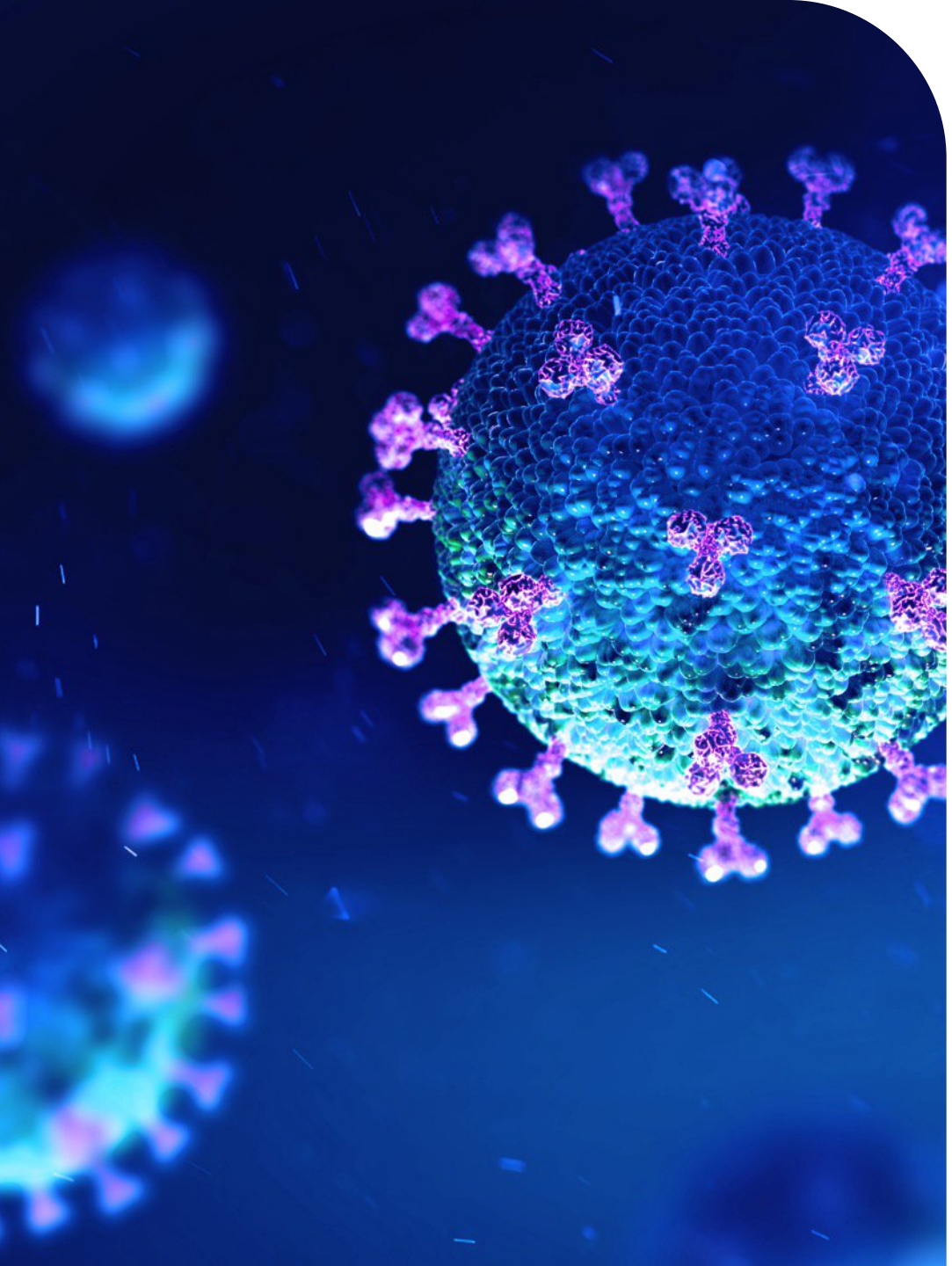


Cerba Research

Tackling Tomorrow's Threats: Innovation to address emerging pandemic potential influenza and re-emerging poliovirus

Leon de Waal, PhD, Cerba Research

World Vaccine Congress Washington 2026

How can Cerba Research support Vaccine Research?

Contract Research

Assay Development / Validation

Pre-Clinical Services

Non-Clinical Efficacy (Animal rule)

Global Sample Logistics

Specialty Central Virology Laboratory Testing, including: virus culture, virus titration, microneutralization, genotyping, phenotyping, sequencing, molecular diagnostics, serology, molecular biology and molecular pathology

A global speciality and centralized laboratory service provider with more than 50 years experience.

Specialty services in **Virology** & **Immunology** through its specialty BU Cerba Research in the Netherlands.

One stop shop combining routine and specialty testing for intervention strategies against wide range of targets.

Ranging from exploratory/pre-clinical testing to support of Phase III studies and Global Logistics.

Our Virology and Vaccine Center of Excellence

Rijswijk

- Sequencing Center of Excellence
- Next Generation Sequencing, Long Read Sequencing
- Bio-informatics
- FDA compatible data analysis pipelines
- Multiple isolation and PCR platforms
- Real Time qPCR
- ddPCR

Schaijk

- Preclinical facility for SPF, Conventional, BSL2 and BSL3(+)
- Wide Range of different preclinical animal models (wild-type and GMO)
- Solutions for any kind of experimental set up and early development
- Dedicated Team of Scientists and Study Directors
- Polio High Containment Facility (GAPIV) for clinical sample testing

Rotterdam

- Clinical Trial Support for Infectious Diseases
- Virology Laboratory Services
- Immunology & Biomarker Analysis
- Custom Assay Development & Validation
- Biobanking & Logistics
- Dedicated team of Scientists and Consultants

CUTTING EDGE LAB

BSL 2 BSL 3

State-of-the-Art Facilities

Cerba Research

Influenza virus

Emerging pandemic potential influenza virus

Influenza virus

Family *Orthomyxoviridae*

80-120nM particle

Single-stranded segmented -RNA genome

8 gene segments encoding 10 major proteins

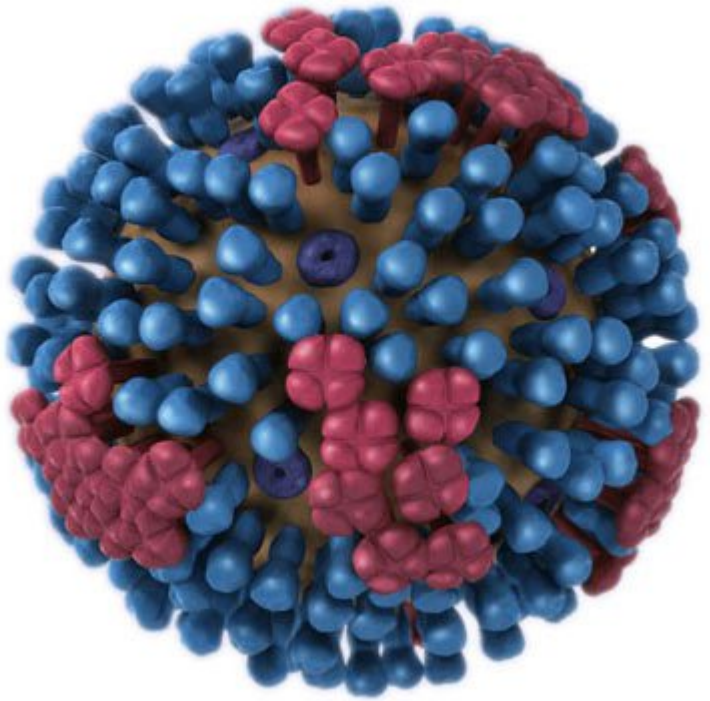
4 genotypes:

Influenza A virus

Influenza B virus

Influenza C virus

Influenza D virus



Source: www.cdc.gov

Influenza virus: pathogenesis

Seasonal influenza virus:

Antigenic drift => yearly update of seasonal vaccine

Symptomatic infection: mild / upper respiratory tract infectionpneumonia

Estimated CFR: <0,1%

Pandemic potential influenza virus

Antigenic shift => no pre-existing immunity / zoonotic

Symptomatic infection:

mild / upper respiratory tract infection

pneumonia

Estimated CFR: varies, but can go up to >45% (H5N1)

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Influenza virus: pandemics

Spanish Flu (H1N1) – 1918-1919

Asian Flu (H2N2) – 1957-1958

Hong Kong Flu (H3N2) – 1968-1969

Swine Flu (pH1N1) – 2009-2010

What's next?

Cumulative number of confirmed human cases† for avian influenza A(H5N1) reported to WHO, 2003-2026

Country	2003-2009*		2010-2014*		2015-2019*		2020-2024*		2025		2026		Total	
	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths	cases	deaths
Australia	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Azerbaijan	8	5	0	0	0	0	0	0	0	0	0	0	8	5
Bangladesh	1	0	6	1	1	0	0	0	3	0	0	0	11	1
Cambodia	9	7	47	30	0	0	16	6	18	9	0	0	90	52
Canada	0	0	1	1	0	0	1	0		0	0	0	2	1
Chile	0	0	0	0	0	0	1	0	0	0	0	0	1	0
China	38	25	9	5	6	1	3	1	1	0	0	0	57	32
Djibouti	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Ecuador	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Egypt	90	27	120	50	149	43	0	0	0	0	0	0	359	120
India	0	0	0	0	0	0	1	1	2	2	0	0	3	3
Indonesia	162	134	35	31	3	3	0	0	0	0	0	0	200	168
Iraq	3	2	0	0	0	0	0	0	0	0	0	0	3	2
Lao People's Democratic Republic	2	2	0	0	0	0	1	0		0	0	0	3	2
Mexico	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Myanmar	1	0	0	0	0	0	0	0	0	0	0	0	1	0
Nepal	0	0	0	0	1	1	0	0	0	0	0	0	1	1
Nigeria	1	1	0	0	0	0	0	0	0	0	0	0	1	1
Pakistan	3	1	0	0	0	0	0	0	0	0	0	0	3	1
Spain	0	0	0	0	0	0	2	0	0	0	0	0	2	0
Thailand	25	17	0	0	0	0	0	0	0	0	0	0	25	17
Turkey	12	4	0	0	0	0	0	0	0	0	0	0	12	4
United Kingdom of Great Britain and Northern Ireland	0	0	0	0	0	0	5	0	1	0	0	0	6	0
United States of America**	0	0	0	0	0	0	68	1	3	0	0	0	71	1
Viet Nam	112	57	15	7	0	0	2	1	1	0	0	0	130	65
Total	**468**	**282**	**233**	**125**	**160**	**48**	**102**	**10**	**30**	**12**	0	0	**993**	**477**

CFR >45%

Source: www.who.int

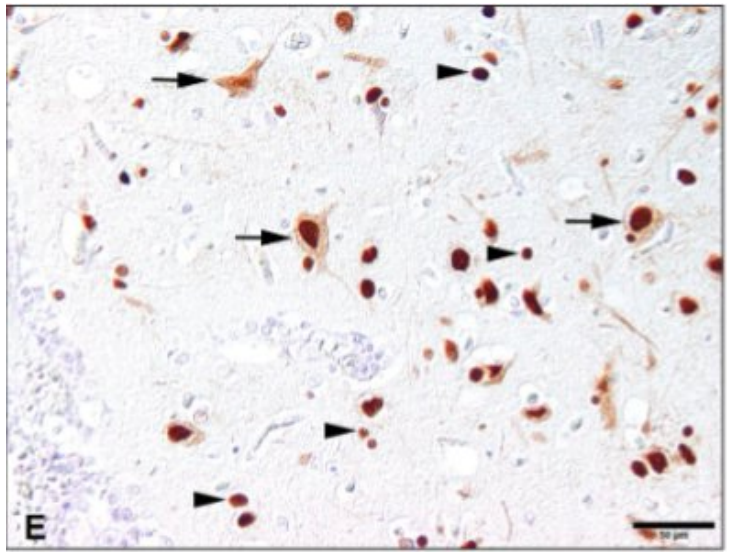
Cerba Research

Influenza virus: what's next?

Highly pathogenic avian influenza A virus (HPAIV) H5N1 infection in two European grey seals (*Halichoerus grypus*) with encephalitis

Monica Mirolo [a], Anne Pohlmann [b], Ann Kathrin Ahrens [b], Bianca Kühl [c], Ana Rubio-Garcìa [d], Katharina Kramer [e], Ulrike Meinfelder[f], Tanja Rosenberger [f], Hannah Leah Morito [a], Martin Beer [b], Martin Ludlow [a], Peter Wohlsein [c], Wolfgang Baumgärtner [c], Timm Harder [b] and Albert Osterhaus [a,d]

[a]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Foundation, Hannover, Germany; [b]Friedrich-Loeffler-Institute, Greifswald-Insel Riems, Germany; [c]Department of Pathology, University of Veterinary Medicine, Foundation, Hannover, Germany; [d]Sealcentre Pieterburen, Pieterburen, Netherlands; [e]Landeslabor Schleswig-Holstein, Neumünster, Germany; [f]Seehundstation Friedrichskoog, Friedrichskoog, Germany



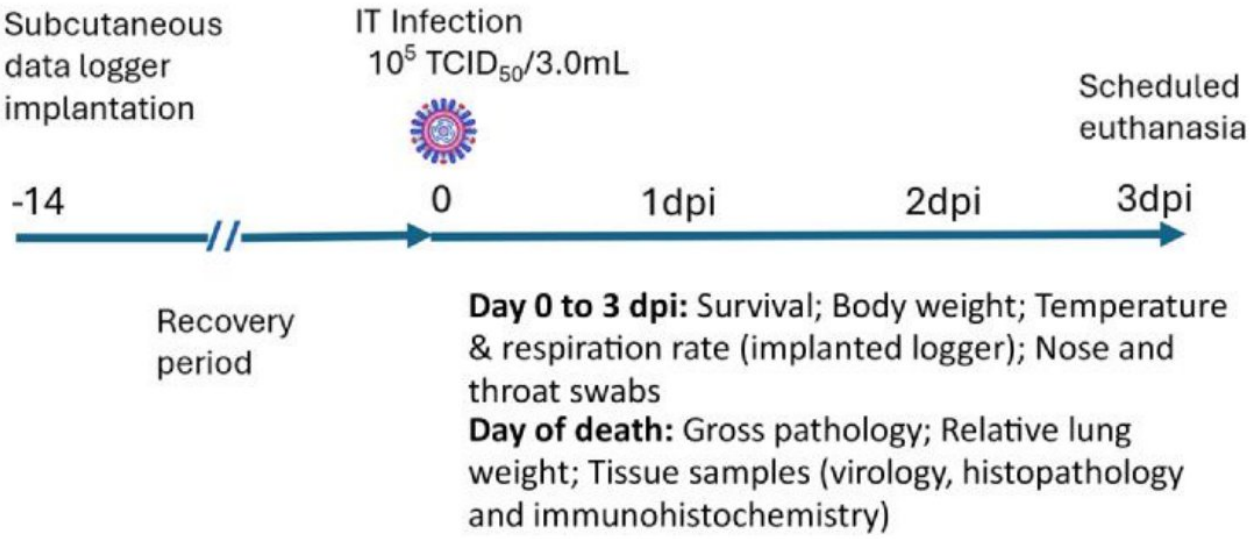
Mirolo et al, Emerg Microbes Infect, 2023

Influenza virus: preclinical model development (design)

Highly Pathogenic Avian Influenza Virus A/H5N1 Subclade 2.3.4.4b Isolated from a European Grey Seal (*Halichoerus grypus*) Is Highly Virulent in Ferrets

Kate Guilfoyle,[1,a] Monica Mirolo,[2,a] Leon de Waal,[1] Geert van Amerongen,[1] Guido van der Net,[1] Theresa Störk,[3] Mara Sophie Lombardo,[3] Wolfgang Baumgärtner,[3] Ásgeir Bjarnason,[4] Hekla Bryndis Jóhannsdóttir,[4] Guus Rimmelzwaan,[2] Martin Ludlow,[2] and Albert Osterhaus[2]

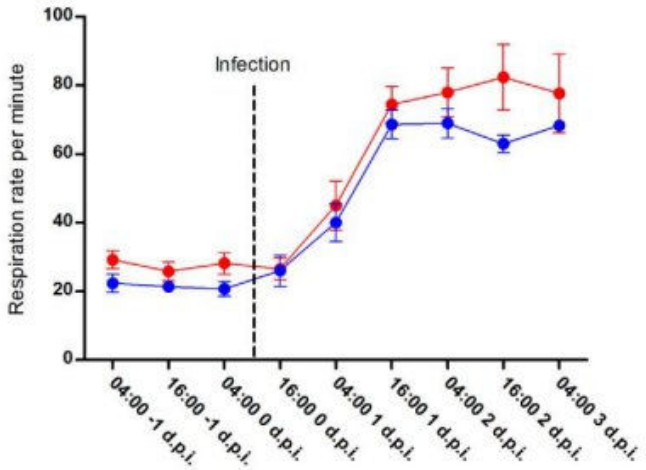
[1]Preclinical Speciality Services, Cerba Research (Formerly Viroclinics Xplore), Schaijk, The Netherlands; [2]Research Center for Emerging Infections and Zoonoses, University of Veterinary Medicine, Hannover, Germany; [3]Department of Pathology, University of Veterinary Medicine, Hannover, Germany; and [4]Star-Oddi, Ltd, Gardabaer, Iceland



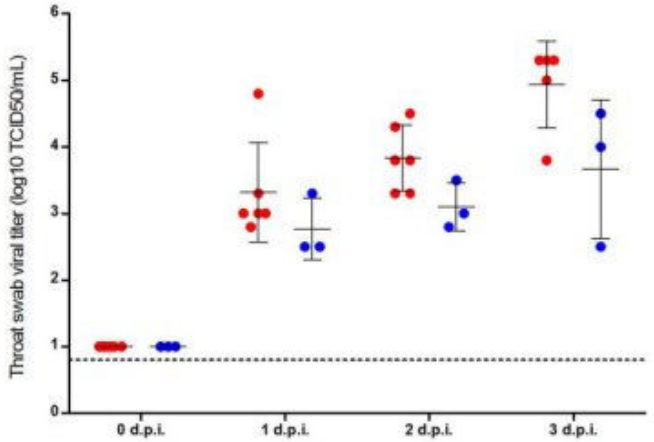
Guilfoyle et al, JID, 2025

Influenza virus: preclinical model development (Results)

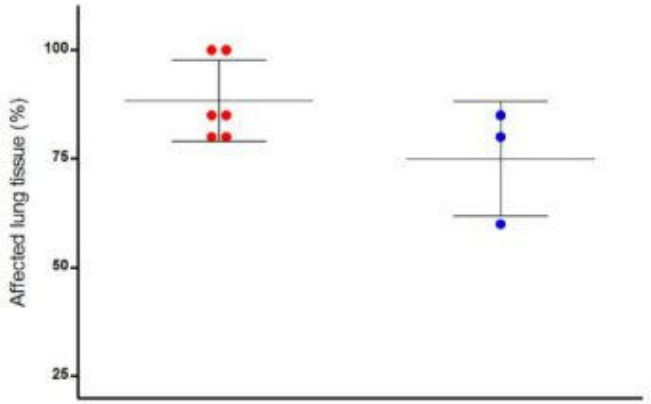
Respiratory rate



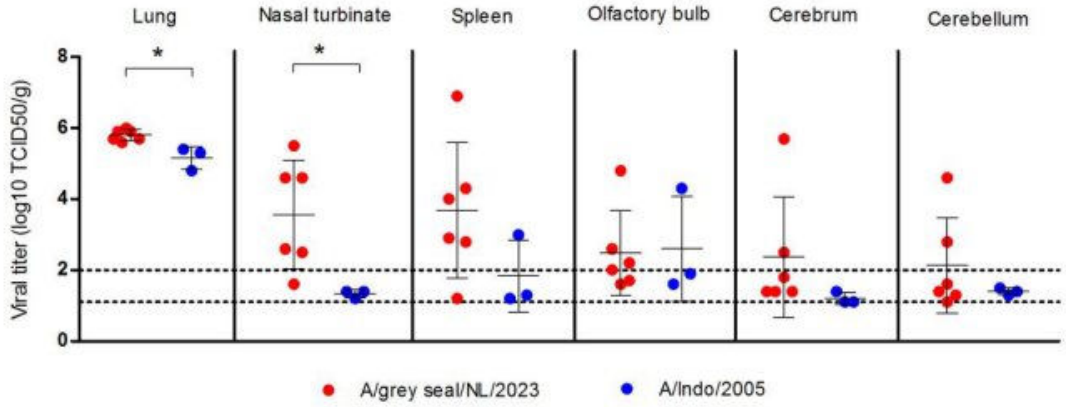
Virology throat



Gross pathology



Virology tissue



Guilfoyle et al, JID, 2025

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Influenza virus: preclinical => clinical development

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Influenza virus: serology

	Microneutralization (MN) Assay	ELISA (Enzyme-Linked Immunosorbent Assay)
Purpose	Measures **functional** virus-neutralizing antibodies	Measures **binding** antibodies (not necessarily functional)
What It Detects	Antibodies that **neutralize live virus** and block infection	Antibodies that **bind to viral antigens** (e.g., spike protein)
Biological System	Live virus + cells (e.g., Vero cells)	**In vitro** only (no live virus required)
Readout	Cytopathic effect, viral replication, or reporter signal, colorimetric/fluorescent signal (OD value)	Colorimetric/fluorescent signal (OD value)
Time Required	2–5 days depending on virus replication	Few hours (rapid)
Biosafety Requirements	Requires BSL-2 or BSL-3 or BSL-4 depending on the virus	BSL-1 or BSL-2
Quantitative?	Yes (e.g., neutralizing titer like NT_{50})	Yes (e.g., antibody concentration, OD or titer)
Functional Relevance	High – reflects protective immunity	Moderate – indicates exposure or immune response

ELISA is better for speed, scale, and early surveillance. MN is better for accuracy and functional immunity.

Both must be used in combination during a pandemic:
ELISA for **screening and monitoring**,
MN for **confirming neutralizing capability** of antibodies.

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Influenza virus: assays for infectivity

Assay Type	Description	Output
Plaque Assay	Visualizes virus-induced cell death (plaques)	PFU/mL
$TCID_{50}$	Estimates dose to infect 50% of cells	Infectious dose (log scale)
Focus Forming Assay	Uses antibodies to stain infected cells	Focus-forming units (FFU)
Endpoint Dilution	Serial dilution to find infection limit	ID_{50} or ID_{90}
Reporter Gene Assay	Detects infection via luminescent or fluorescent reporters	Relative light/fluorescence

Applications:

- Vaccine development
- Antiviral drug screening
- Viral vector quantification
- Quality control in biomanufacturing

Choosing the Right Assay Depends On:

*The **virus** and its ability to cause CPE*

*Whether you're measuring **entry** or **full replication***

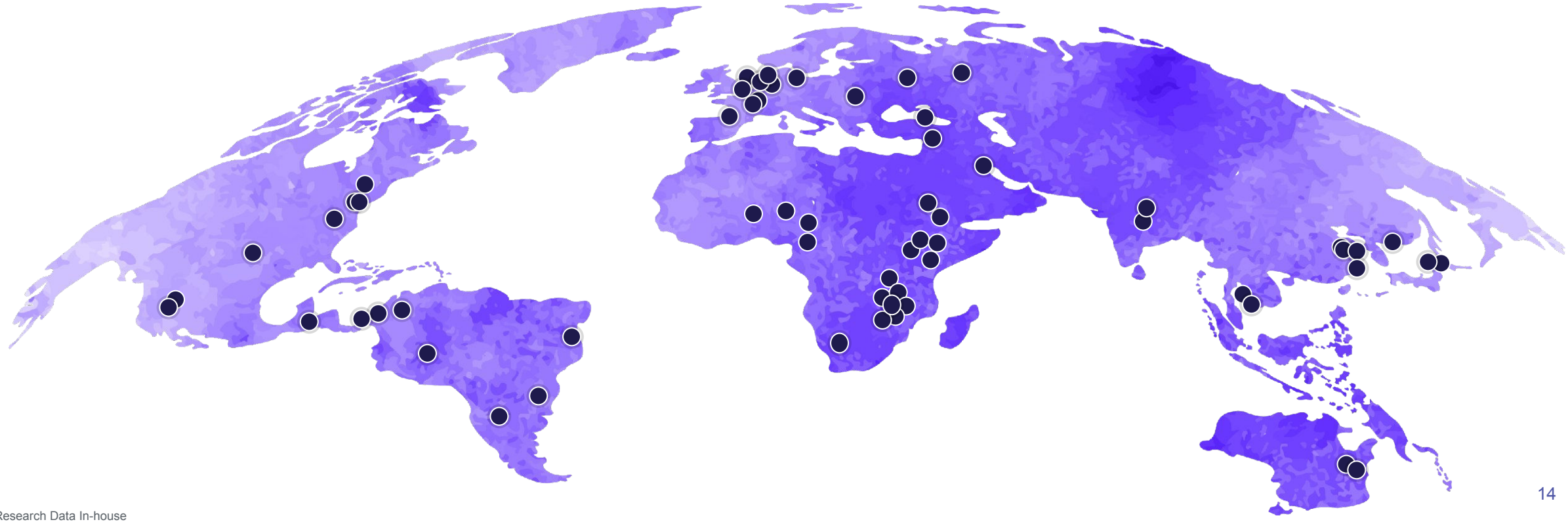
*The **biosafety level (BSL)** of the virus*

*Need for **high-throughput** and/or regulatory **validation**.*

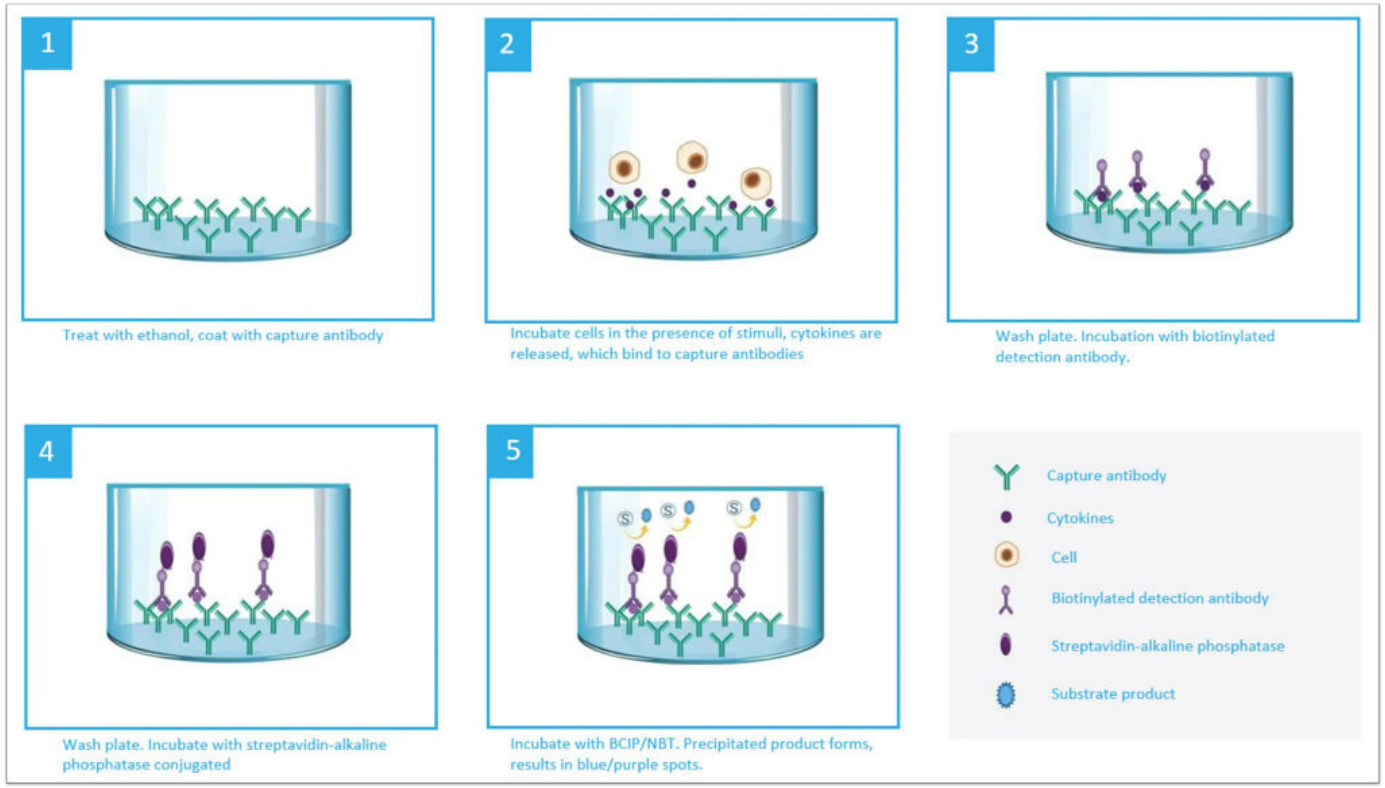
Influenza virus: cell-mediated immunity

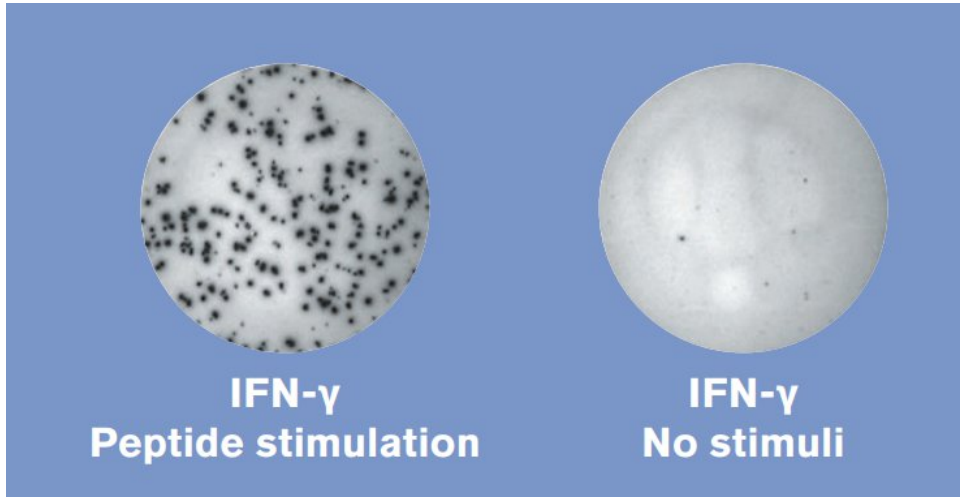
45+ processing labs in America, Europe, Africa, Asia, and Australia

- Premium logistics
- Short turnaround times (8-48Hrs)
- High quality standards
- Extensive network and expanding
- Standardized SOP and on-site training



ELISpot

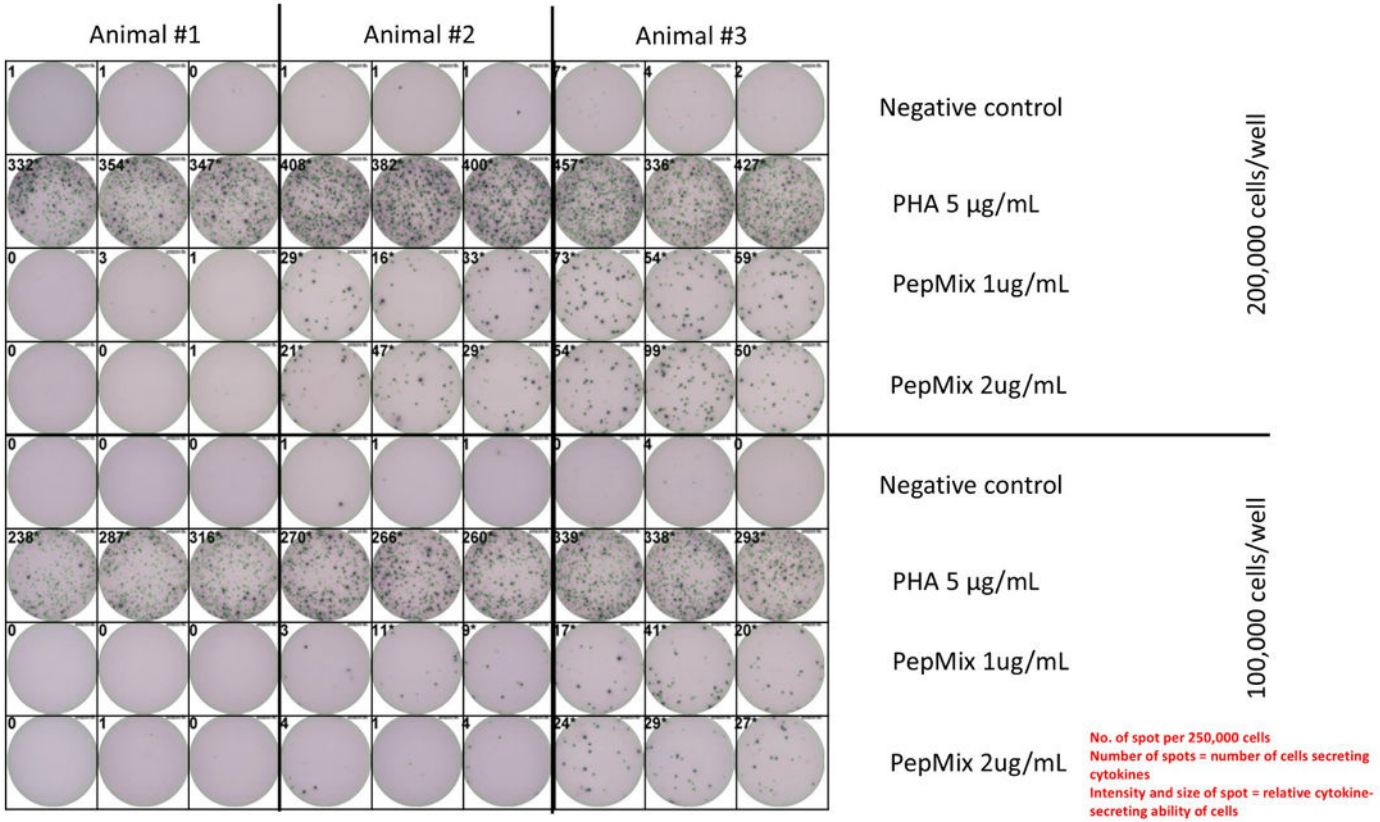


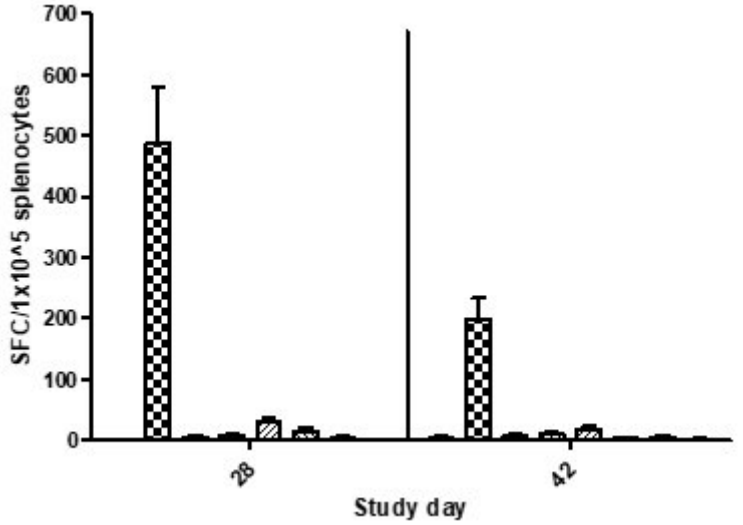


The cytokine ELISpot assay has also become a standard tool in the development and monitoring of new vaccines and vaccine candidates.

ELISpot: Preclinical IFN-γ detection

The aim of this study was to determine the IFN-γ responses in peripheral blood mononuclear cells (PBMCs) following influenza infection in ferrets using ELISpot method.





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Re-emerging poliovirus

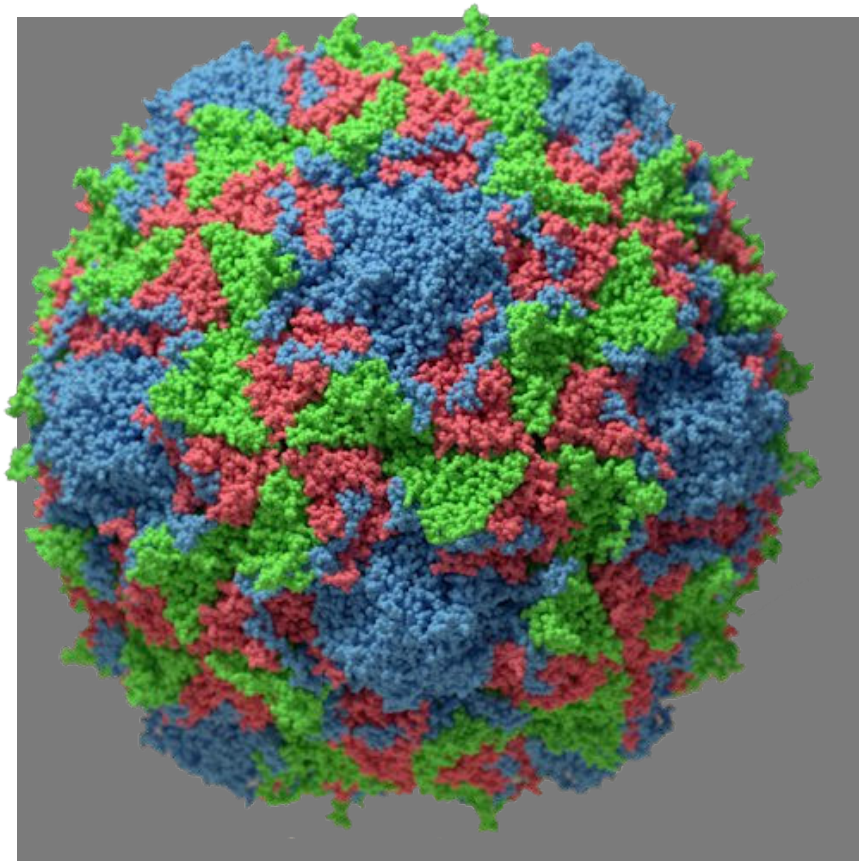
Poliovirus (PV)

Family *Picornaviridae* > Genus *Enterovirus* > Species *Enterovirus Coxsackiepol*

30nM particle

Single-stranded +RNA genome

7.5 kb genome



Source: www.virology.ws

3 serotypes/genotypes:

Poliovirus 1 (PV1)

Poliovirus 2 (PV2)

Poliovirus 3 (PV3)

Polio vaccines

Jonas Salk, 1955

Inactivated viruses of all 3 serotypes

Injected, requires skilled personnel

More expensive

Jonas Salk administering inactivated vaccine (1954)
Source:Jonas Salk - Wikipedia

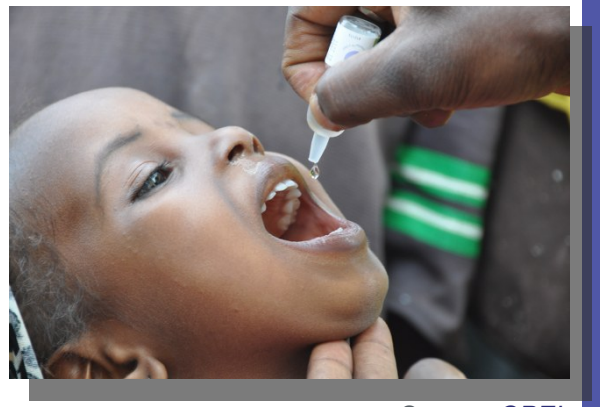
Oral polio vaccine (OPV)

Albert Sabin, 1963

Live-attenuated viruses of all 3 serotypes

Easily administered

Cheaper



Source: GPEI

Albert Sabin administering live-attenuated vaccine
Source:Jonas Salk and Albert Bruce Sabin | Science History Institute

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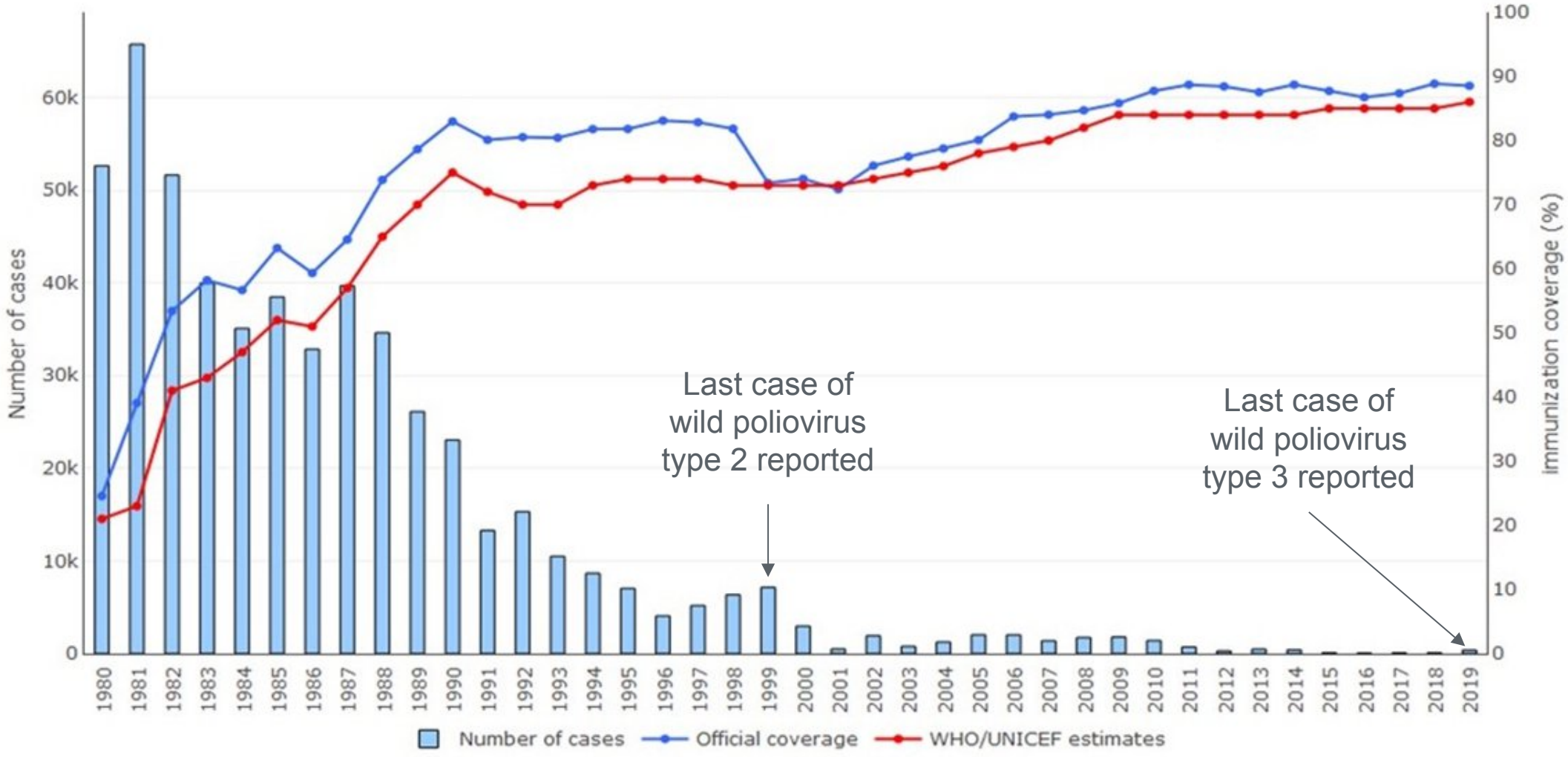
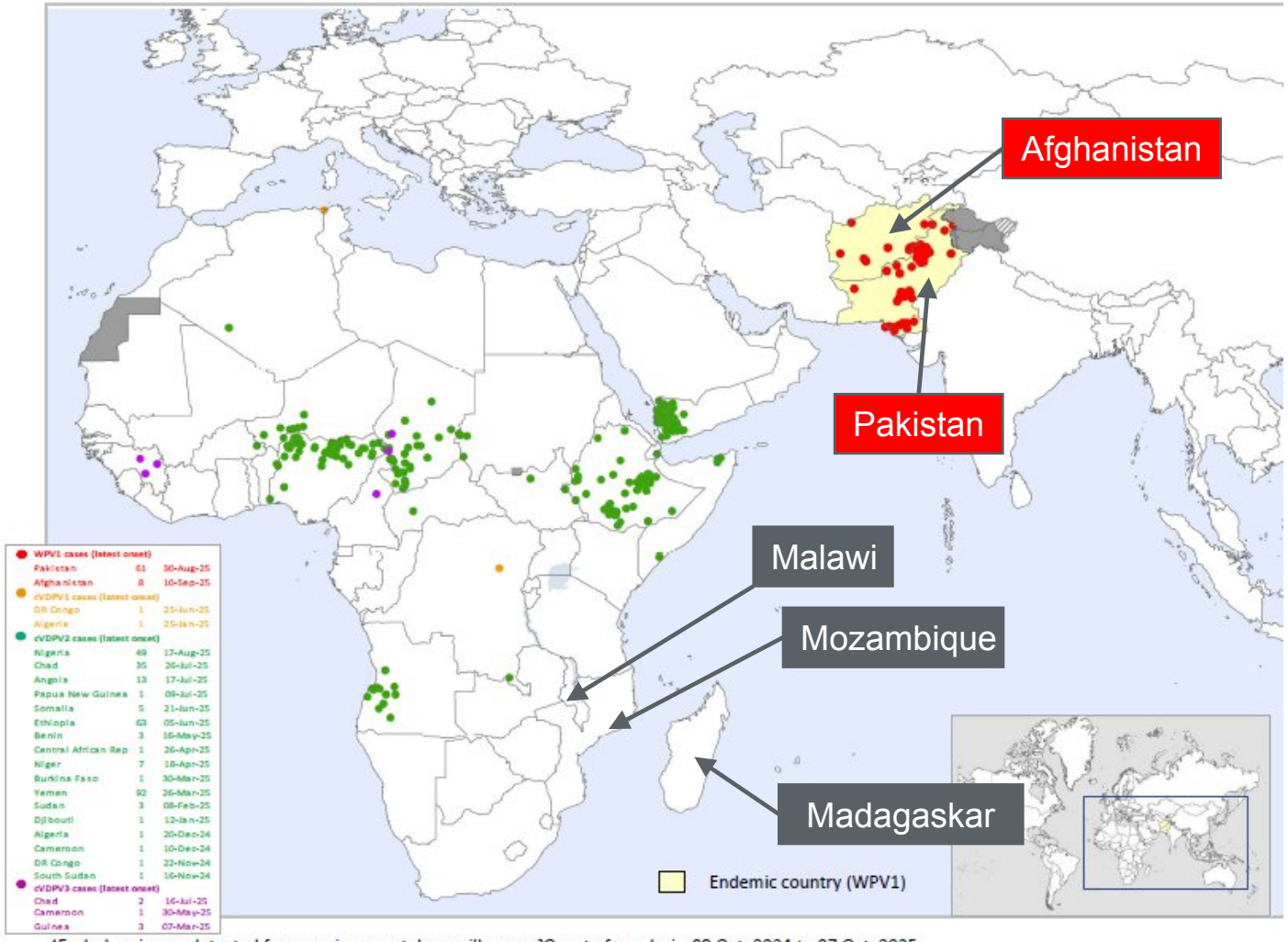
Poliovirus eradiation



Poliovirus epidemiology



Circulating vaccine-derived poliovirus

- cVDPV1 cases
- cVDPV2 cases
- cVDPV3 cases
- Wild poliovirus type 1 cases

[1]Excludes viruses detected from environmental surveillance; [2]Onset of paralysis: 08 Oct. 2024 to 07 Oct. 2025

Polio vaccines

- **Inactivated polio vaccine (IPV)**
- Jonas Salk, 1955
- Inactivated viruses of all 3 serotypes
- Injected, requires skilled personnel
- More expensive

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- Albert Sabin, 1963
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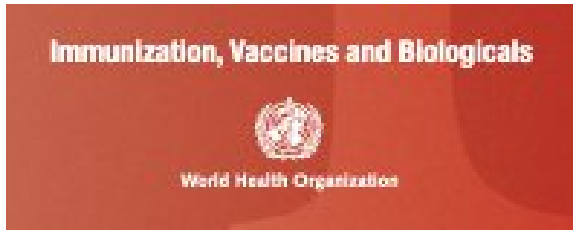

World Health Organization

STANDARD OPERATING PROCEDURE

NEUROVIRULENCE TEST
OF TYPES 1, 2 OR 3 LIVE ATTENUATED POLIOMYELITIS
VACCINES (ORAL) IN TRANSGENIC MICE SUSCEPTIBLE
TO POLIOVIRUS

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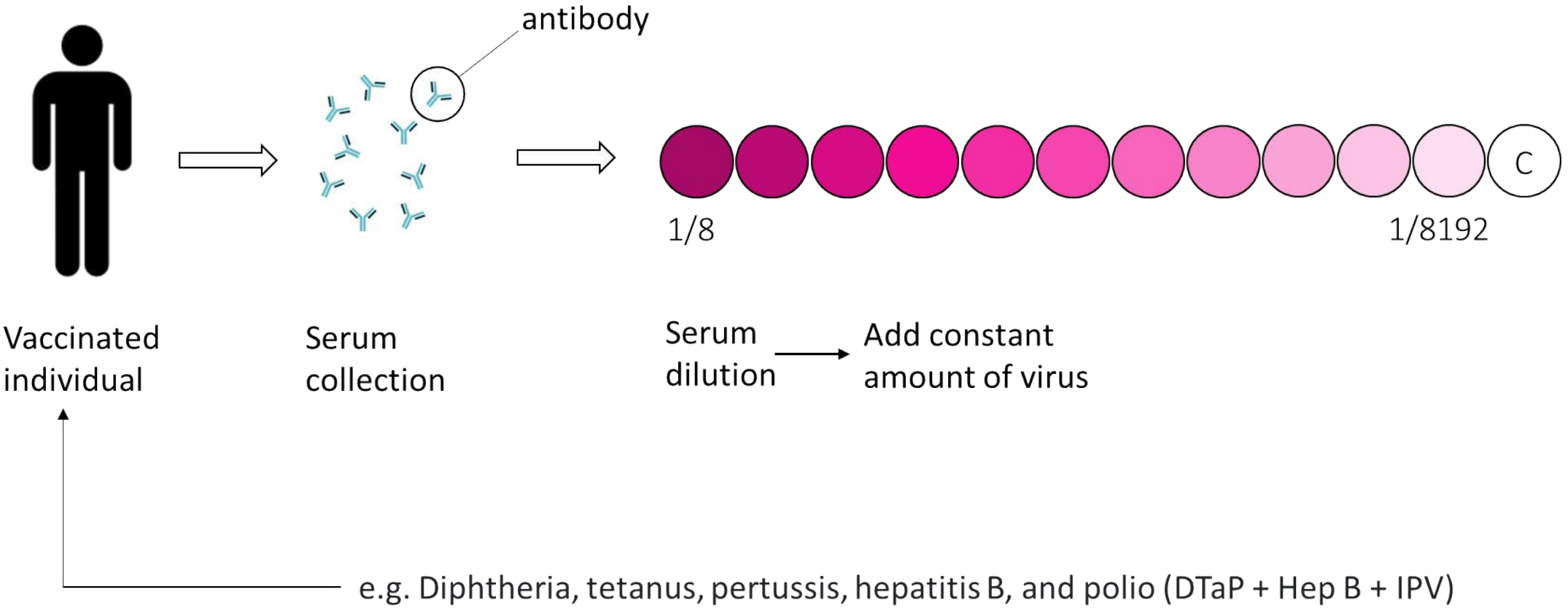
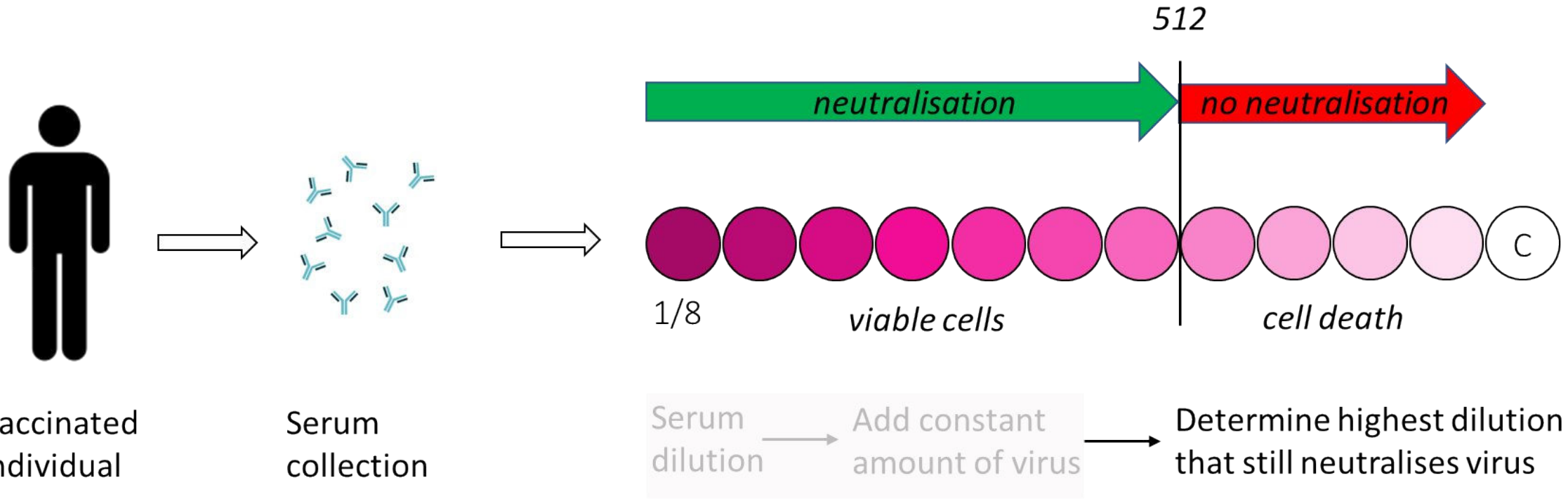
Serological testing after IPV vaccination



Serological testing after IPV vaccination



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Safety testing after (n)OPV vaccination

Vaccinated individual (OPV)

Excretion and spread to others in close contact

Passive immunization

community with insufficient vaccine coverage

Prolonged circulation and spread, reacquired neurovirulence

Vaccine-derived poliovirus outbreak

World Health Organization

STANDARD OPERATING PROCEDURE

NEUROVIRULENCE TEST OF TYPES 1, 2 OR 3 LIVE ATTENUATED POLIOMYELITIS VACCINES (ORAL) IN TRANSGENIC MICE SUSCEPTIBLE TO POLIOVIRUS

Cerba Research

Safety testing after (n)OPV vaccination

Vaccinated individual (OPV)

Excretion and spread to others in close contact

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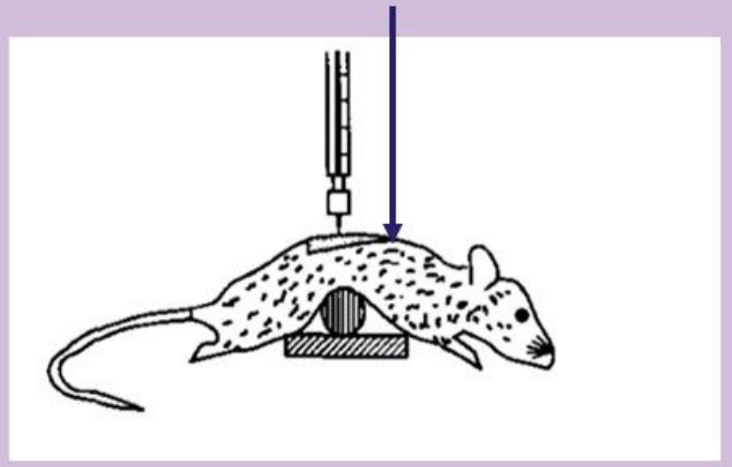
STANDARD OPERATING PROCEDURE

NEUROVIRULENCE TEST OF TYPES 1, 2 OR 3 LIVE ATTENUATED POLIOMYELITIS VACCINES (ORAL) IN TRANSGENIC MICE SUSCEPTIBLE TO POLIOVIRUS

Cerba Research

Safety testing after (n)OPV vaccination

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

World Health Organization

STANDARD OPERATING PROCEDURE

NEUROVIRULENCE TEST
OF TYPES 1, 2 OR 3 LIVE ATTENUATED POLIOMYELITIS
VACCINES (ORAL) IN TRANSGENIC MICE SUSCEPTIBLE
TO POLIOVIRUS

Polio vaccines

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- Easily administered
- Cheaper

Safety and immunogenicity of a reduced-dose inactivated poliovirus vaccine versus a full-dose inactivated poliovirus vaccine in infants in Bangladesh: a double-blind, non-inferiority, randomised, controlled, phase 3 trial

Prasad S Kulkarni, Khalequ Zaman, Sajjad A Desai, Sandesh Bharati, Doli R Goswami, Amina T Sharmeen, Sohel Rana, Warda Haque, Ashish Khandelwal, Satyaprasad Manney, Parikshit Tyagi, Sunil Gairola, Jagdish K Zade, Sambhaji S Pisal, Rajeev M Dhere, Cyrus S Poonawalla, Chris Lamberigts, Varsha Parulekar, Anirudha Vyankatesh Potey

Genetic and phenotypic stability of poliovirus shed from infants who received novel type 2 or Sabin type 2 oral poliovirus vaccines in Panama: an analysis of two clinical trials

Rahnuma Wahid, Laina D Mercer, Tirza De Leon, Rodrigo DeAntonio, Xavier Sáez-Llorens, Andrew Macadam, Konstantin Chumakov, Jeroen Strating, Björn Koel, Jennifer L Konopka-Anstadt, M Steven Oberste, Cara C Burns, Raul Andino, Erman Tritama, Ananda S Bandyopadhyay, Gabriela Aguirre, Ricardo Rüttimann, Chris Gast, John O Konz

Evaluating stability of attenuated Sabin and two novel type 2 oral poliovirus vaccines in children

Rahnuma Wahid[1✉], Laina Mercer[1], Chris Gast[1], Tirza De Leon[2,3], Xavier Sáez-Llorens[3,4], Alan Fix[1], Andrew Macadam[5], Laura Stephens[5], Konstantin Chumakov[6,7], Saskia L. Smits[8], Marta Murreddu[9], Jennifer L. Konopka-Anstadt[10], M. Steven Oberste[10], Cara C. Burns[10], Raul Andino[11], Novilia Sjafri Bachtiar[12], Erman Tritama[12], Ananda S. Bandyopadhyay[13], Gabriela Aguirre[14], Ricardo Rüttimann[14] and John O. Konz[1]

Cerba Research

Innovations

Cerba Research

Life science needs to focus on the 3Rs

The FDA modernisation act 2.0: Bringing non-animal technologies to the regulatory table

Alastair Stewart [a], Delphine Denoyer [a], Xumei Gao [a], Yi-Chin Toh [b]

FDA

NIH to prioritize human-based research technologies

New initiative aims to reduce use of animals in NIH-funded research.

NIH National Institutes of Health
Turning Discovery Into Health

Life science needs to focus on the 3Rs

Reduction

Ex vivo preclinical models

- Susceptibility studies
- Model development
- Efficacy studies:
  - Antivirals
  - Biologicals
  - Vaccines

Replacement

Organoids

- Air-liquid interphase cultures
  - Antivirals
  - Biologicals
- Multi-lineage organoids
  - Immunogenicity of vaccines (tonsil organoids)
  - Safety of nOPV (CNS organoids)
- Sequencing as alternative for neurovirulence testing

Refinement

Optimized animals models

- Alternative HEPs (reduction of discomfort)

New read-out techniques:

- CMI in vaccine research
- MSD platform
- Alternative read-outs:
  - Activity read-out
  - Imaging

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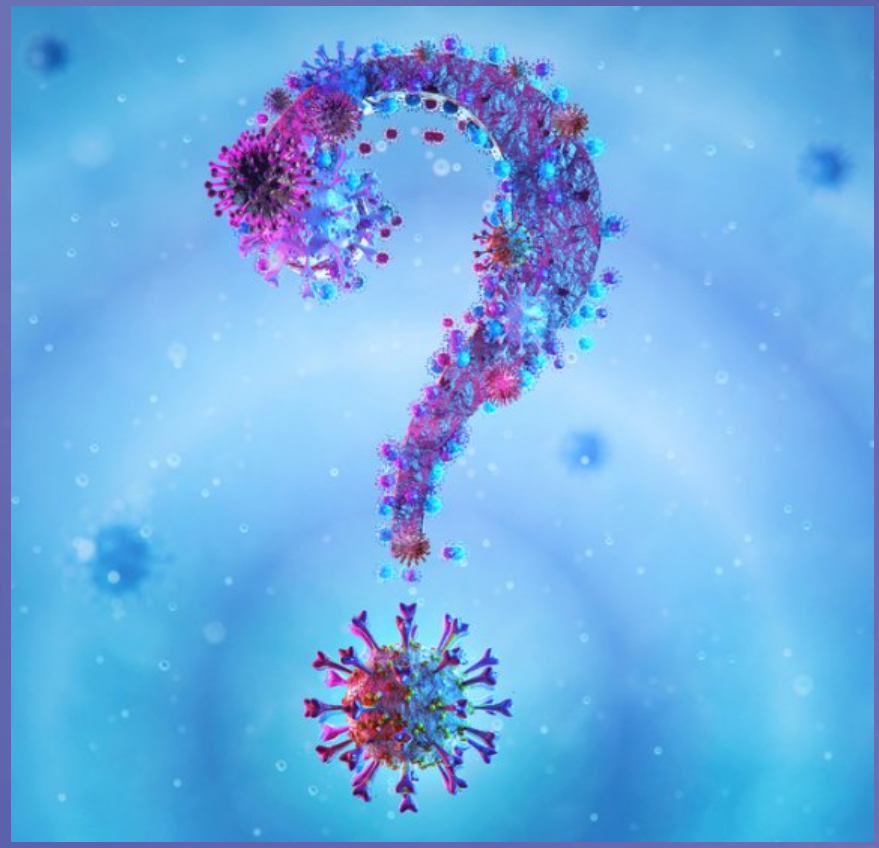

For more information contact

Leon de Waal, PhD

leon.dewaal@cerbaresearch.com

Booth: #208