CENTER FOR VACCINE INNOVATION & ACCESS

Next Generation Sequencing (NGS)-

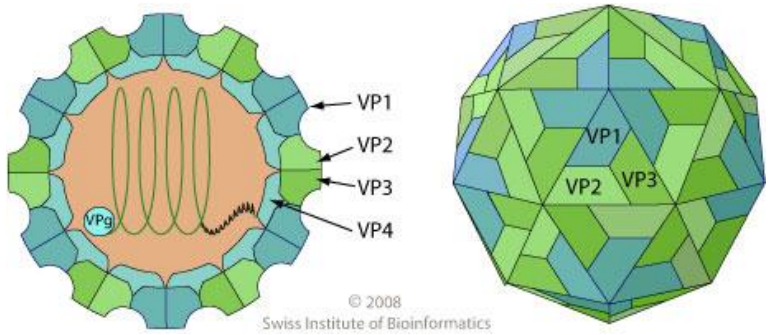
An alternative to animal based neurovirulence testing (NVT) for polio vaccines

Kutub Mahmood PhD

CVIA, PATH

PATH

Polio Virus & Vaccines



- **Enterovirus subgroup,** family Picornaviridae (small RNA viruses)
- 3 serotypes: PV1, PV2, PV3

- **Fecal-oral transmission**
- replication in pharynx (~1 week)
- GI tract -> fecal shedding (several weeks)

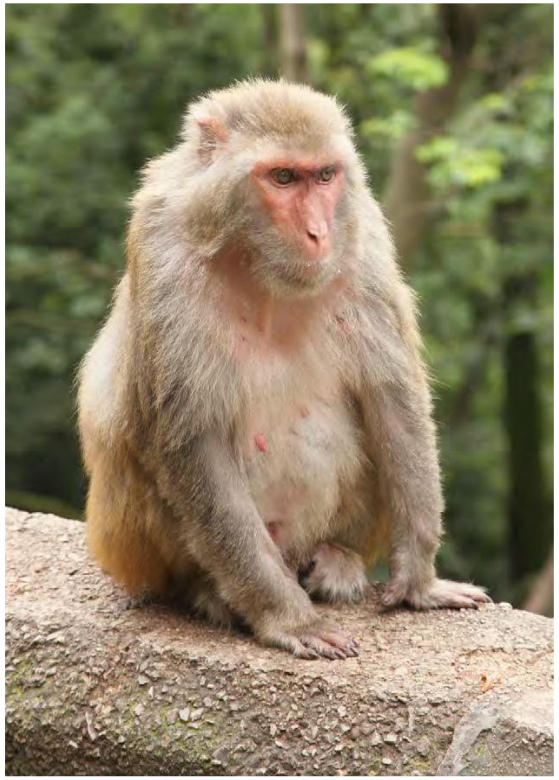
- **Clinical disease 6-20 days post-exposure**
- ~95% asymptomatic
- <1% Paralytic polio, acute flaccid paralysis, possible permanent loss muscle control

- **Preventative Vaccines**
- Oral poliovirus vaccine (OPV) bOPV, mOPV, nOPVs
- Inactivated poliovirus vaccine (IPV) – Salk IPV & Sabin IPV

- **Correlates of Protection**
- Magnitude of antibody response ($\geq$ 1:8 neutralizing antibodies)
- Mucosal IgA (protection from viral shedding, OPV associated)

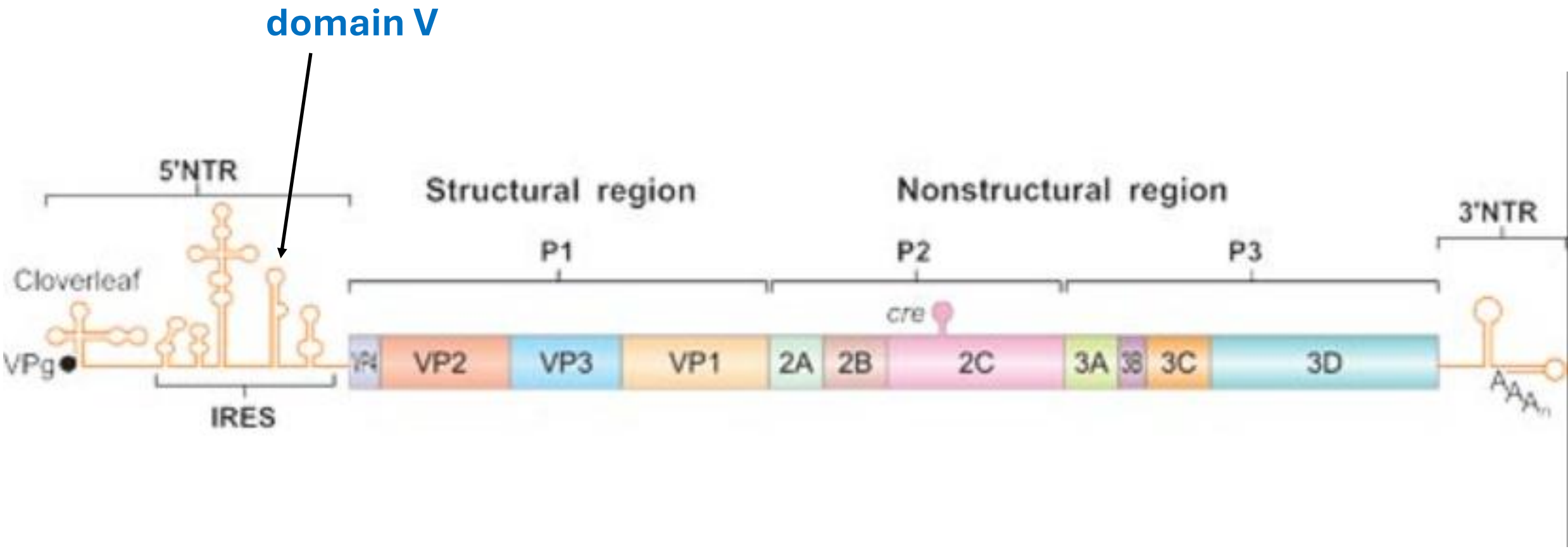
WHO monkey neurovirulence test for polio vaccines

To measure residual virulence of Sabin strains

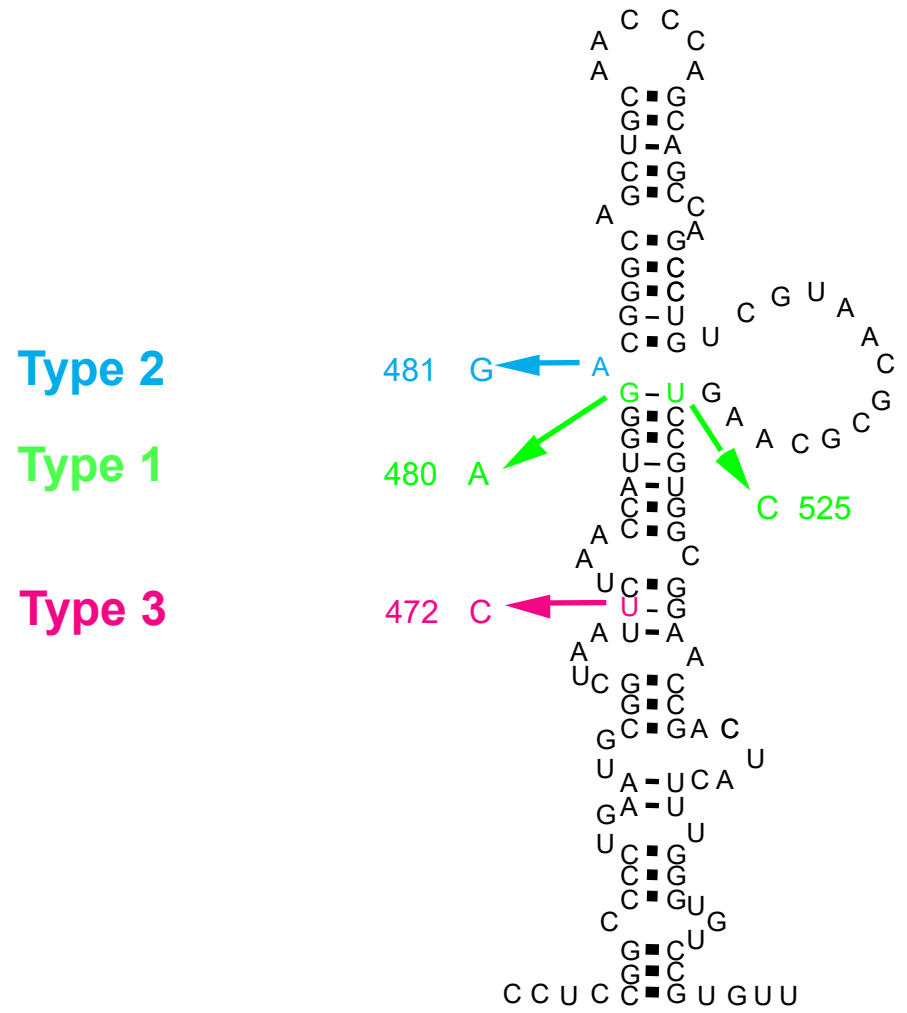


- 24*2=48 monkeys inoculated intra-spinally
- 24 with new vaccine lot and 24 with reference virus
- Observed for 17 days for signs of paralysis
- All monkeys sacrificed for histological examination
- Lesions in CNS are scored and compared
- Vaccine lot “passes” if lesions are not greater than in reference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

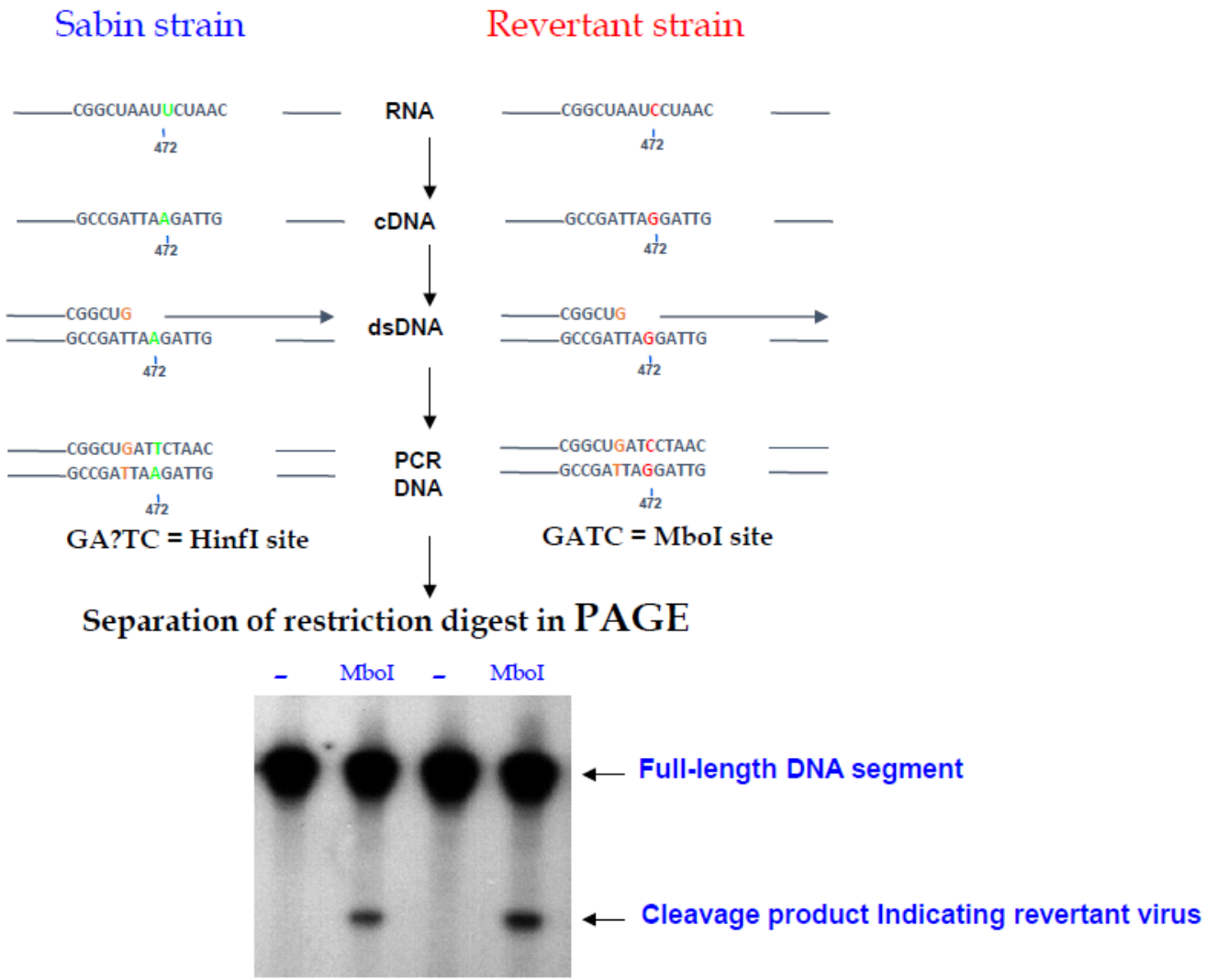
Neurovirulence Determinants in domain V of 5’NTR of Poliovirus Genome



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

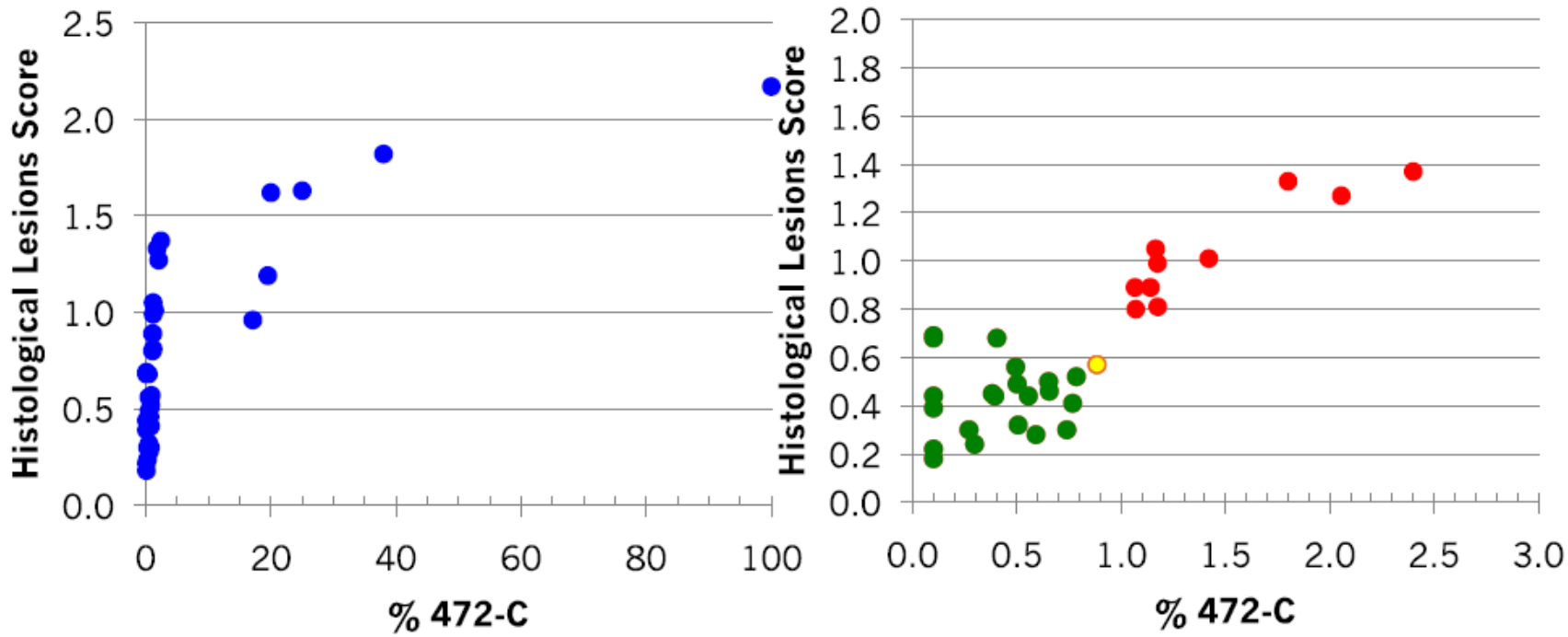
Correlation between amount of virus with altered nucleotide sequence and the monkey test for acceptability of oral poliovirus vaccine

(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)



Regulatory Acceptability of MAPREC by WHO

- 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

Challenges for performing animal NVT

- 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).
  - ~ 48 monkeys or 80 Tg mice are sacrificed for QC release of one monovalent 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
- Handing of live polio virus under containment requirements of GAP III/ IV
- 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.

World Health Organization

WHO/BS/2019.xxxx
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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]
National Institute for Biological Standards and Control (NIBSC),
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

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]
National Institute for Biological Standards and Control (NIBSC),
South Mimms, Potters Bar, Herts, EN6 3QG, UK
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

Current Collaborative Study for use of NGS in routine testing

- **ECBS proposal 2021: Use of whole-genome next generation (deep) sequencing for routine lot release of OPV and quality control of sIPV - developing test protocol and reference reagents.**

- **Status: Collaborative study started in late 2024, now completed**

- **Study Report for submission to WHO ECBS Meeting spring 2026**

- **Aims and Objectives of the Study:**

- The primary aim of this study is to establish reference reagents to be used in NGS methods to monitor the consistency of production of OPV and the characterization of virus seeds and 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 of MAPREC and 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.

- 6 international participants: Indonesia, India, Japan, China, USA and UK. Includes both manufacturers and control laboratories. International meeting was held in September 2025 (3rd-5th Sep), Bangkok, Thailand

International Meeting on QC Animal Assays for Polio Vaccines
Sept 3rd-5th Bangkok, Thailand

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NGS related implementation activities as alternate for animal NVT

- NGS training for the nOPV manufacturers and regulators (May/Aug 2025)
  - BioFarma, BioE, Indonesia BPOM & India DCGI's NCL, Kasauli, India

- PATH hosted Webinar on Use of NGS for vaccine lots testing (Sept17-18th 2025)
  - advt.agnts testing,
  - MNVT

- Alignment with WHO, IABS, EDQM, and other organizations for NGS implementation

- Request for NGS training workshops from other OPV/IPV manufacturers

  - Planned training workshops in 2026

- nOPV team at PATH working with manufacturers to replace MNVT usage with NGS

Collaborative Partners

Medicines & Healthcare products Regulatory Agency

FDA Center for Biologics Evaluation and Research

In Partnership with:

OPV & IPV Manufacturers & National Laboratories

World Health Organization

GATES foundation

This work is funded by the Gates Foundation grant INV-017623

A patron with great love of animals

St. Francis of Assisi

Died 3rd Oct 1228

World Animals Day- Oct 4th


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