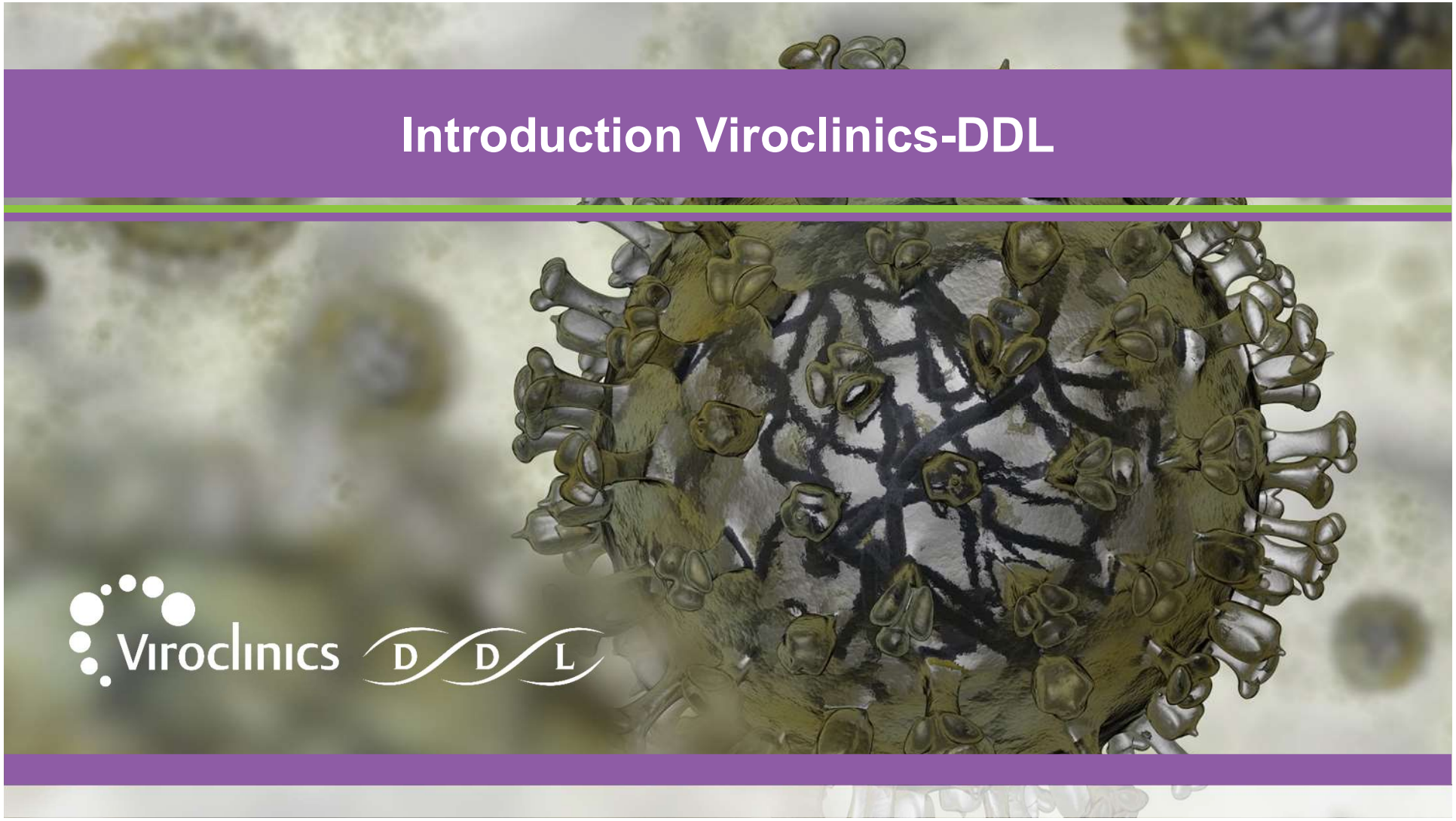
Webinar: Polio Analytical Services

Agenda

- Introduction Viroclinics-DDL
- Background Polio
- GAP III Regulations
- Analytical assays in a Polio High Containment Facility
- Q & A

Viroclinics DDL

Introduction Viroclinics-DDL



European HQ and Laboratory Sites

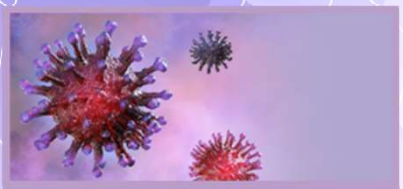
Viroclinics
Clinical diagnostics laboratory. Support staff and corporate HQ location.

Viroclinics Xplore
Early stage and preclinical services. Capabilities at BSL2 and BSL3(+) level.

DDL Diagnostic Laboratory
Focused on molecular diagnostics, molecular biology and molecular pathology.

Bridged Assays
Based in Shanghai, central virology lab service for phase II, III and IV clinical trials.

Our Therapeutic Fields

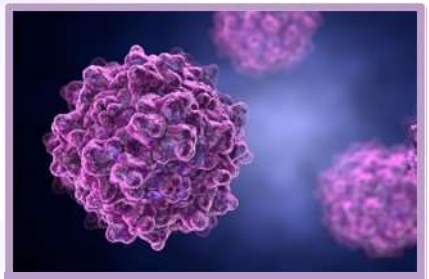


Infectious Diseases

Respiratory viruses
Influenza
RSV
MERS-CoV
SARS-CoV
SARS-CoV-2 (COVID-19)

Blood-borne viruses
Hepatitis B, C, D
HIV

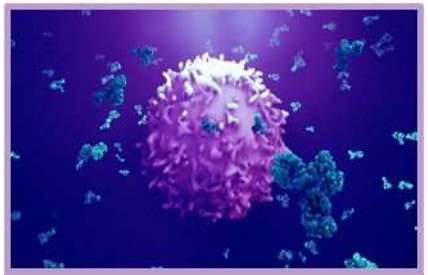
Other viral targets
HPV, **Polio**, Dengue, Chikungunya ... and more



Gene Therapy

Adeno-associated viruses
AAV-2
AAV-9
… and more

Bacteriology



Immuno-Oncology

Under development

Microbiome

BSL 3 Facilities

CUTTING EDGE LAB

BSL 2

BSL 3

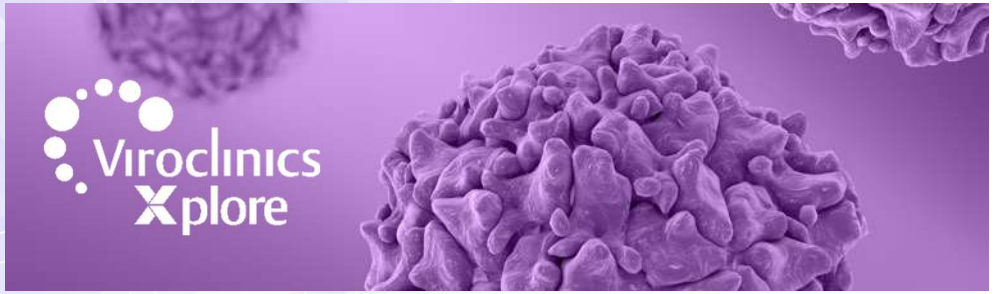
State-of-the-Art Facilities

In-house preclinical and clinical laboratories

DEDICATED SCIENTISTS & CONSULTANTS

Our Capabilities - Preclinical Services

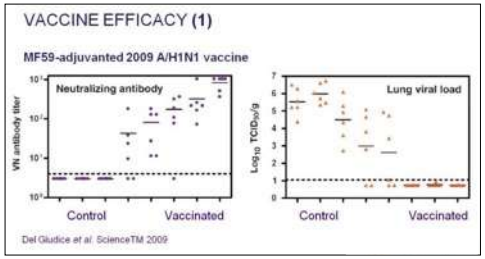
- 1600 m^2
- SPF, Conventional, BSL2 and BSL3(+)
- GAP Ⅲ Polio Certificate of Participation
- Negatively-pressurized isolator cages
- Range of different species
- Solutions for any kind of experimental set up: challenge and transmission studies

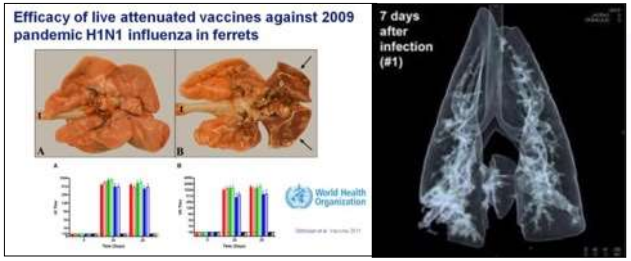


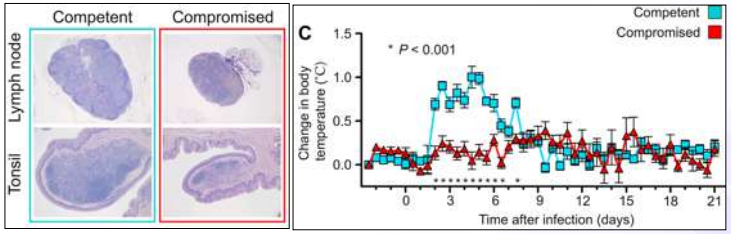
Our Capabilities - Preclinical Services

Type of research
• Vaccine Efficacy
• Passive Immunization
• Immunogenicity
• Pharmaco Kinetics
• Immunopathology
• Pathogenicity
• Antiviral Therapy
• Antiviral Prophylaxis
• Safety
• Drug Delivery
• Resistance / Mutants
• Transmission
• …(*more*)

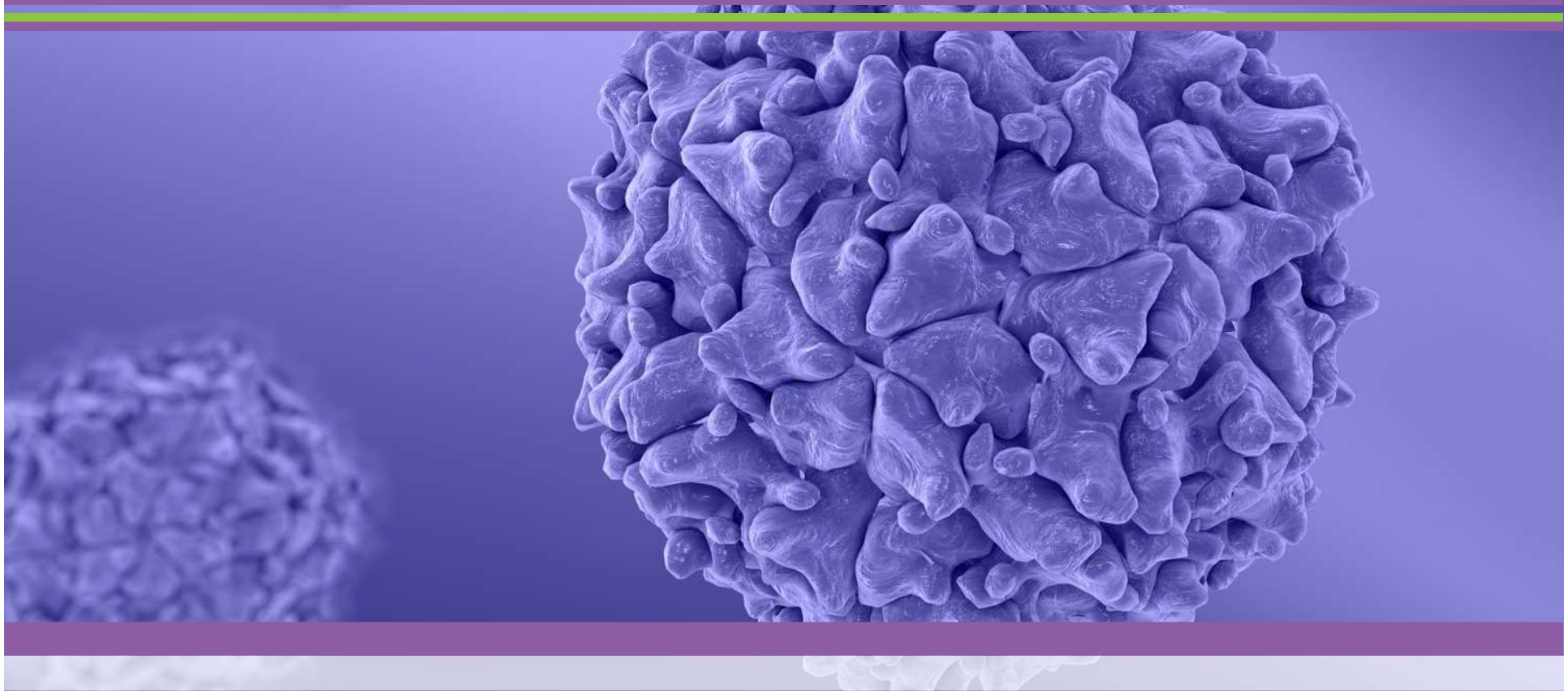
Read Outs
• Virus Titers
• Virus Copies
• Antibody
• CMI
• Genomics
• Pathology
• Histopathology
• Mortality
• Clinical Signs
• Imaging
• …(*more*)







Background Polio



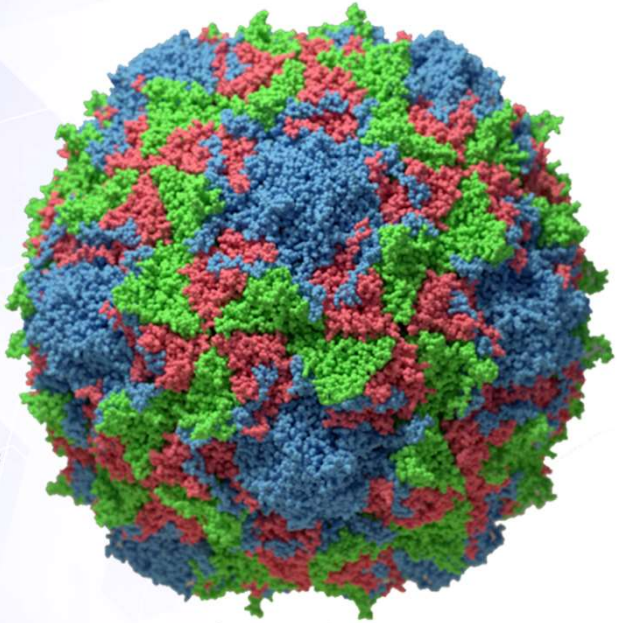
Poliovirus

Picornaviridae

(+) ss RNA genome

~7500 nucleotides

30 nm



3 serotypes:

Poliovirus 1 (PV1)
Poliovirus 2 (PV2)
Poliovirus 3 (PV3)

Wild poliovirus (WPV)

Circulating Vaccine derived poliovirus (cVDPV)

Image: www.virology.ws


polio
global
eradication
initiative

Polio vaccines

Inactivated polio vaccine (IPV)

- Jonas Salk, 1955
- Contains inactivated viruses of all 3 serotypes
- Prevents disease
- More expensive
- Injectable, requires skilled personnel

The "Bilthoven Unit," designed by the Dutch microbiologists van Hemert (pictured) and van Wezel, generated large quantities of poliovirus for vaccine production in the 1960s.

Oral polio vaccine (OPV)

- Albert Sabin, 1963
- Contains attenuated viruses of all 3 serotypes
- Prevents disease
- Cheaper
- Easily administered, passive immunization

Oral polio vaccination with attenuated virus

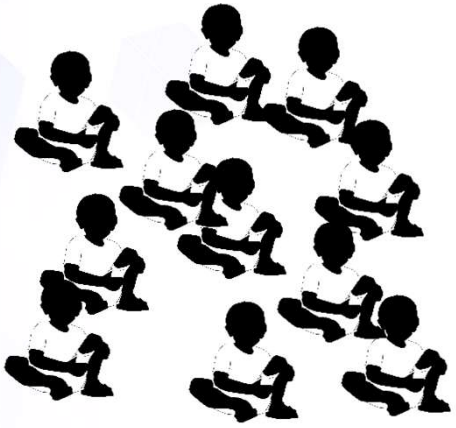
replication in the intestine

Vaccination of naïve individual

Immunized individual

Oral polio vaccination with attenuated virus

Vaccinated individual



Excretion and spread to others in close contact

Passive immunization

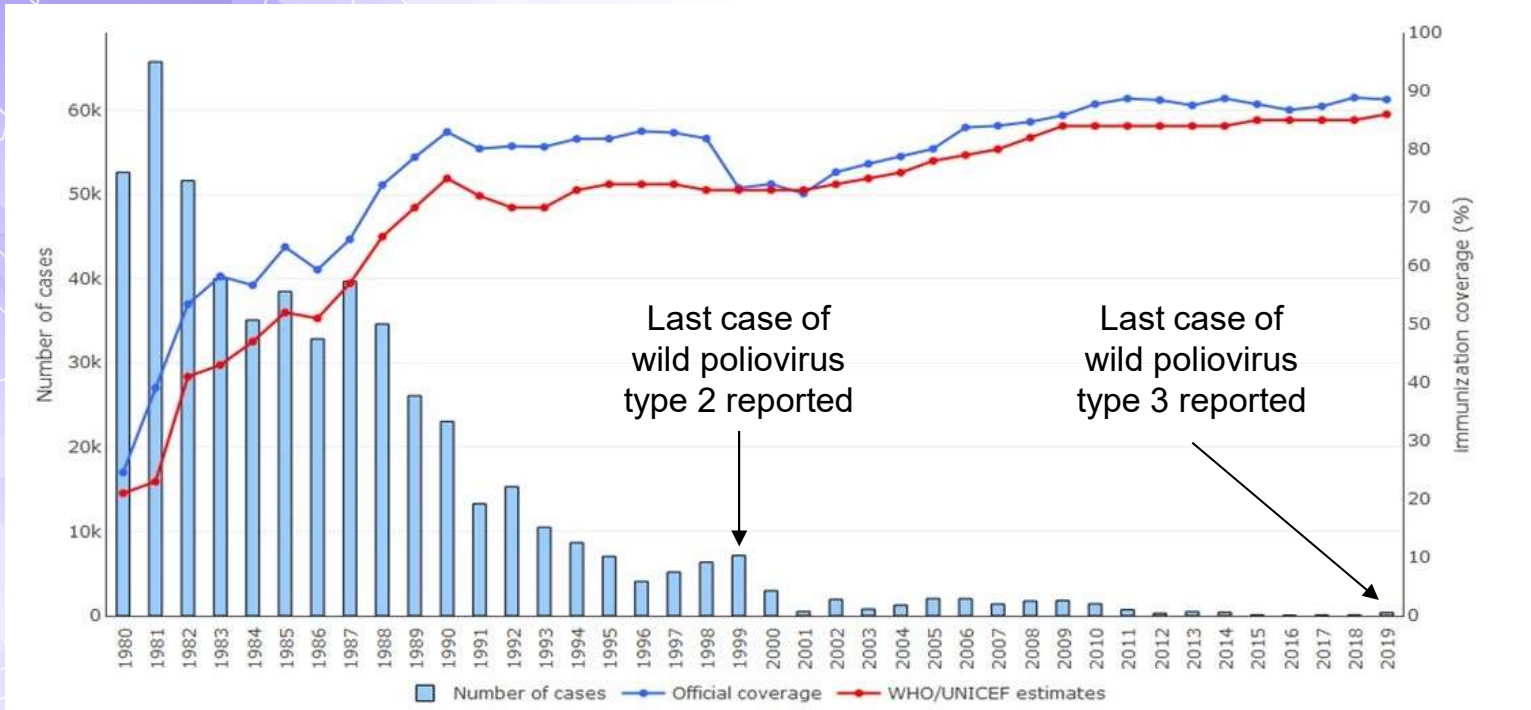
Circulating vaccine-derived poliovirus

community with insufficient vaccine coverage

Prolonged circulation and spread, reacquired neurovirulence

Vaccine-derived poliovirus outbreak

Poliomyelitis Global annual reported cases



Source: www.who.int

Polio eradication

2019

Two out of three wild poliovirus strains eradicated

Global eradication of wild poliovirus type 3 declared on World Polio Day

24 October 2019

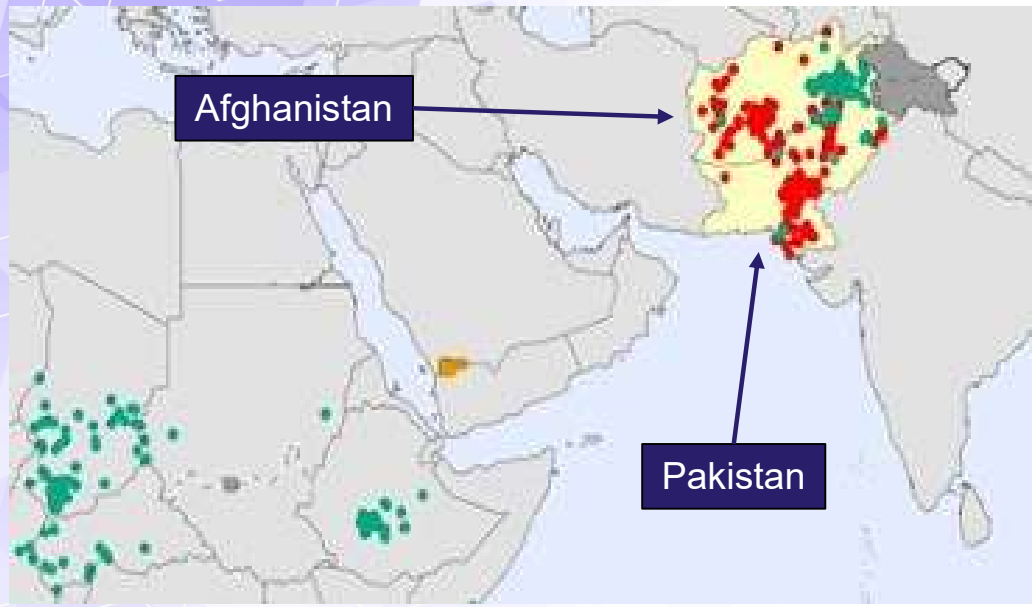
In an historic announcement on World Polio Day, an independent commission of experts concluded that wild poliovirus type 3 (WPV3) has been eradicated worldwide. Following the eradication of smallpox and wild poliovirus type 2, this news represents a historic achievement for humanity.

2020

Africa Kicks Out Wild Polio

On August 25, 2020, the African Region was officially certified as wild poliovirus-free. The Centers for Disease Control and Prevention (CDC) has served as the lead implementing partner for U.S. efforts to eradicate polio, playing a pivotal role for three decades in helping African countries and the continent reach this milestone. The goal of our work has been to protect all children from the dangerous, sometimes deadly, yet entirely vaccine-preventable consequences of polio transmission and outbreaks.

Risks and challenges for polio eradication



Spread of wild poliovirus from endemic countries

- WPV1 cases
- cVDPV2 cases

Risks and challenges for polio eradication

Vaccine derived poliovirus outbreaks

- Lack of basic health infrastructure that limits vaccine distribution
- Neglect of polio vaccination programs

cVDPV1 cases

cVDPV2 cases

Risks and challenges for polio eradication

Laboratory escapes

Belgium: Live polio virus solution accidentally released into local water

by ROBERT HERRIMAN

September 12, 2014 Europe 19 Comments

Polio virus escaped twice from laboratories

TNN | Oct 6, 2004, 01:25 IST

HEALTH

POLIO VIRUS FOUND IN SEWAGE AT PUBLIC HEALTH INSTITUTE

By Janene Pieters on Wednesday, 2 September 2020 - 11:50

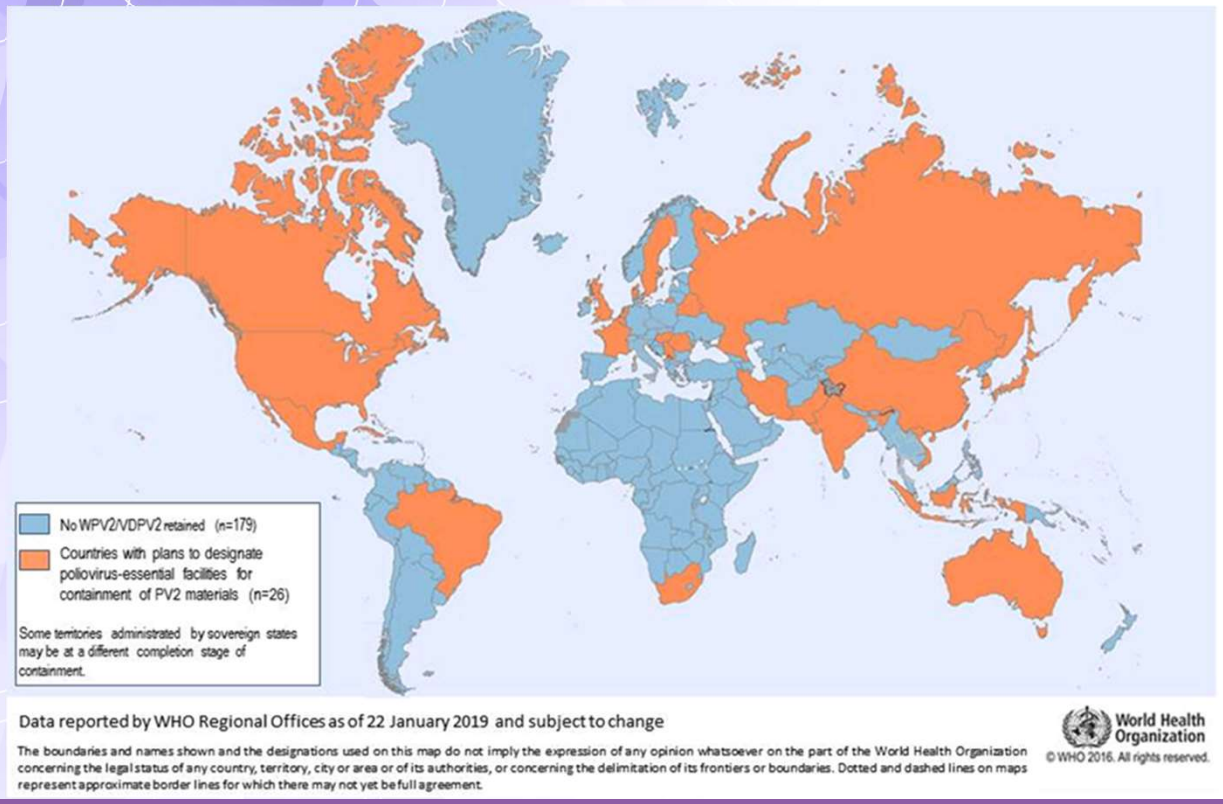
Employee vaccine manufacturer infected with polio virus

Publication date 05/02/2017 - 00:00

GAP III

WHO Global Action Plan to minimize poliovirus facility-associated risk after type-specific eradication of wild polioviruses and sequential cessation of oral polio vaccine use.

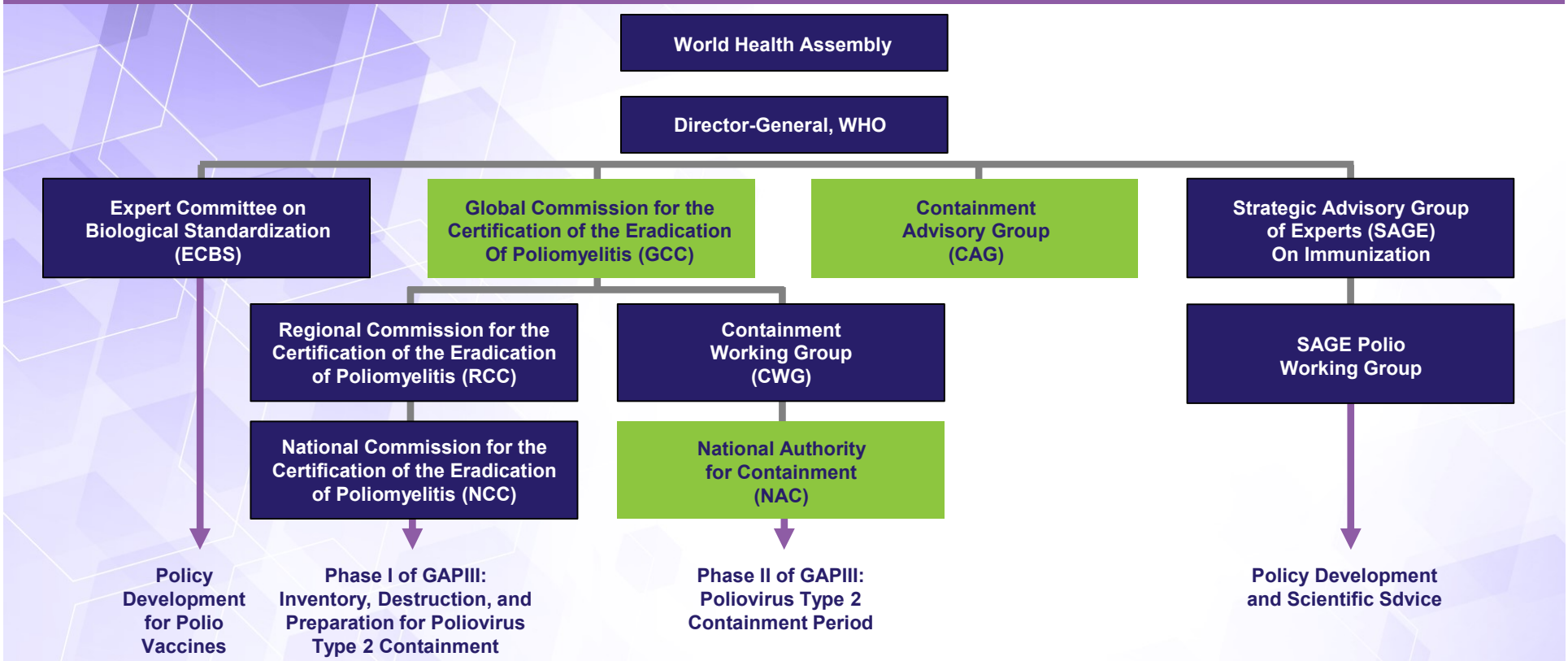
Poliovirus type 2, type 3 materials



26 countries plan to retain poliovirus type 2/3 materials in 78 designated poliovirus-essential facilities (PEFs)

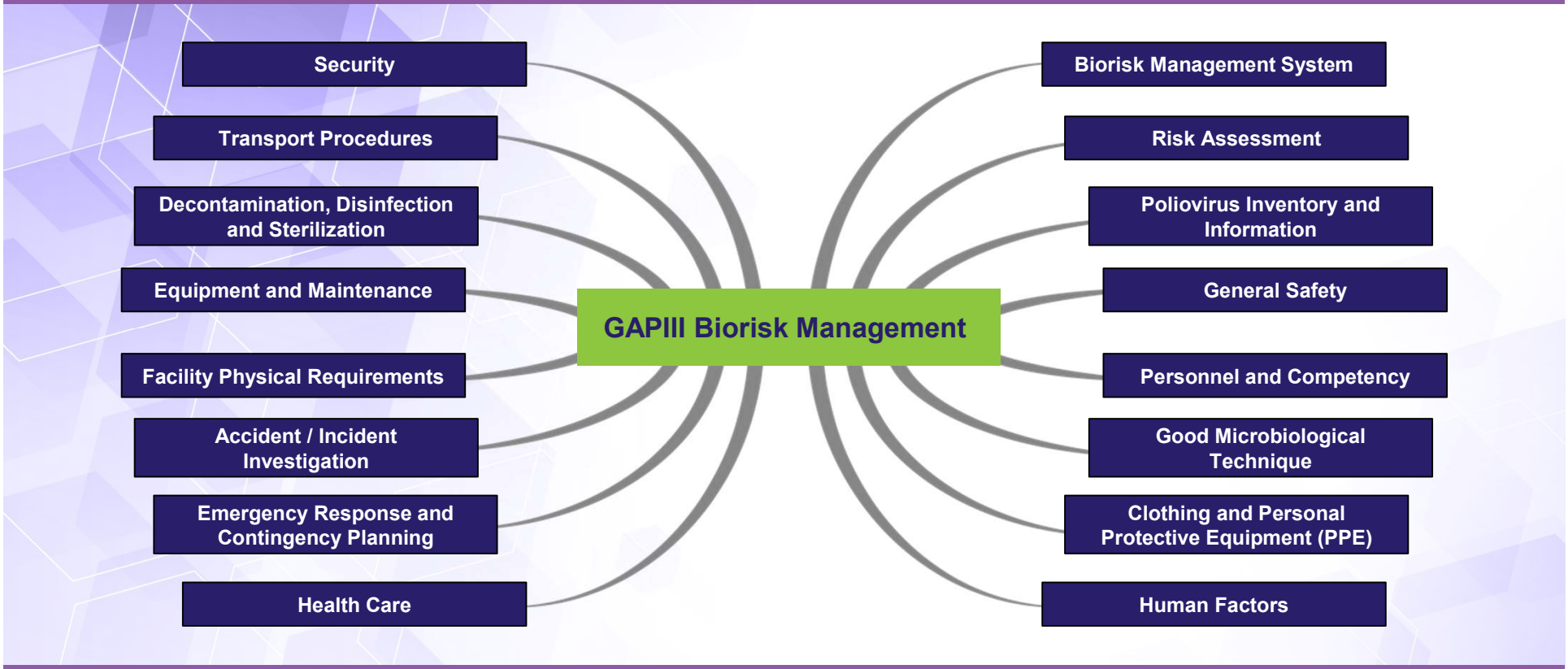
Viroclinics Xplore has established a GAP Ⅲ Polio Certificate of Participation

Containment



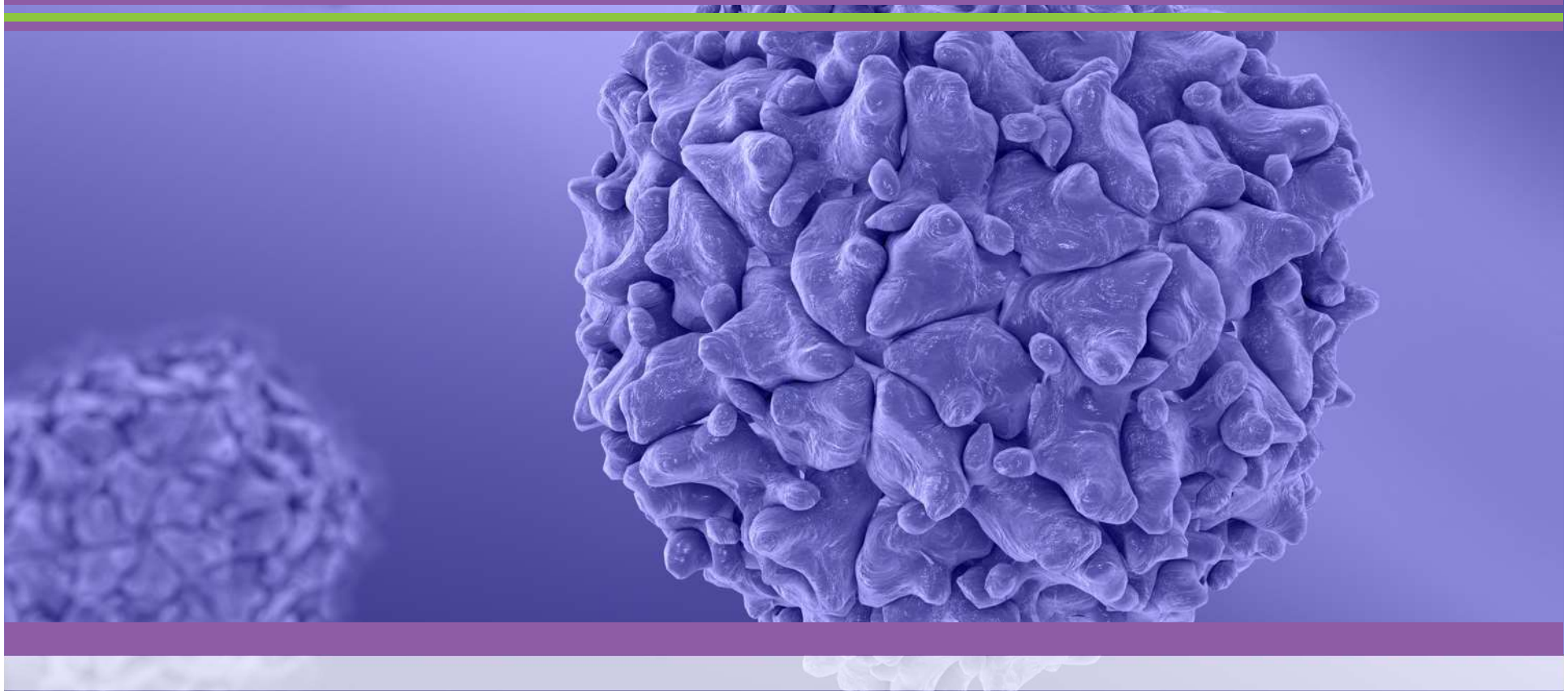
Adapted: World Health Organization 2019

BioRisk Management



Adapted: World Health Organization 2019

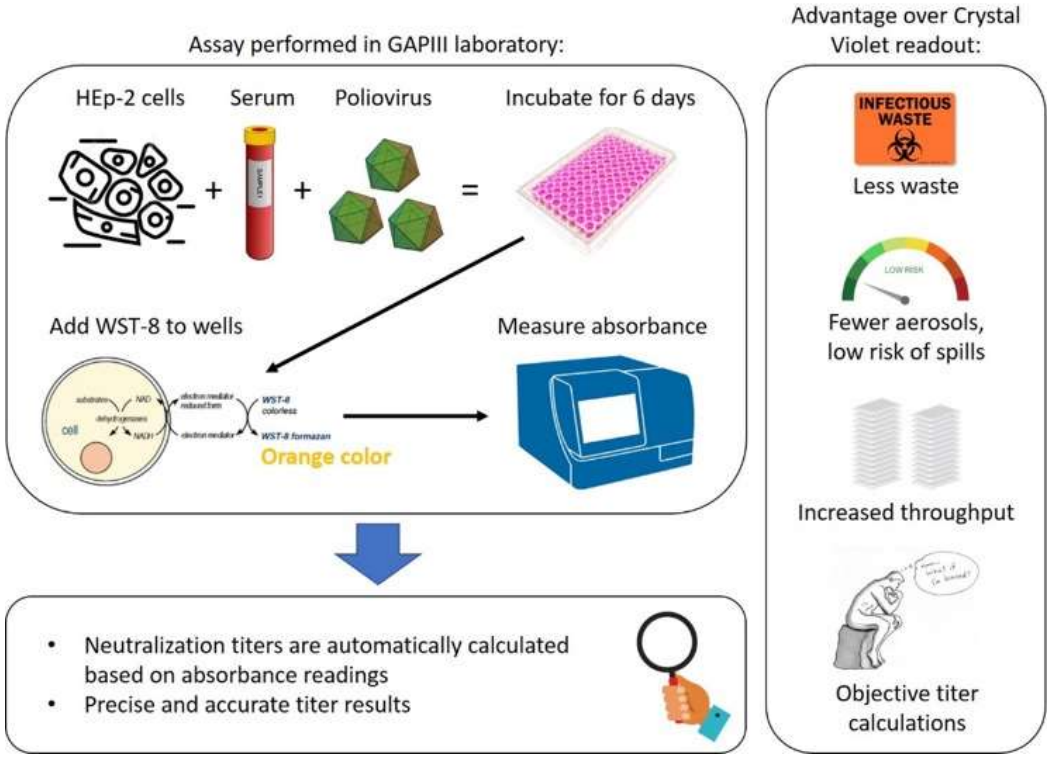
Analytical assays in a Polio High Containment Facility



Assays

- Virus neutralization
- Quantitative virus detection
- Preclinical testing
- Neurovirulence testing
- Deep sequencing

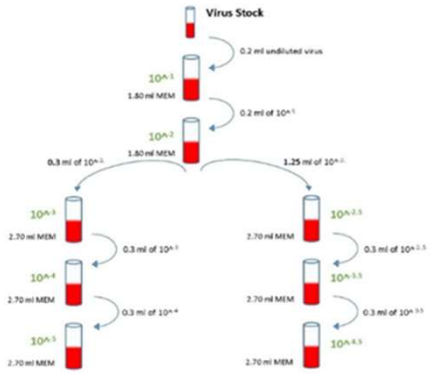
Virus neutralization



Titration in cell culture and preparation of inocula

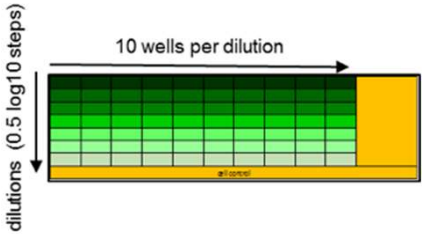
1. Dilution of samples

1 log10 and 0.5 log10 dilution steps



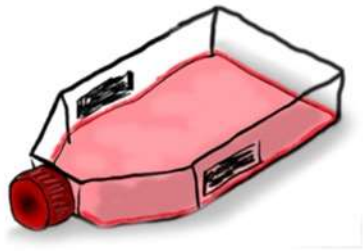
2. Transfer of dilutions

To 96-well plate



3. Addition of Hep2 cells

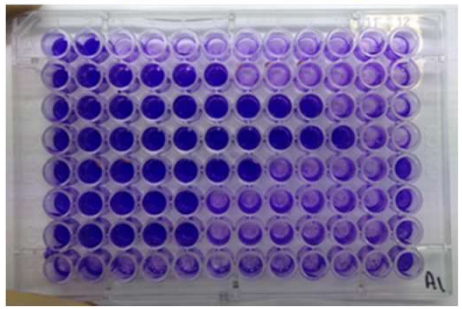
To 96-well plate



4. Incubate

- At 35˚C, without CO_2
- Using sealing films for 96-well plates
- Incubate for 6 days

Crystal violet staining



WST-8

Outlined in the WHO Recommendations to assure the Quality, Safety and Efficacy of Live Attenuated Poliomyelitis Vaccine

Mouse Model for Neurovirulence Testing

TgPVR21 mouse line

CIEA
SINCE 1952
CENTRAL INSTITUTE FOR EXPERIMENTAL ANIMALS
3-25-12 Tonomachi, Kawasaki-ku, Kawasaki, 210-0821 JAPAN
Phone: +81-44-201-8510 Fax:+81-44-201-8511

Certificate

Viroclinics Biosciences
Marconistraat 16
3029 AK Rotterdam
The Netherlands

Date : 26th September, 2016

Central Institute of Experimental Animals (CIEA) for WHO has an obligation to provide suggestions for appropriate maintenance and use of TgPVR mice to prevent the escape of TgPVR mice as a possible Porio virus carrier. Especially, for a new facility (country) to start maintenance of TgPVR mice, CIEA must check a sketch map and pictures of animal facility and Standard Operating Procedure (SOP) before using TgPVR mice.

According to the World Health Organization (WHO) guideline reported in November, 1992, the inspection of your animal facility (Landerd Campus: Nistelrooise baan 3, 5374 RE Schaijk,The Netherlands) has been performed. For the inspection, the following 4 major points of WHO guideline, 1) Animal facility, 2) Rearing cage, 3) Rack, 4) Care and management of TgPVR mice were examined.

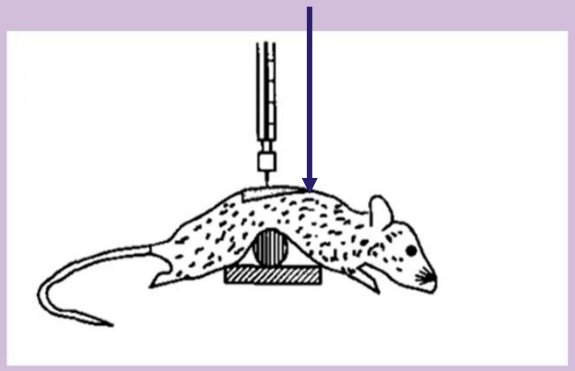
As the result of the inspection, we approved that your animal facility was appropriate for maintenance and use of TgPVR mice.

Sincerely yours,

Ryuta Nomura,
Executive Director
Central Institute for Experimental Animals

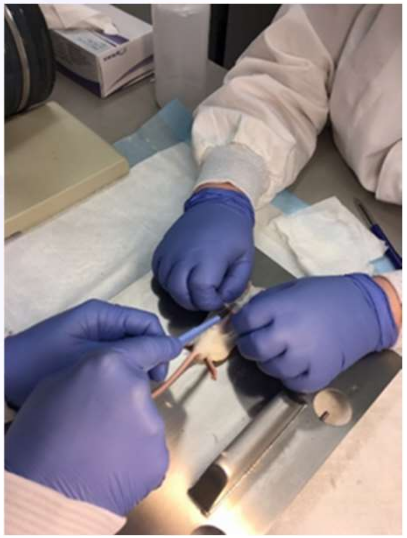
Neurovirulence Test

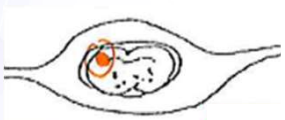
Needle inserted between the spinous process and the first lumbar vertebrae



Stages	Physical signes		
Normal	Grips the edge of the cage	Walks normally on the grid and on a flat surface	Full ability to move limbs forward
Weak	Unable to grip the edge of the cage	Walks normally on the grid and on a flat surface	Full ability to move limbs forward
Paresis/ Partial paralysis	Unable to grip the edge of the cage	Limbs fall through the grid more than once while walking and toes curl repeatedly while walking on a flat surface	At least a partial to move limb forward
Paralysis	Unable to grip the edge of the cage	No use of limb on grid or flat surface	Inability to move the limb forward

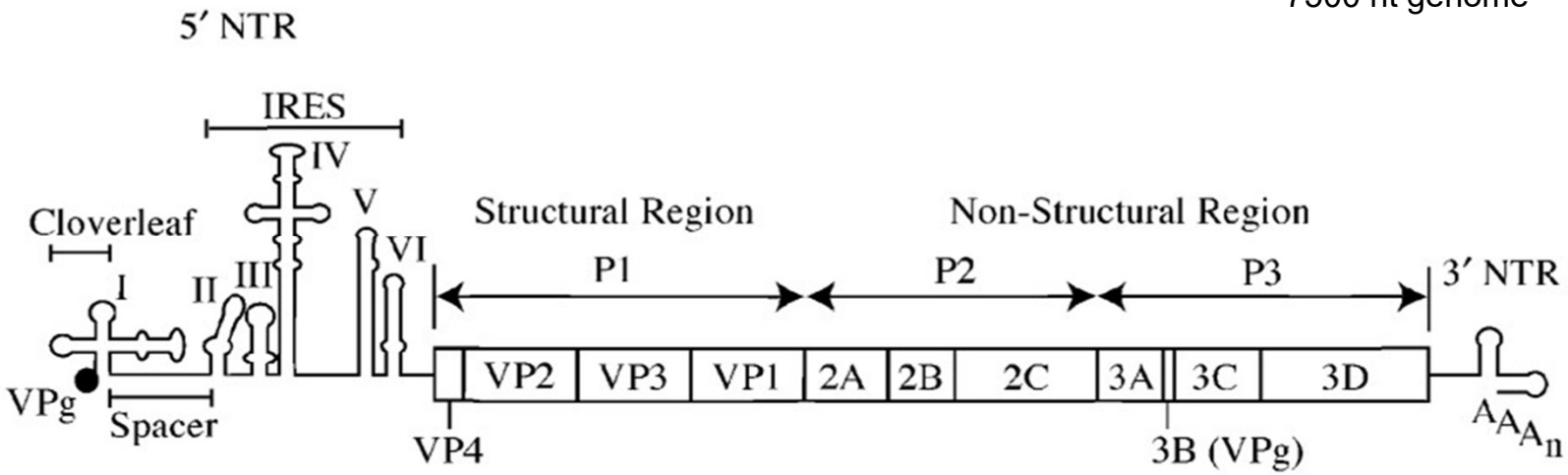
Intraspinal Injection Training with Indian Ink





Poliovirus Genome

- (+) ssRNA virus
- ~7500 nt genome



Wikipedia: “Poliovirus”

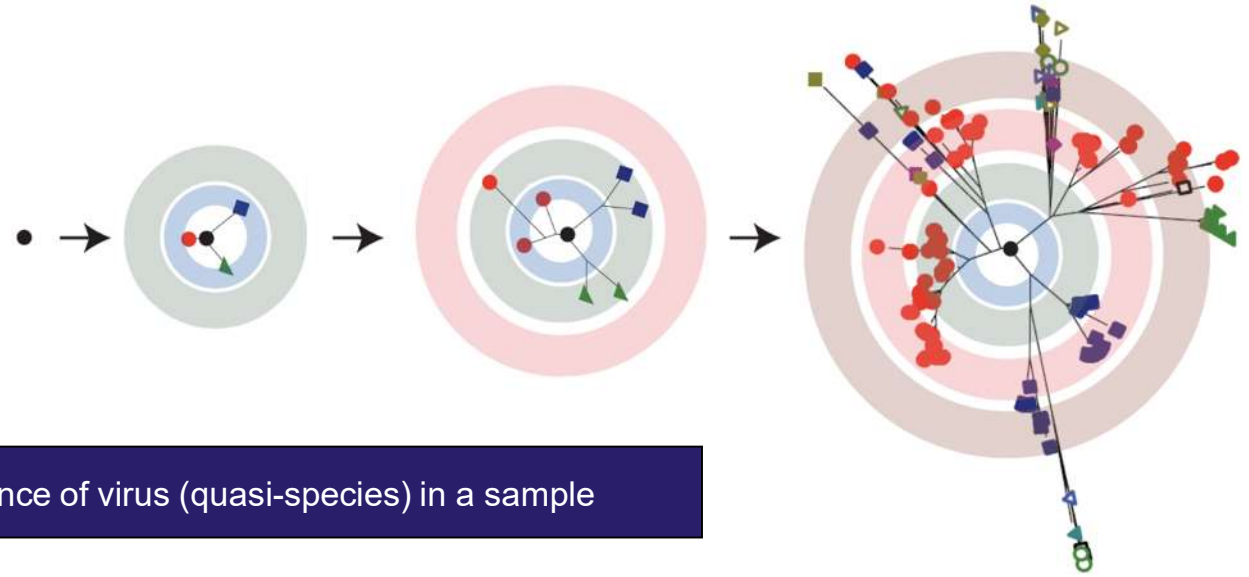
Quasi-Species

Polymerase (RdRP, $3D^{pol}$) lacks proofreading activity

- Mutations
- Recombination

→ Evolution
→ Pathogenesis
→ Escape mutations / treatment resistance

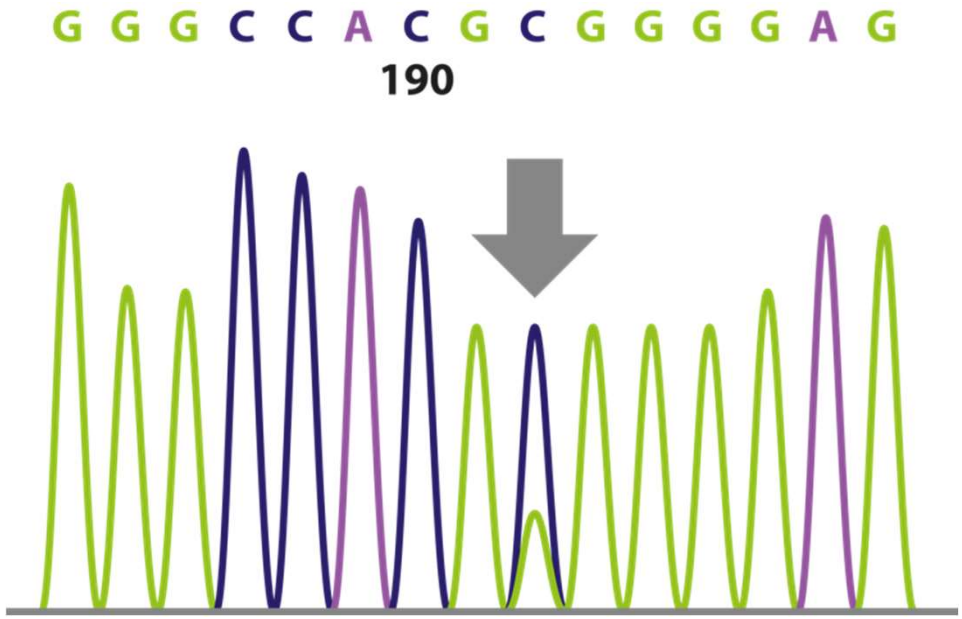
'Quasi-species'



Determine sequence of virus (quasi-species) in a sample

Lauring & Andino, PLoS Path, 2010

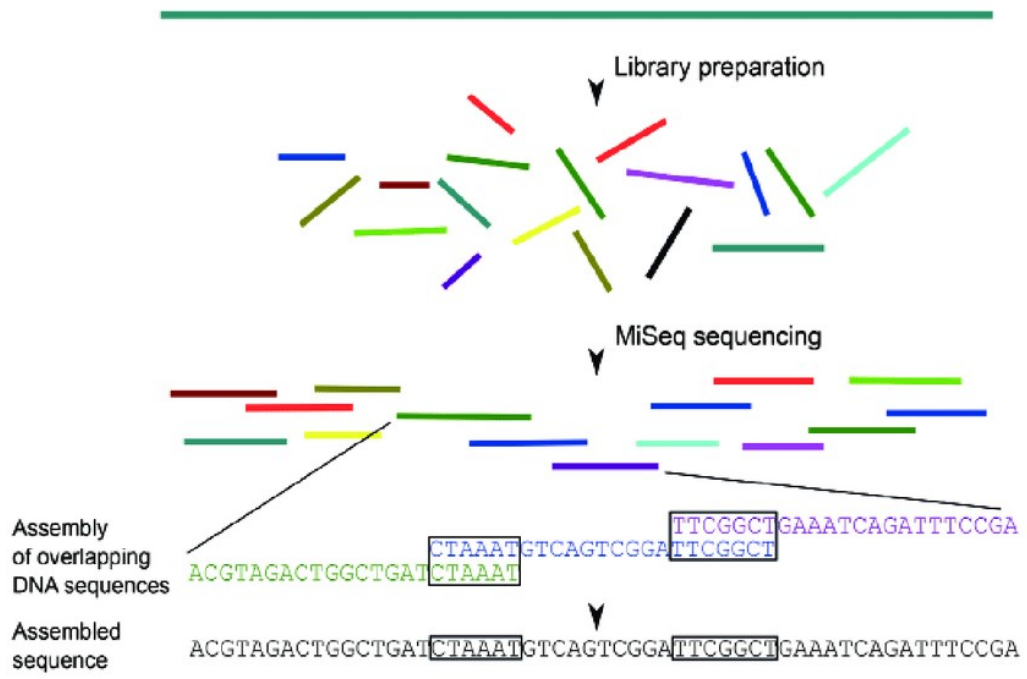
Sequencing Techniques: 1. Sanger Sequencing



- Determine consensus sequence
  → average of all genomes in a pool
- (sensitive and quantitative) determination of variants or mutations **not possible**

Sequencing Techniques: 2. Next-Generation Sequencing (Illumina Technology)

- Sequence **thousands** of genomes **individually** per run
  → Deep sequencing
- Most common base at each position
  → Consensus sequence
- Differences between individual genomes
  → Variant

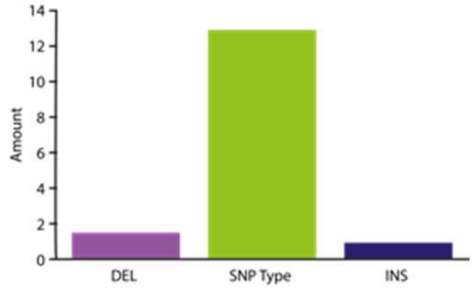


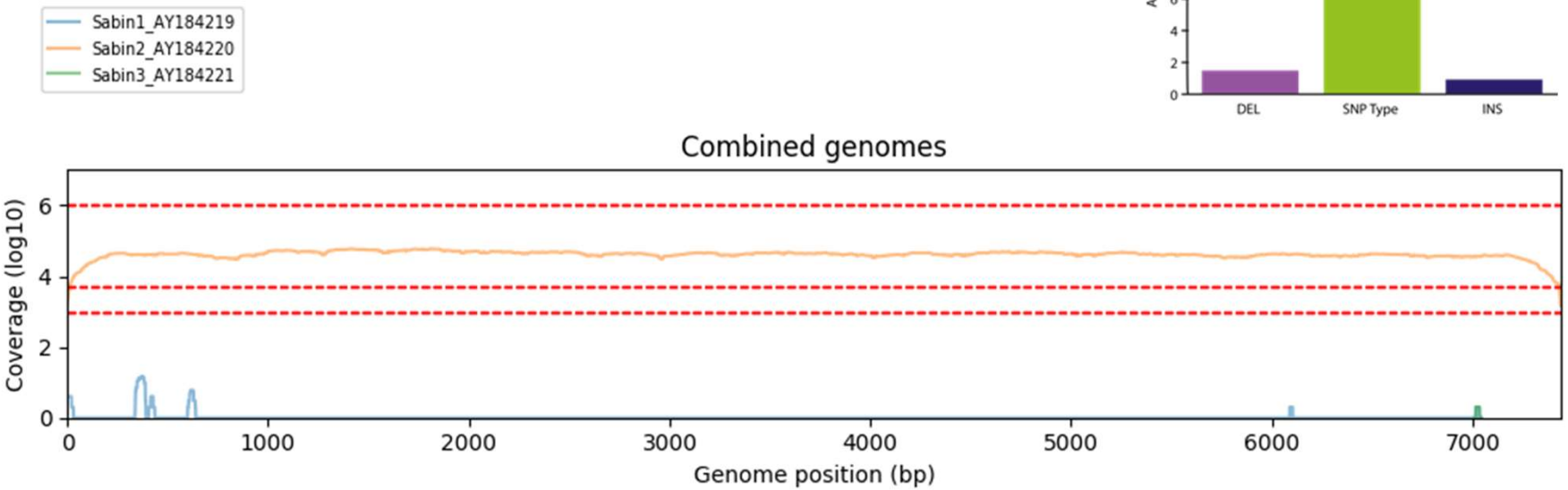
Poliovirus Next-Generation Sequencing at Viroclinics

- Samples - Clinical samples (e.g. stool)
  - Cell culture isolates
  - Vaccine lots
- Lysis → in GAPIII facility
- RNA isolation, cDNA synthesis & whole genome amplification
- Library preparation
- Illumina sequencing
- Data analysis using in-house pipeline

Results

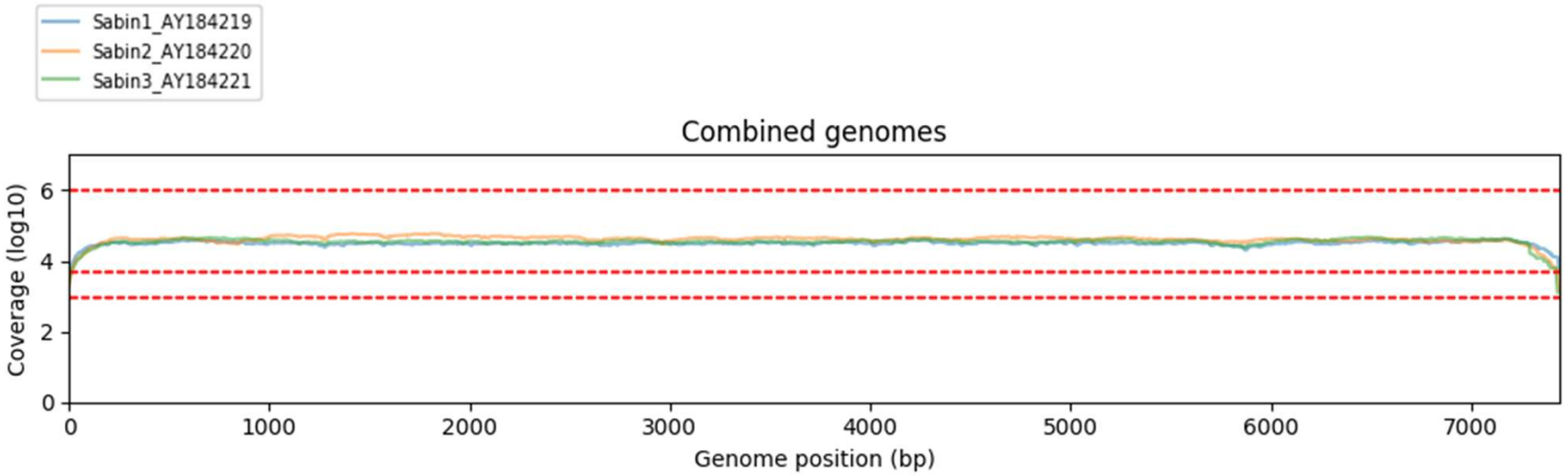
- **Mapping to reference (single/multiple references)**
- **Variant calling**





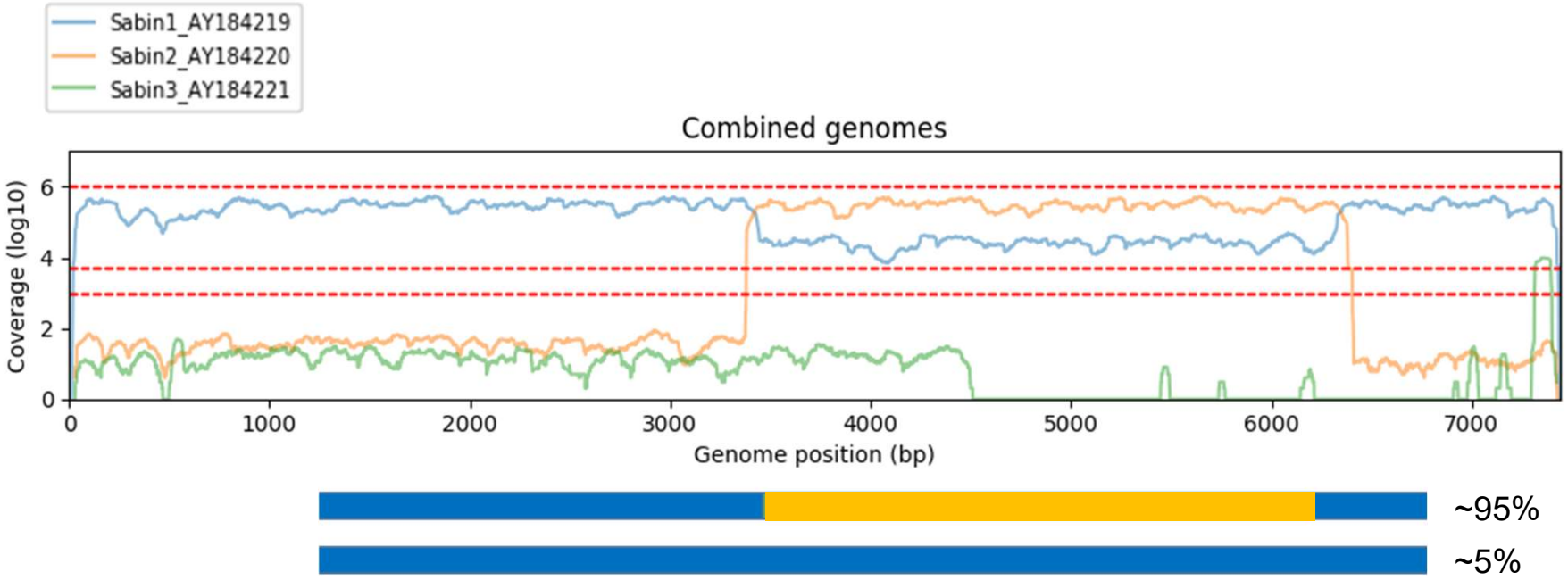
Results (Complex Samples)

Mixed samples:



Results (Complex Samples)

Recombinants between types:



Our Track Record

Post Study Sample Storage

Sample integrity guaranteed with back up systems and 24/7 security and remote monitoring

Polio High Containment Facility

- Virus titration/quantification using fully validated CCID50 protocols
- Neurovirulence testing through a certified and validated assay in transgenic mice (TgPVR21)
- Virus neutralization fully validated on 6 strains
- Transgenic mouse model available for preclinical testing
- Mutational analysis of (whole genome) poliovirus through Next Generation Sequencing
- Top class logistical services to get the samples to our facility
- Storage of samples and (vaccine) virus stocks
- Research and Development

18,000 ft^2 Preclinical Laboratory Area

Viroclinics contributes to polio eradication by offering its services to Vaccine manufacturers

Questions?

Contact:
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Consultant BD
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For additional info, please visit:
www.viroclinics.com

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DDL Diagnostic Laboratory

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