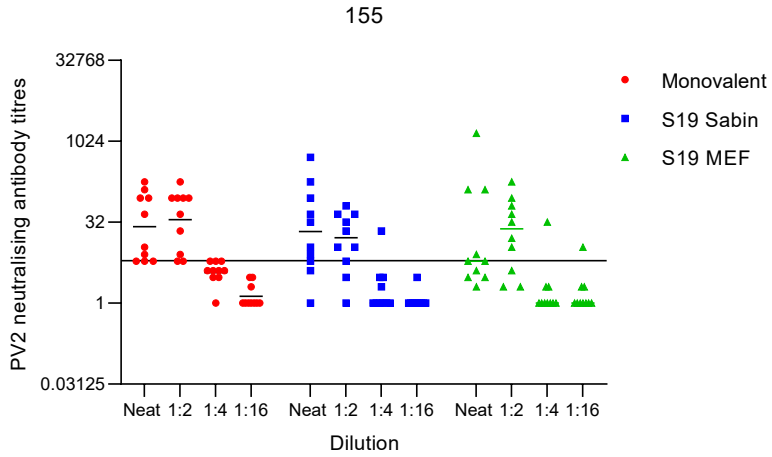
Sample	Dilution	Response	Validity	Relative potency
PAK5388	Neat	1/10	No	N/A
	1:2	3/10		
	1:4	1/10		
	1:16	0/10		
Mahoney	Neat	9/10	Yes - All criteria met	0.89189
	1:2	8/10		
	1:4	8/10		
	1:16	1/10		
S19 Sabin	Neat	7/10	Yes - All criteria met	0.435262
	1:2	7/10		
	1:4	4/10		
	1:16	1/10		

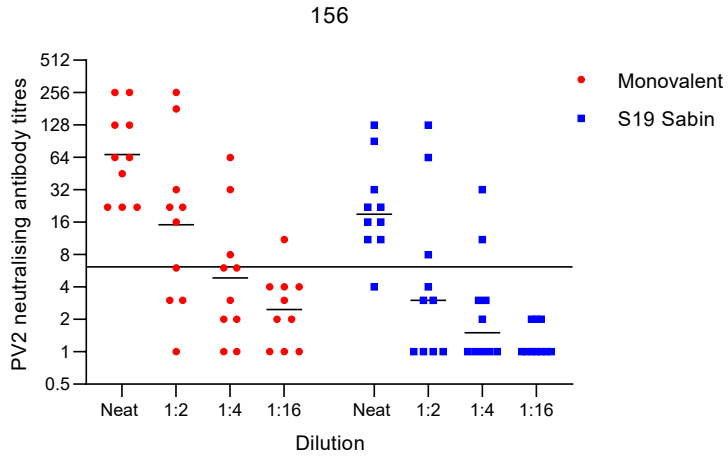
Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	9/10	Yes - All criteria met	1.02992
	1:2	8/10		
	1:4	8/10		
	1:16	1/10		
S19 Mahoney	Neat	7/10	Yes - All criteria met	0.715583
	1:2	7/10		
	1:4	4/10		
	1:16	1/10		

Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	10/10	Yes - All criteria met	1.06575
	1:2	10/10		
	1:4	6/10		
	1:16	2/10		

Different challenge viruses – PV type 2





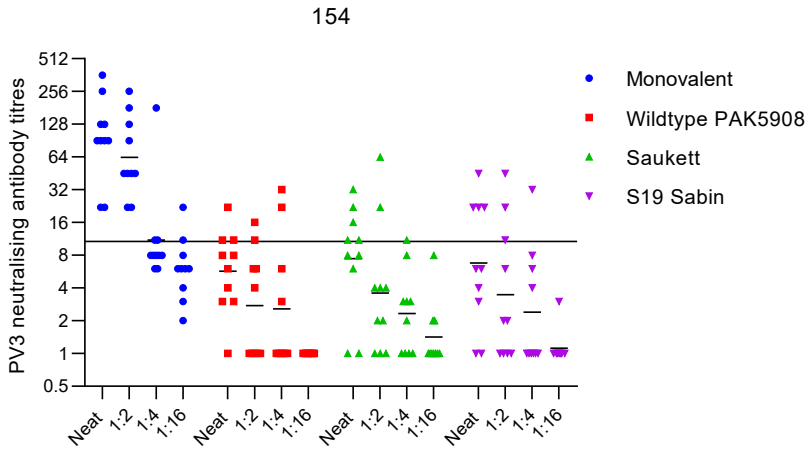


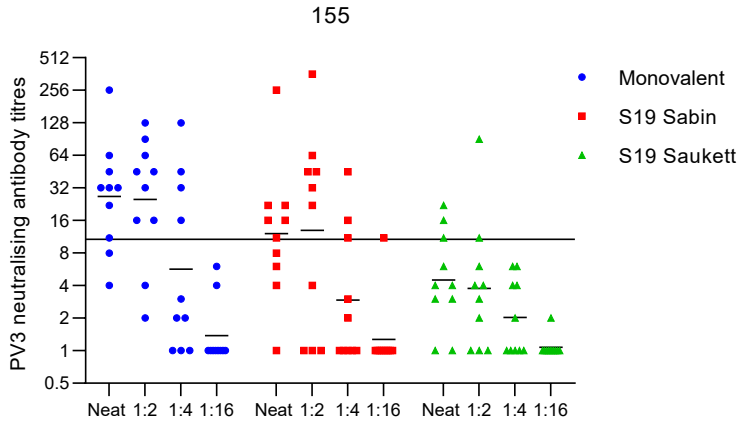
Sample	Dilution	Response	Validity	Relative potency
MEF	Neat	6/10	Yes - All criteria met	1.45139
	1:2	6/10		
	1:4	1/10		
	1:16	0/10		
VDPV2	Neat	0/10	No	N/A
	1:2	0/10		
	1:4	1/10		
	1:16	0/10		
S19 Sabin	Neat	3/10	Yes - All criteria met	1.0286
	1:2	5/10		
	1:4	1/10		
	1:16	0/10		

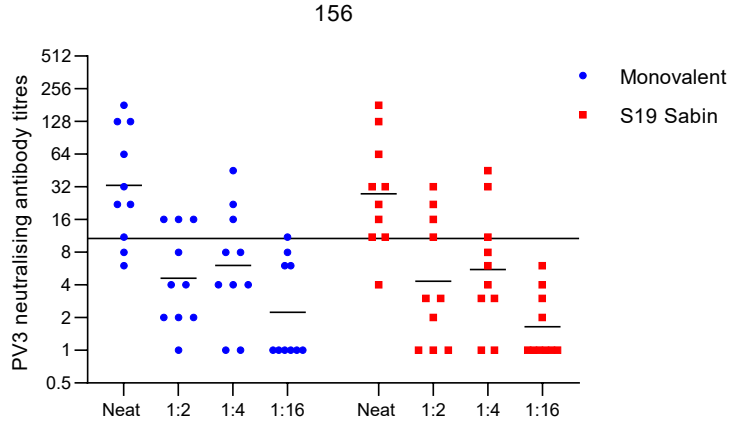
Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	9/10	Yes - All criteria met	1.00622
	1:2	8/10		
	1:4	8/10		
	1:16	1/10		
S19 MEF	Neat	7/10	Yes - All criteria met	0.795646
	1:2	7/10		
	1:4	4/10		
	1:16	1/10		

Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	9/10	Yes - All criteria met	0.576575
	1:2	3/10		
	1:4	2/10		
	1:16	0/10		

Different challenge viruses – PV type 3







Sample	Dilution	Response	Validity	Relative potency
PAK5908	Neat	3/10	No	N/A
	1:2	2/10		
	1:4	2/10		
	1:16	0/10		
Saukett	Neat	5/10	Yes - All criteria met	0.194373
	1:2	2/10		
	1:4	1/10		
	1:16	0/10		
S19 Sabin	Neat	4/10	Yes - All criteria met	0.187167
	1:2	3/10		
	1:4	1/10		
	1:16	0/10		

Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	6/10	Yes - All criteria met	0.690997
	1:2	6/10		
	1:4	3/10		
	1:16	1/10		
S19 Saukett	Neat	3/10	No	N/A
	1:2	2/10		
	1:4	0/10		
	1:16	0/10		

Sample	Dilution	Response	Validity	Relative potency
S19 Sabin	Neat	9/10	Yes - All criteria met	1.05751
	1:2	4/10		
	1:4	3/10		
	1:16	0/10		

Outline of Collaborative Study

- 5 global laboratories invited to participate;
    - These are from 3 different countries

- Participants have been requested to conduct three independent rat potency tests using 12/104, 17/160 and 17/130 as test references.

- Perform the tests according to the current guidelines in the EP

    10 rats per dilution per reference / product tested

    4 dilutions to be used to obtain valid results for all three serotypes

- Determine the neutralisation titre of each of the rat sera against their own in-house challenge polioviruses.
- In addition, test all 3 sabin S19 strains provided in the study pack as challenge viruses.
    - This will require preparation of their own sabin S19 stocks before use.

S19 strains

- S19 strains are polioviruses that replicate in tissue culture but are unlikely to replicate at all in humans should they be exposed even to large amounts.

- The strains are genetically stable and include a portfolio of strains containing the capsid proteins (and thus having the antigenic properties) of the Sabin live attenuated vaccine strains or the wild strains used most commonly in the production of inactivated polio vaccine.

- Asking the participants to use the S19 strains in the collaborative study will allow us to assess inter laboratory variability.
    - It will also allow assessment of the suitable of S19 strains to be used as challenge viruses.

- They provide a safer alternative to other strains used.
    - Can be used at BSL2 containment.

Summary and Discussion

- The *in vivo* potency rat test is an essential requirement for the analysis of preclinical vaccine immunogenicity.
- If compared to a licensed product, the *in vivo* rat potency test may be able to assist in the selection of D-antigen content in dose-finding studies in humans.
- Establishing a sIPV reference for the rat potency test will allow manufacturers to develop their own in-house assays using a validated reagent.
- More data is required for the assessment of sIPV *in vivo* immunogenicity compared to *in vitro* antigenicity.
- Participants will hopefully send data by Q2 / Q3 of 2024 and submitted to ECBS Spring 2025.

Acknowledgements

Elaine Pegg
Peter Rigsby
Laura Stephens
Javier Martin
Collaborative study coordinators

MHRA
Regulating Medicines and Medical Devices

PATH

Kutub Mahmood

Collaborative study participants

Session V. Ensuring vaccine stability and production consistency

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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Considerations for stability and consistency of polio vaccines

WHO workshop on implementation of international standards for quality control of polio vaccines including OPV and IPV

31 Oct- 2 Nov 2023, Jakarta, Indonesia

Catherine Milne and Laurent Mallet, EDQM

Presentation Outline

- Stability
  - Definitions
  - General considerations
  - Choice of stability indicating parameters and use
  - Stability notes from the TRSs - OPV, nOPV, IPV, sIPV
  - Intermediates and cumulated age
  - Regulatory approval and lifecycle
- Consistency
  - Definitions
  - General considerations
  - Relevant Parameters OPV, nOPV, IPV, sIPV
- Monitoring
  - Use of appropriate Reference Material
  - OOS versus trend analysis
- Adapting to change
- Conclusions

Stability Definitions

- **Stability of vaccines**
  - Stability is the ability of a vaccine to retain its chemical, physical, microbiological and biological properties within specified limits throughout its shelf-life.

- **Stability tests**
  - A series of tests designed to obtain information on the stability of a vaccine in order to define its shelf-life and utilization period under specified packaging and storage conditions and to monitor this throughout the product lifecycle.

Stability Definitions

- **Stability-indicating parameters:**
  - Direct or indirect indicators of vaccine efficacy or safety demonstrated in clinical trials. Used to assess product suitability throughout the shelf-life.
  - Determination of these parameters should result in quantitative values with a detectable rate of change. Qualitative parameters such as sterility could also be considered but cannot be included in the statistical analysis.
- **Shelf-life:**
  - The period of time during which a vaccine, if stored correctly, is expected to comply with the specification as determined by stability studies on a number of batches of the product.
  - The shelf-life is used to establish the expiry date of each batch. Shelf-life is used for the final product (FP); storage period is used for the intermediates.

General considerations

OPV, IPV, nOPV, sIPV – are vaccines like any other and subject to

WHO GL on Stability Evaluation of Vaccines, Annex 3, TRS No 962

Sections on:

- Stability evaluation at different stages of production and use
- Regulatory considerations
- Design of studies and statistical considerations
- Data analysis

Considerations for the control temperature chain (CTC) programme are found in: **WHO GL on stability evaluation of vaccines under extended controlled temperature conditions TRS No 999**

Also refer to: **ICH guidance (Q1 series and Q5C)**

Choice of stability indicating parameters and use

- Ideally they reflect the link between quality and efficacy or safety as demonstrated in clinical trials.
- Identified during the development of a vaccine, taking into account a potential link between biological activity (e.g. toxicity or potency) and safety and efficacy
  - For example, upper and lower potency specifications for vaccines reflect the link of vaccine potency both with the minimum dose used to demonstrate the efficacy in clinical trials and the maximum dose shown to be safe.
- A stability profile with stability-indicating parameters is defined
- Stage(s) of testing determined and criteria of acceptance set
- Testing programme established to ensure adequate monitoring
- These elements should be approved by the NRA

Choice of stability indicating parameters and use

Potency:

- Live attenuated vaccines e.g. OPV, nOPV
  - Titre can be directly studied on the intermediate and/or final lot
- Inactivated vaccines e.g. wIPV, sIPV
  - Potency/content assays are used but are only relevant if demonstrated to be stability indicating – this is a critical consideration for method development in particular for in vitro assays where the stage of testing (and presence of other components) may impact their relevance

Parameters other than potency-indicating ones should also be considered since they indicate changes in vaccine quality with unknown effects on efficacy and safety e.g. appearance, pH....

OPV/nOPV Stability – from the OPV TRS

- Shelf-life of FP and storage time of process intermediates is established based on real-time, real-condition stability studies and approved by the NRA
- Accelerated thermal stability tests may provide additional information and aid in assessing comparability when there are manufacturing changes
- Formulation of the vaccine should minimise potency loss through the shelf-life and potency at release should ensure that the minimum potency is reached at end of shelf-life; as demonstrated in at least 3 consecutive final lots
- Ongoing monitoring post- licensure recommended to support shelf-life specifications and refine the stability profile

wIPV/sIPV Stability – from the IPV TRS

- Performed at stages where intermediate is stored e.g. single harvests, inactivated purified monovalent pool, trivalent bulk, final bulk and final lot – each should have a defined shelf life
- Defined and selected according to stage of production
- Where manufacturing involves only formulation of the final bulk from trivalent bulks supplied by another manufacturing establishment and the filling of final containers, stability data on the trivalent bulks should be generated if the storage container or storage conditions are changed, and the shelf-life until use should be established by the manufacturer performing the final fill.

Intermediates and cumulative age

- Vaccine production involves production of intermediates e.g. harvests, bulks, etc. many of which are stored for logistical reasons before processing
- Stability testing should be performed on the intermediates at different stages of production and proposed storage periods validated and included in the dossier
- The stability of the characteristics of a final product should be guaranteed during the whole shelf-life, irrespective of the age of the intermediates at the time they are used in the production process
- Total age of all components at the end of shelf-life is considered as cumulative age of the product. In practice, stability data on the final product should include the data generated on the intermediates of different ages used in the final formulation.
- Complete stability data covering the total cumulative age of all the antigens in a vaccine may not be available before approval of storage periods and the shelf-life or approval of their extension. Nevertheless, manufacturers are encouraged to collect such data on a continuous basis and to report them to the national regulatory authorities.

Regulatory approval and lifecycle

- A stability protocol is an important element of the manufacturer's dossier and should include all tests performed to support the shelf-life of the vaccine in question.
- Given that the assurance of stability is a continuous process, the dossier submitted for licensing needs to be supplemented with the data from stability studies completed afterwards
- Data provided for licensure should be generated on the lots representative of the intended manufacturing scale production as well as of the final formulation using validated methods.
- Changes in manufacturing will necessitate additional stability studies and regulatory approval

Consistency Definitions

- **Consistency of vaccines**
  - Vaccines produced under defined conditions which lead to the reproduction of the physical and biological characteristics and properties batch to batch within defined limits and which are ideally linked to the quality, safety and efficacy determined in clinical studies
- **Consistency tests**
  - A series of tests designed to measure critical quality parameters indicative of consistency which contribute to verify each batch is within the accepted batch-to-batch variability for the given parameter

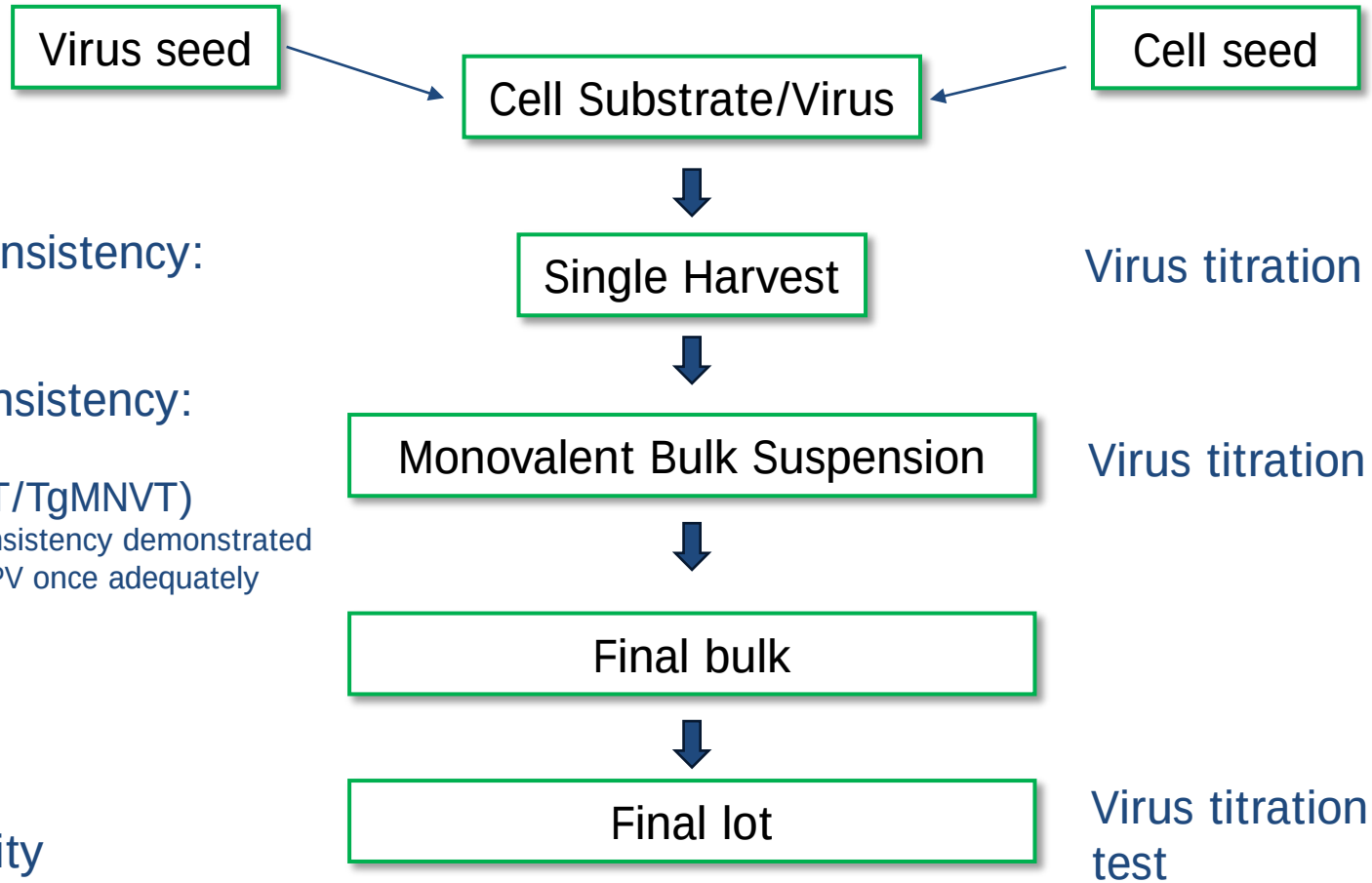
Consistency – General considerations

- Parameters measured should be identified during product development and characterisation.
- Consistency parameters should ideally be relevant to potency, safety, or efficacy
- Acceptable limits are defined based on link to clinical data
- Method and process performance and their variability should also be taken into consideration when defining the acceptable limits
- All should be approved by the NRA

Relevant parameters OPV, nOPV consistency – from the OPV TRS

- Potency: Virus titration versus reference
  - Single harvest, monovalent bulk, final lot
- Genomic/molecular consistency
  - MAPREC/HTS (OPV), HTS (nOPV) – compared to seed lot or reference preparation
  - Single harvest, monovalent bulk
- Neurovirulence *in vivo* (MNVT/TgMNVT)
  - Performed on monovalent bulk
    - May be omitted for nOPV after sufficient characterisation and NRA approval
    - Ultimately may be replaced with HTS for OPV provide sufficient validation and NRA approval
- Thermal stability test
  - Potency test performed on sample stored at 37°C for 48 hours; loss of potency from 'normal' sample is within approved limits e.g. not greater than 0.5 log $CCID_{50}$/human dose
  - Thermal stability should be considered as a vaccine characteristic that provides an indicator of consistency of production in the context of lot release
  - The thermal stability test is not designed to provide a predictive value of real-time stability, but to test for conformity with a defined specification for a tested vaccine.
  - Performed on final lot
- Stability profile from stability studies from intermediates and final lot can also reflect consistency

OPV/nOPV Consistency Schematic



Genomic/molecular consistency:
MAPREC/HTS (OPV), HTS (nOPV)

Virus titration

Genomic/molecular consistency:
MAPREC/HTS (OPV), HTS (nOPV)

Neurovirulence *in vivo* (MNVT/TgMNVT)
- omitted for nOPV once molecular consistency demonstrated
- Replaced by full genome HTS for OPV once adequately validated

Virus titration

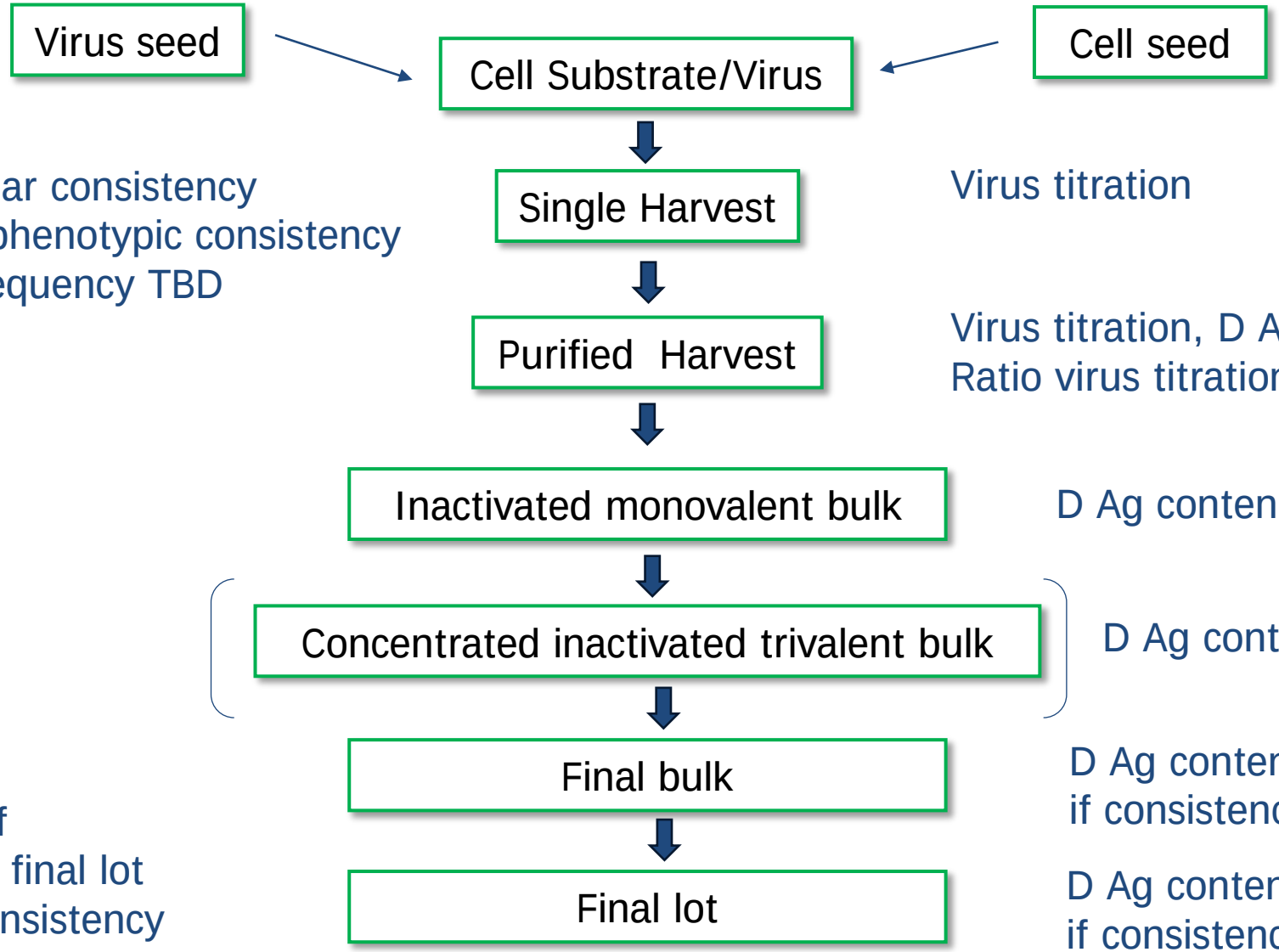
Virus titration, Thermal Stability test

Stability profile from stability studies from intermediates and final lot can also reflect consistency

Relevant Parameters IPV, sIPV Consistency – from IPV TRS as amended

- Molecular consistency/phenotypic consistency (sIPV) – MAPREC or genomic analysis preferred but may be MNVT/TgMNVT
  - Purified monovalent pools – consistency of sequence with consensus and seed lots – may be reduced to a proportion/year after consistency demonstrated
- Virus titration
  - Single harvest , purified monovalent pools
- Protein content
  - Purified monovalent pools, final lot (may be omitted after consistency demonstrated)
- D antigen content
  - Purified monovalent pools, Trivalent bulk, Final bulk, Final lot (may be omitted if done on the final bulk)
  - The ratio between virus titre (per millilitre) and D-antigen content (per millilitre) of purified monovalent pools prior to inactivation should also be established for each poliovirus type during product development and should be monitored during commercial production.
- Kinetics of inactivation
  - Inactivated monovalent pools
- In vivo potency
  - Final bulk (may be omitted after consistency demonstrated)
- Stability profile from stability studies from intermediates and final lot can also reflect consistency

IPV/sIPV Consistency Schematic



sIPV: molecular consistency preferred or phenotypic consistency – in vivo – frequency TBD

Virus titration

Virus titration, D Ag content, Total protein, Ratio virus titration/D Ag content

D Ag content, Inactivation kinetics

D Ag content

D Ag content, in vivo potency: omitted if consistency demonstrated

D Ag content, protein content: omitted if consistency demonstrated

Stability profiles of intermediates and final lot can also reflect consistency

Monitoring – Use of appropriate Reference Materials

- Use of reference materials (in particular for potency/content) is of critical importance in the interpretation of the data generated in stability and consistency studies. Reference materials should be calibrated against the International Standard when available

- A monitoring programme should be put in place to ensure the stability of the in-house reference standard and the comparability of its subsequent replacement

Monitoring – Out of specification versus out of trend

Specifications should be established for the parameters monitored for stability and consistency.

- Any confirmed out of specification (OOS) results should be considered non-compliant
  - ➢ Appropriate regulatory actions should ensue and an investigation into the root cause should be carried out and, once identified, corrected

In addition,

- Trend analysis of quantitative data (both stability and consistency) is expected
- Appropriate statistical analysis is used to identify out of trend data
- A significant shift in trend may not lead to an OOS situation but should be investigated to identify the cause and any necessary corrective actions

Adapting to change

Change in production process, control methods, reference material will require specific actions to ensure that the control strategies for stability and consistency remain suitable

- Comparability studies after process change help to ensure that the process changes do not impact quality attributes linked to potency and safety (refer to ICH Q5E)
- Change of reference material will require bridging studies to compare values of the same material tested with the old and new reference to verify there is no shift in value introduced due to the new reference
- Change in method may require running the old and new methods in parallel for a period to allow definition of suitable specifications – this is particularly relevant, for example, for potency tests

Conclusions

- Quality is built in during product and process development and characterisation
- Stability and consistency testing are important elements to ensure the ongoing quality and safety of poliomyelitis vaccines
- Use of relevant methods to assess critical quality parameters linked to clinical outcomes is key to a successful strategy

Thank you for your attention

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Key principles for stability evaluation

Tong Wu, Ph.D.
Vaccine Quality Division 3, Health Canada
Oct 31 – Nov 2, 2023, Jakarta, Indonesia

Regulatory expectations for stability studies

A licensed vaccine is expected to remain efficacious or effective at the end of shelf-life when stored under the recommended conditions. The proposed shelf-life should be supported by:

- Product-specific stability data from lots that are representative of commercial product.
  - Stability characteristics of a vaccine is influenced by starting materials, manufacturing process and formulation.
- Real time real condition stability data (under the proposed storage conditions over the entire storage period)
  - Rates of vaccine decay at different temperatures may not follow Arrhenius model → conclusion based on extrapolation of stability data at different temperatures may not be reliable.
  - Decay rates may differ over shelf-life (e.g., beginning, middle or end).
- Using product-release model (**WHO TRS 999, Annex 5**), not compliance model, for data analysis.

Using a battery of stability-indicating assays

The battery of assays have the capacity to detect changes of quality attributes that are predictive of vaccine clinical performance. For example:

- Potency for IPV
  - D-antigen ELISA
  - Rat potency: variable and less sensitive for detecting antigen decay and not recommended for routine use.
- Potency for OPV
  - Virus titre
- Additional quality attributes for polio vaccines
  - Appearance
  - pH
  - Sterility
  - Container closure integrity

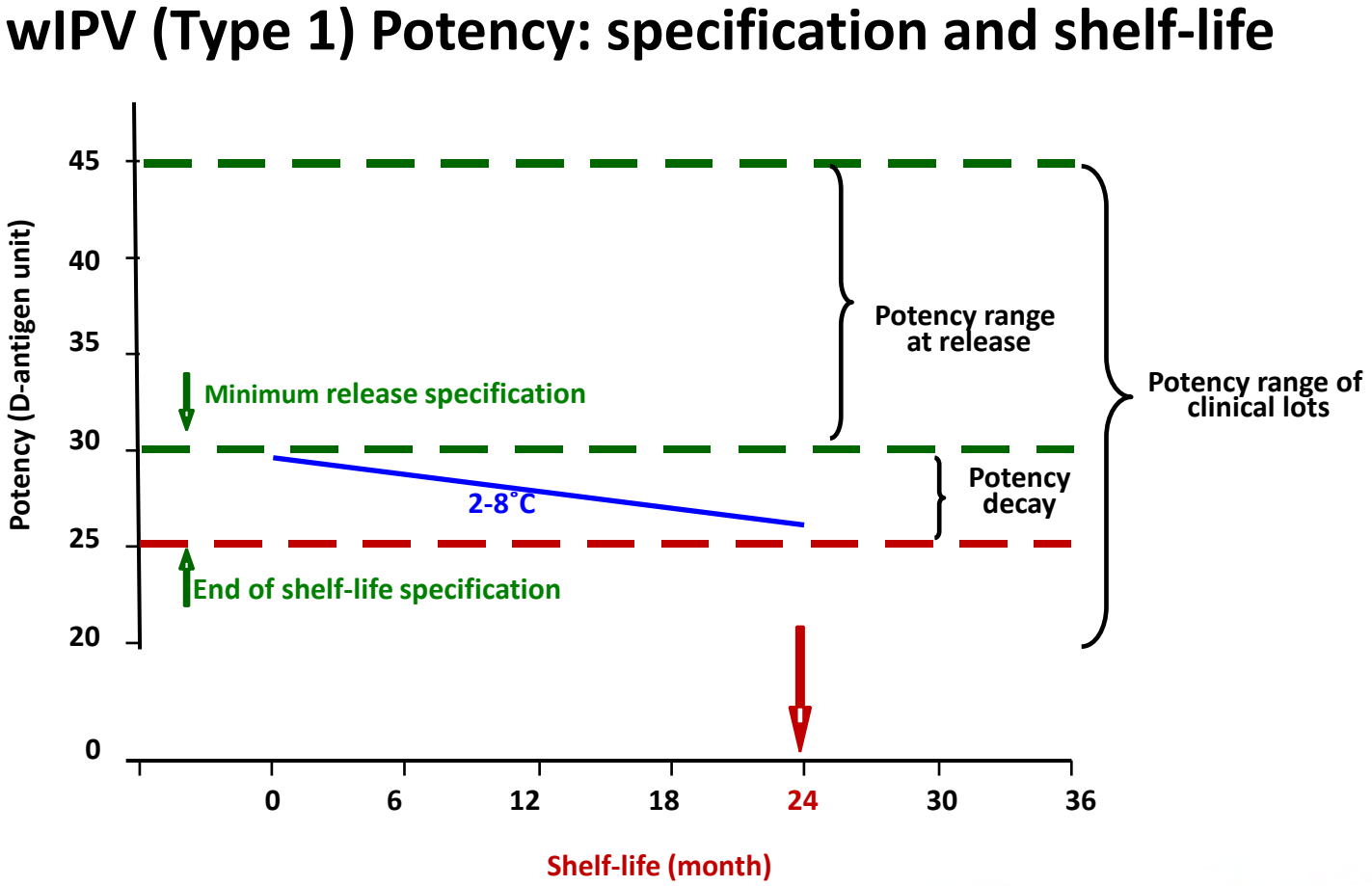
Setting appropriate specifications

- Setting different release and end of shelf-life specifications for quality attributes (e.g., potency) that change during storage.
- The specifications at the end of shelf-life (e.g., minimum potency) should be based on characteristics of vaccine lots demonstrated to be safe and efficacious or immunogenic in clinical studies (Patient-Centric Specifications).
  - Dose ranging studies are critical to support the minimal potency specification at the end of shelf-life.
- The specifications at release should be
  - sufficient to ensure that lots meet the end-of-shelf-life specifications throughout entire shelf-life, considering product decay and assay variability.
  - within manufacturing capability to avoid high rate of lot failure.

Data analysis

- Compliance model (based on whether stability lots meet or fail specifications at each testing point) cannot ensure vaccine clinical performance.
  - For example, OPV lots with high virus counts at release are expect to meet the end of shelf-life specification for a longer period, which may not be applicable to lots with lower virus counts at release.
- Apply product-release model (WHO TRS 999, Annex 5)
  - Using appropriate statistical analysis to determine the rate of change over entire storage period with confidence (usually 95%).
  - Ensuring worst case lots (approaching specification at release) also meet end of shelf-life specification

Product-release model: relationship between specifications and shelf-life



Product-release model: relationship between specifications and shelf-life (cont.)

Minimum Release Specification = End of shelf-life specification + decrease (IPV D-antigen content or OPV virus count) during entire recommended storage period (upper bound with 95% confidence level)*

*** logarithmic transformation (log-transformation) of potency data typically permits analysis of stability data by linear regression.**

Accelerated stability data as stability characteristics

- Vaccine degradation is typically enhanced at higher temperatures.
- The decay rate of a vaccine within a short period (e.g., 4 weeks) at a temperature higher than the recommended long-term storage is a useful stability characteristics (forced degradation curve).
- Forced degradation characteristics of a vaccine can be used to support stability comparability in cases of manufacturing changes post-licensure.
  - WHO TRS 1045 recommends thermal stability study of final OPV lot at 37˚C for 48 hours → an indicator of production consistency.
  - IPV is relatively stable (Type 1 of wIPV is more thermal sensitive) → Forced degradation characteristics of Type 2 and 3 wIPV based on D-antigen content may require temperatures higher than 37˚C.

Summary

- Vaccines may undergo degradation when stored under the recommended long-term storage condition, and this is typically enhanced at higher temperatures.
- To ensure vaccine remain efficacious or effective at the end of shelf-life, stability studies should consider the following:
  - set different specifications for release and end of shelf-life for quality attributes that change during storage.
  - determine rates of decay under long-term storage using statistical modeling (e.g., linear regression analysis).
  - Shelf-life is based on product-release model.
- Forced degradation characteristics can be used to support stability comparability assessment when introducing manufacturing changes post-licensure.

References

- Guidelines on Stability Evaluation of Vaccines, Annex 3, TRS No 962 (https://www.who.int/publications/m/item/guidelines-on-stability-evaluation-of-vaccines)
- Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions, Annex 5, TRS No 999 (https://www.who.int/publications/m/item/ectc-annex-5-trs-no-999)