July 2nd 2024

IABS Webinar on ‘Global Availability of Critical Reagents for Biologicals Testing. Current Status, Challenges and Possible Solutions’

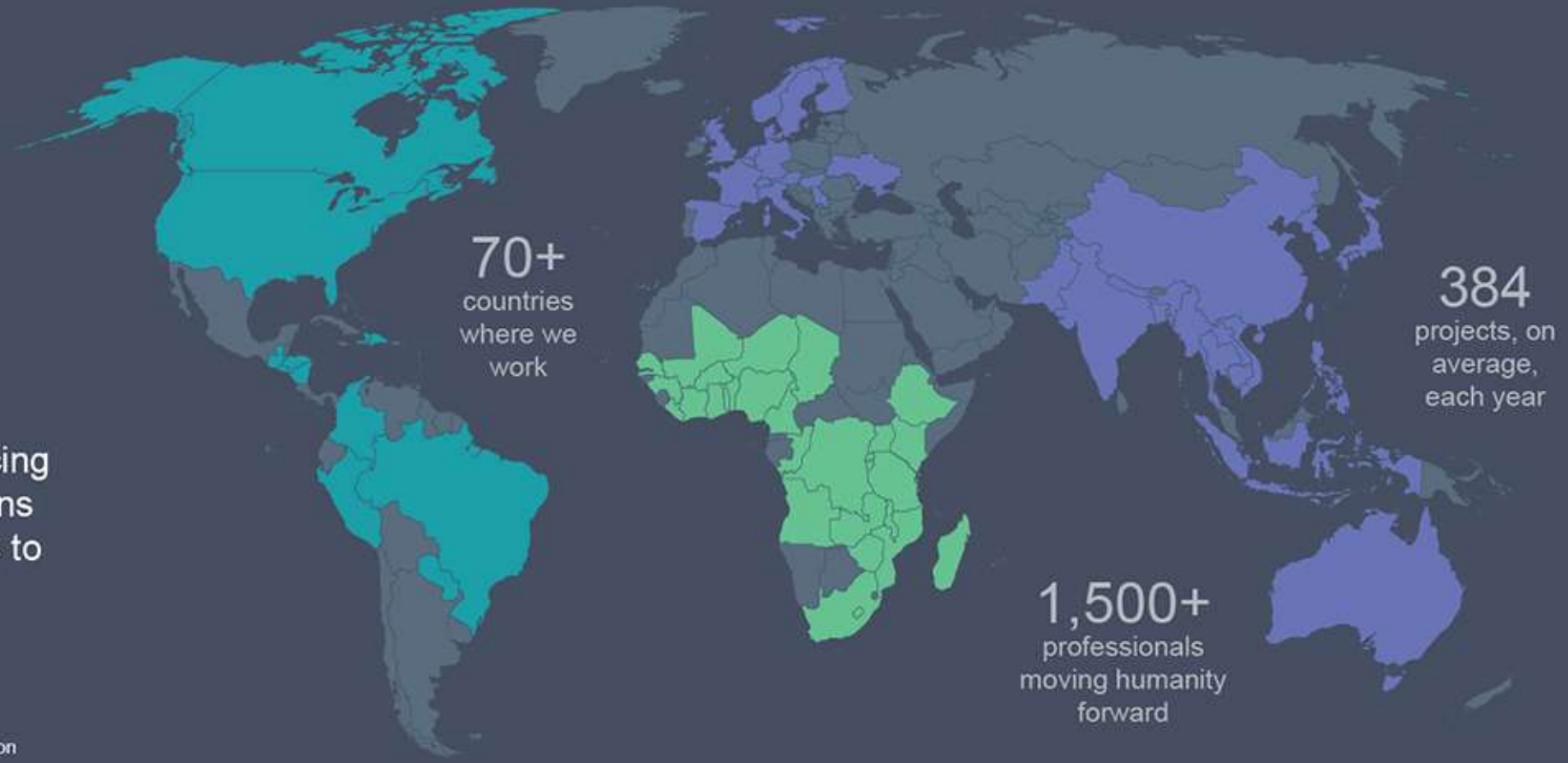
Case study: PATH efforts on Critical/Universal Reagents and Reference Standard Development.

Kutub Mahmood PhD,
Scientific Director,
Center for Vaccine Development and Access (CVIA)

PATH

Unprecedented impact

With the help of local and global partners, PATH generates evidence, advances innovation, and strengthens local capacity to improve health in countries and communities experiencing disproportionate burdens of disease and barriers to well-being.



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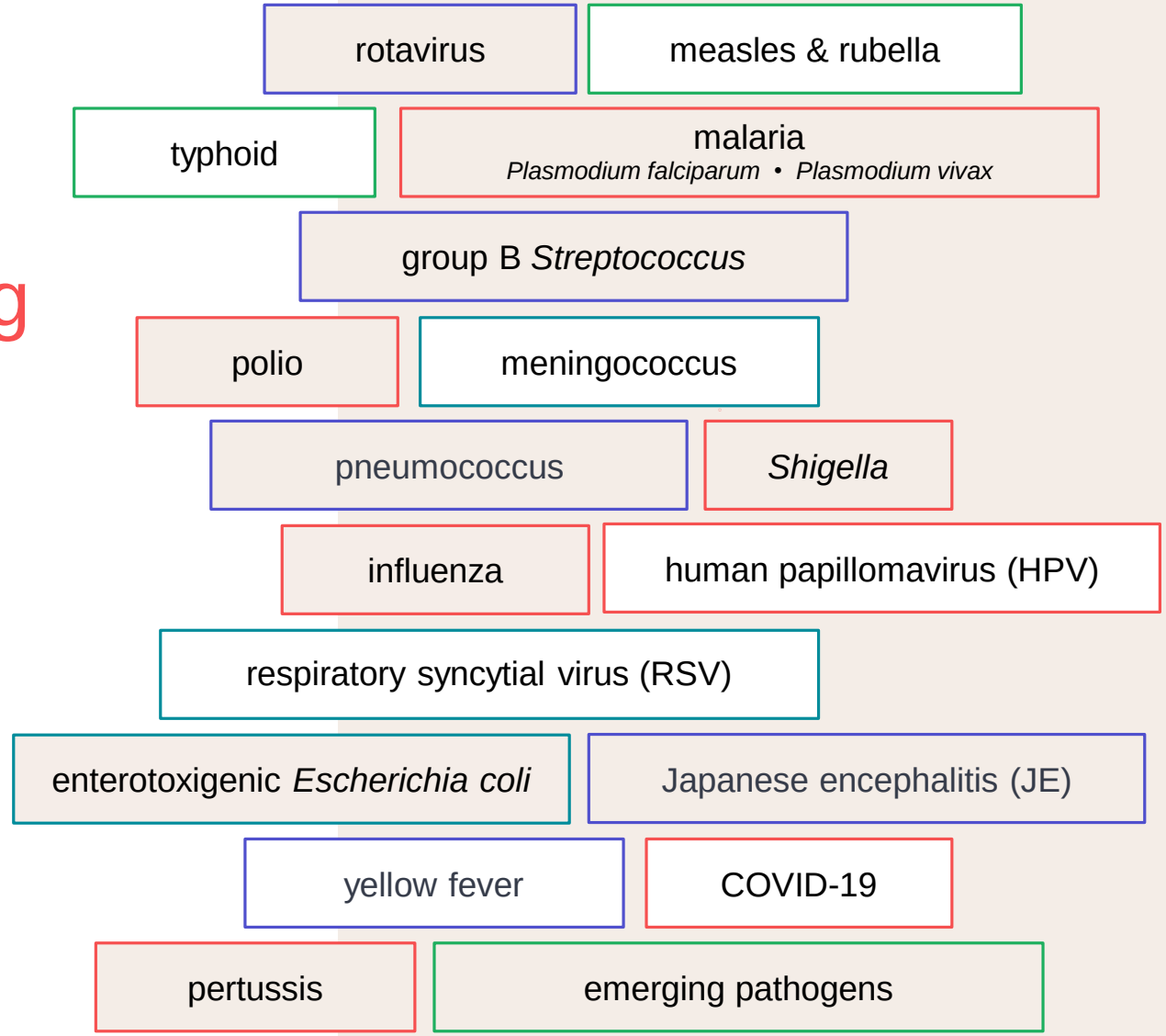
PATH's Center for Vaccine Innovations and Access (CVIA) develops and delivers lifesaving vaccines to women, children, and communities around the globe.

Our breadth of experience – from dangerous childhood illnesses to emerging threats like COVID-19 – uniquely equips us to contribute to bold solutions against any disease.



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Over the last three decades our work has resulted in more than 24 vaccines commercialized, more than half of which have achieved WHO prequalification or EUL.

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Case Study with Potency Assay & Reagents

Challenges

- Development of a potency assay is often overlooked by new vaccine developers
  - Critical for long term success of every vaccine
  - Traditional default technology is an *in vivo* assay typically in mice
- PATH is committed to 3Rs to minimize animal utilization and provides technical support to LMIC manufacturers to develop in vitro potency methods
- Method development - ELISA methodology for alum adsorbed vaccine antigens. We have developed an inhibition ELISA (iELISA) where the monoclonal reagent is treated with the aluminum adsorbed vaccine and the unadsorbed fraction of antibody is measured in an ELISA plate (preadsorbed with antigen)

Opportunity

Supporting development and availability of critical reagents for potency and other quality control testing is a critical part of the solution to improve vaccine supply and access.

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Support for LMIC Manufacturers – Global Availability and Supply of Critical Reagents

- LMIC manufacturers cannot always get access to a reliable supply of reagents for use in long term potency assay work

- PATH has been committed, with the support of the Bill and Melinda Gates foundation to supply critical reagents to LMIC at minimal or no cost for commercial use

Product type	Description	Distributor
PCV antibodies	29 Serotype specific MABs and hybridomas	NIBSC/MHRA
OV16 humanized Ig	Recombinant fully human Ov16 IgG4 control	BioRad
Malaria antigens	Recombinant plasmodium proteins HRP-W2, HRP2-ITG, LDH-Pf, LDH-Pv, LDH-Hu, LDH-Po, LDH-Pm	University of Queensland
G6PD antibodies	Hybridomas and raised monoclonal antibodies.	PATH
Malaria antibodies	Hybridomas to HRP2 antigen.	Precision
COVID benchmarking panel	300 panels of COVID-19 samples comprised from clinical sources.	FIND/WHO
Meningococcal antibodies	*Creating MABs to meningococcal serogroups Ia, Ib, II, III, V and VII.*	*In process*
HPV antibodies	14 MABs against different HPV strains for use in commercial vaccine release and stability testing.	PATH
Group B strep antibodies	*PATH is developing hybridomas and antibodies to GBS for serotypes Ia, Ib, II. III, V, VII*	*In process.*
COVID reagents	• **CR-3022 and Hu-Fc receptor tagged ACE-2 receptor** • **Wuhan specific antibody** • **Delta specific antibody** • **Omicron specific antibody** • **Cross reactive antibody** **Available for research and commercial manufacturers use in vaccine release and stability testing.**	PATH NIBSC/MHRA
Rotavirus antibodies	15 antibodies to rotavirus P4, P6 and P8 antigens for research use.	PATH
Sabin IPV	**HuMabs generated and developed "universal reagents" and endorsed by WHO ECBS**	PATH NIBSC/MHRA

PATH Support for Supply of Critical COVID Reagents

- **SARS-COV2 ‘S’-protein cross-reactive monoclonal antibody CR-3022 and human-Fc tagged ACE-2 receptor – 2020/21**
  - **Currently, this reagent used in potency assay fo S-protein based vaccines.**
    Example: Butantan, Brazil
- **Need for specific S-protein reactive mab and human-Fc tagged ACE-2 receptor – 2022/23**
  - **Wuhan specific antibody**
  - **Delta specific antibody**
  - **Omicron specific antibody**
  - **Cross reactive antibody**

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IPV – (Salk IPV, Sabin IPV)

Background on IPV potency assay

- The potency of IPV is measured *in vitro* using ELISA assays and *in vivo* using rat neutralization assays
- The use of reference standard vaccines is essential
- An International Standard for cIPV is available
- The standard human dose for cIPV consists of the following:
  - 40 D- Ag units for poliovirus type 1
  - 8 D-Ag units for poliovirus type 2
  - 32 D-Ag units for poliovirus type 3

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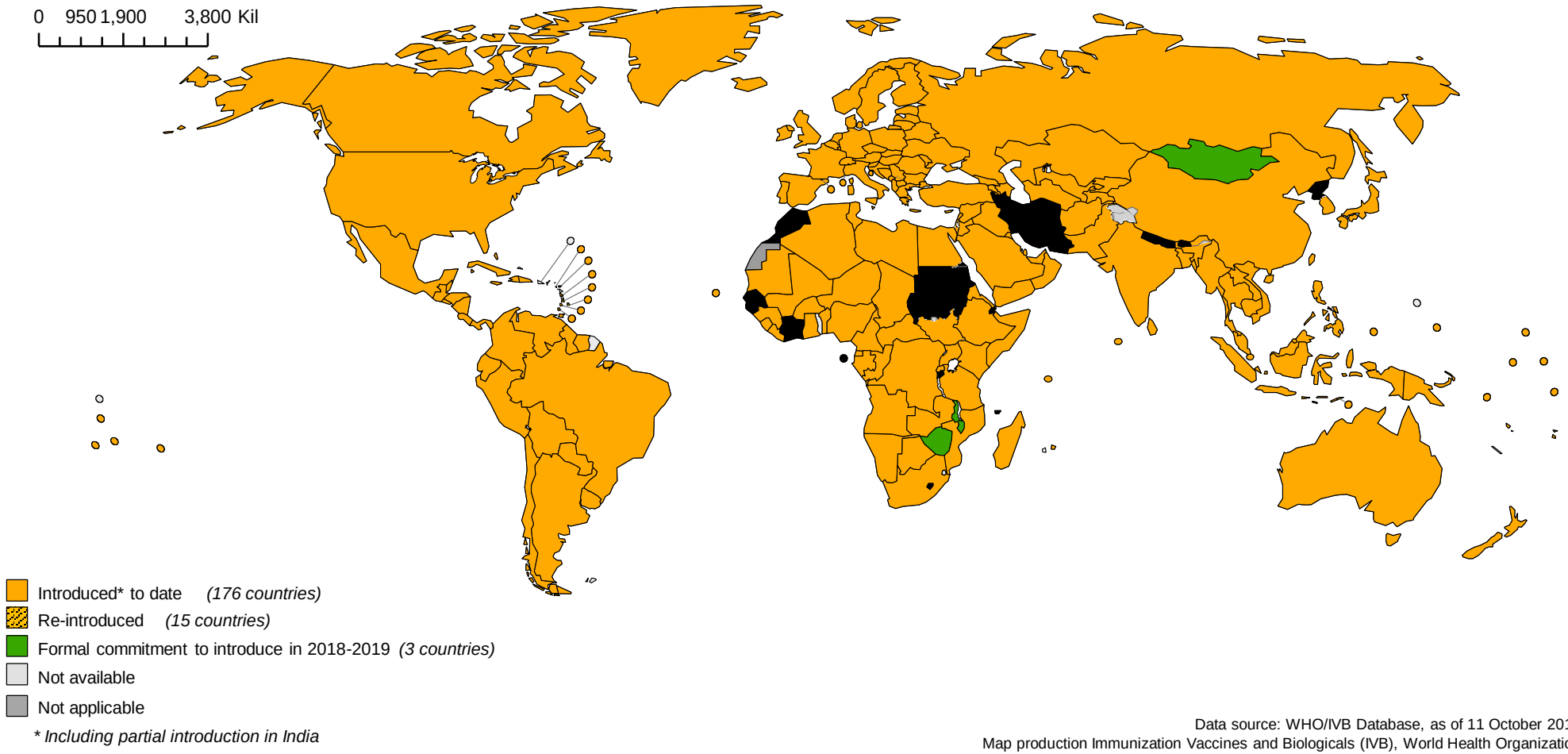
Countries using IPV vaccine to date and formal decision to introduce



Data source: WHO/IVB Database, as of 11 October 2018
Map production Immunization Vaccines and Biologicals (IVB), World Health Organization

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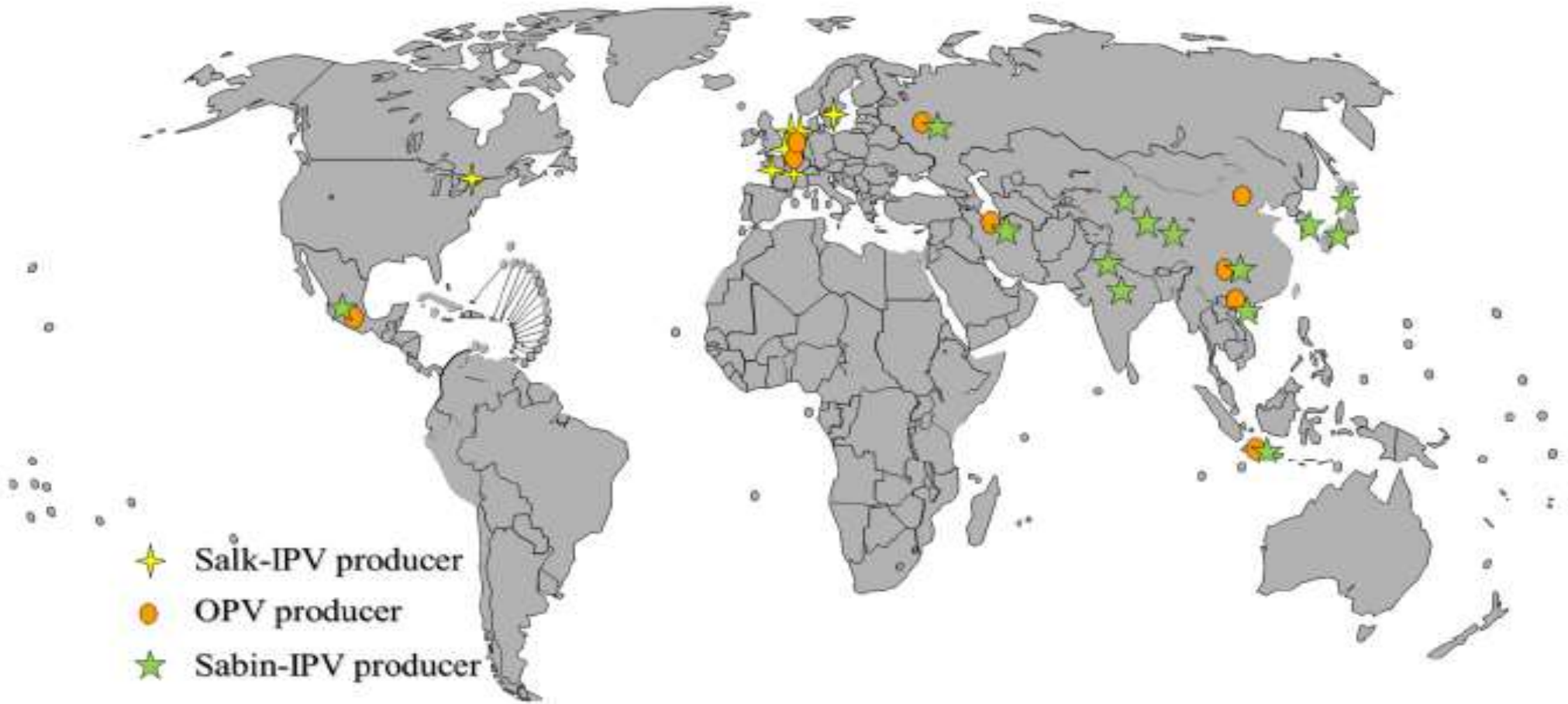
Polio vaccine manufacturers



IPV Supply and Demand Scenario to 2030

Availability of affordable IPV supply is essential to enable a switch to a full two-dose IPV schedule and facilitate adoption of hexavalent vaccine in line with country product preferences.

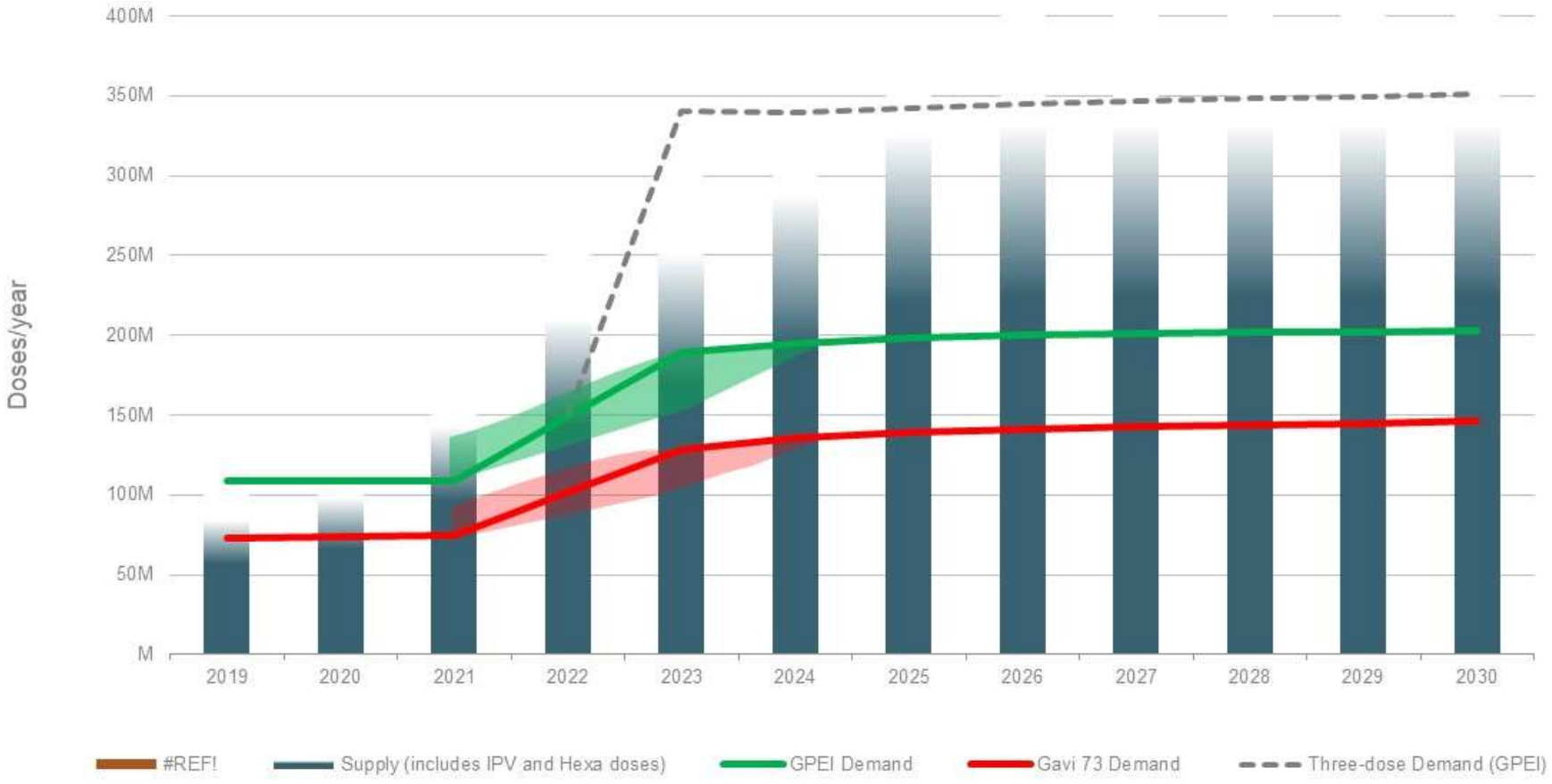


Chart shows base demand scenario for Gavi 73 and GPEI countries, and a gradual transition to a two-dose schedule (assumes fIPV using countries maintain this schedule)

Shaded area reflects potential range of demand:

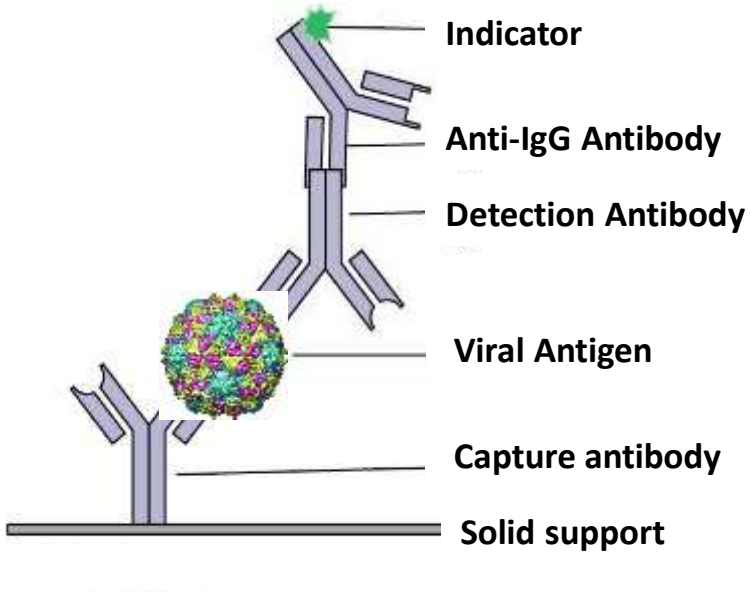
- Higher demand if some countries move to a two-dose schedule earlier given supply availability
- Lower demand if move to two-doses is delayed due to polio eradication timelines and later OPV withdrawl
- Demand changes could also be driven by switches between use of fIPV and full-dose products, and IPV funding

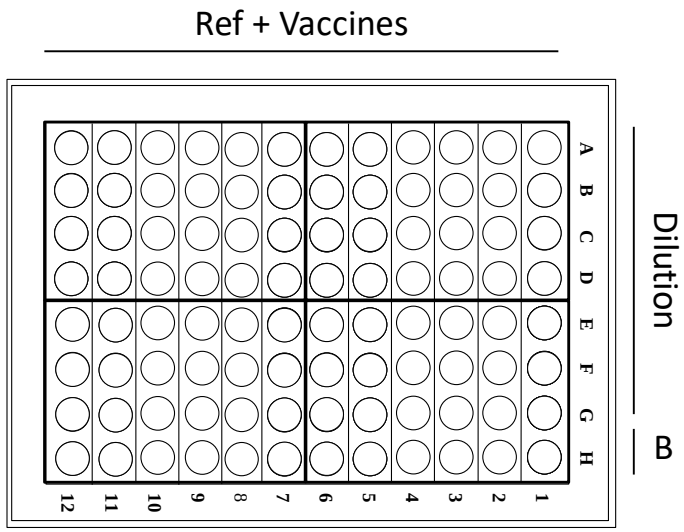
Hypothetical three-dose scenario reflects demand if all GPEI countries were to switch to a 3-dose hexavalent

Note: Analysis assumes gradual switch to a 2-dose IPV schedule in 2022-2023; India, Bangladesh, Sri Lanka, Ecuador and Cuba use fIPV; hypothetical 3-dose demand scenario assumes all GPEI countries move to a 3-dose hexavalent schedule from 2023; supply estimates are risk adjusted.

Source: BMGF analysis, UNICEF website, November 2018

In vitro potency: D-Ag ELISA





Licensed Manufacturer	Type of Vaccine sIPV	Sabin IPV antigen content per dose (D antigen unit for sIPV; sDU)					
		Clinical Studies				Licensed Product	Status
		DU / ds	Low	Medium	High	selected	
Kaketsuken	DTaP-sIPV	Type 1	0.75	1.5	3	**1.5**	Licensed in Japan
		Type 2	25	50	100	**50**	routine immunization
		Type 3	25	50	100	**50**	
Biken/JPRI	DTaP-sIPV	Type 1	0.75	1.5	3	**1.5**	Licensed in Japan
		Type 2	25	50	100	**50**	routine immunization
		Type 3	25	45	67.5	**50**	
Kunming Institute	Standalone	Type 1	15	30	45	**15**	Licensed in China
		Type 2	16	32	64	**32**	
		Type 3	22.5	45	100	**45**	
BBIBP Beijing Bio-Institute Biological Products	Standalone	Type 1		30		**30**	Licensed in China
		Type 2		32		**32**	routine Immunization Sept 2017
		Type 3		45		**45**	

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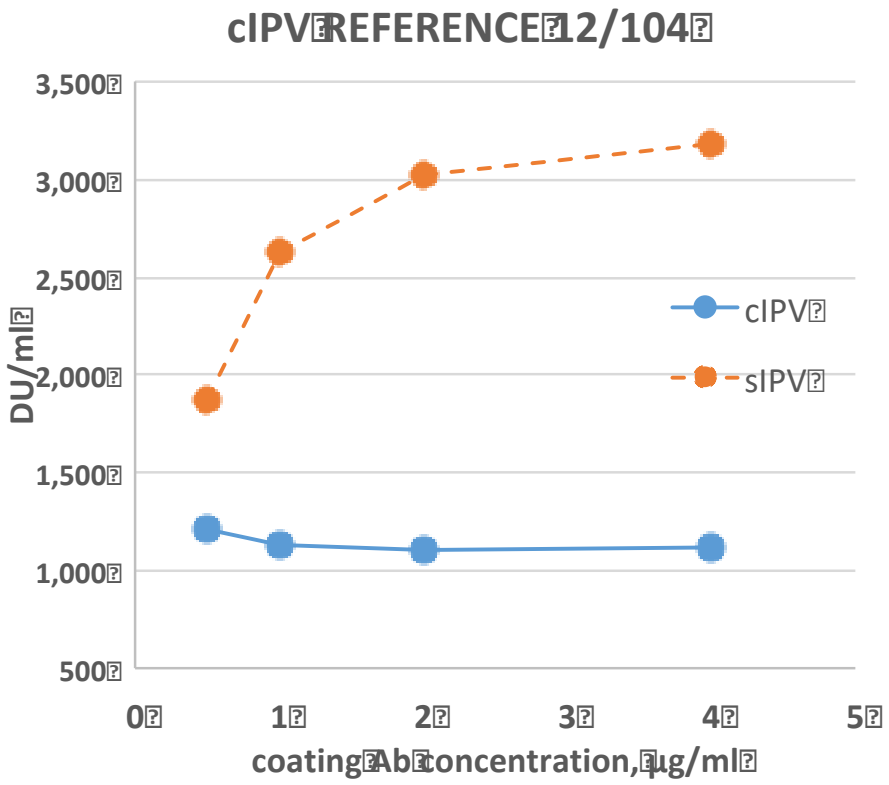
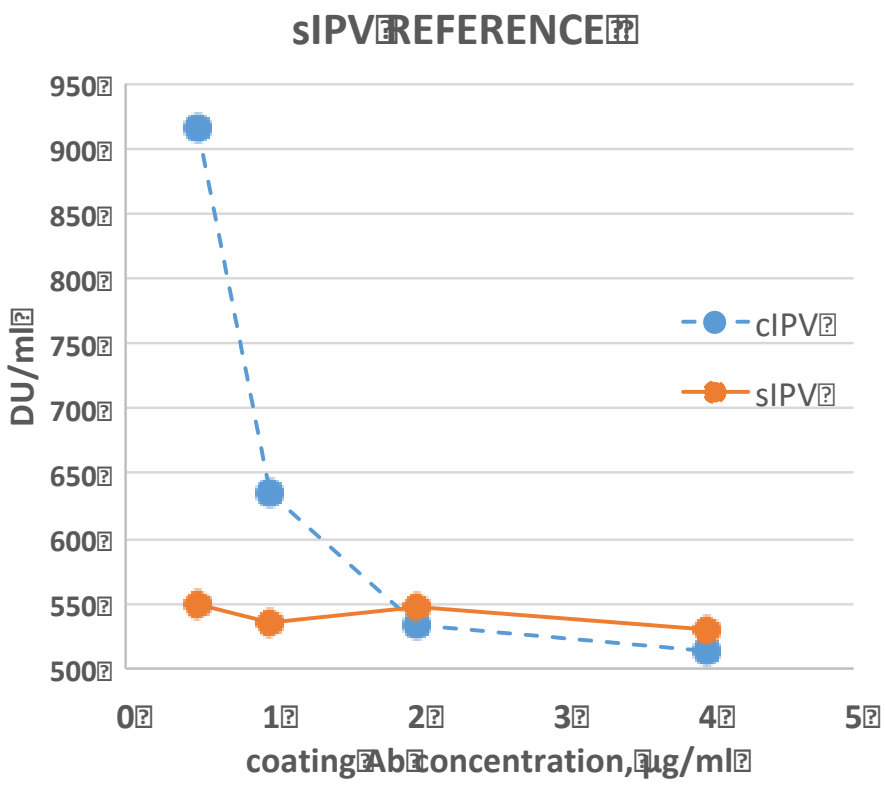
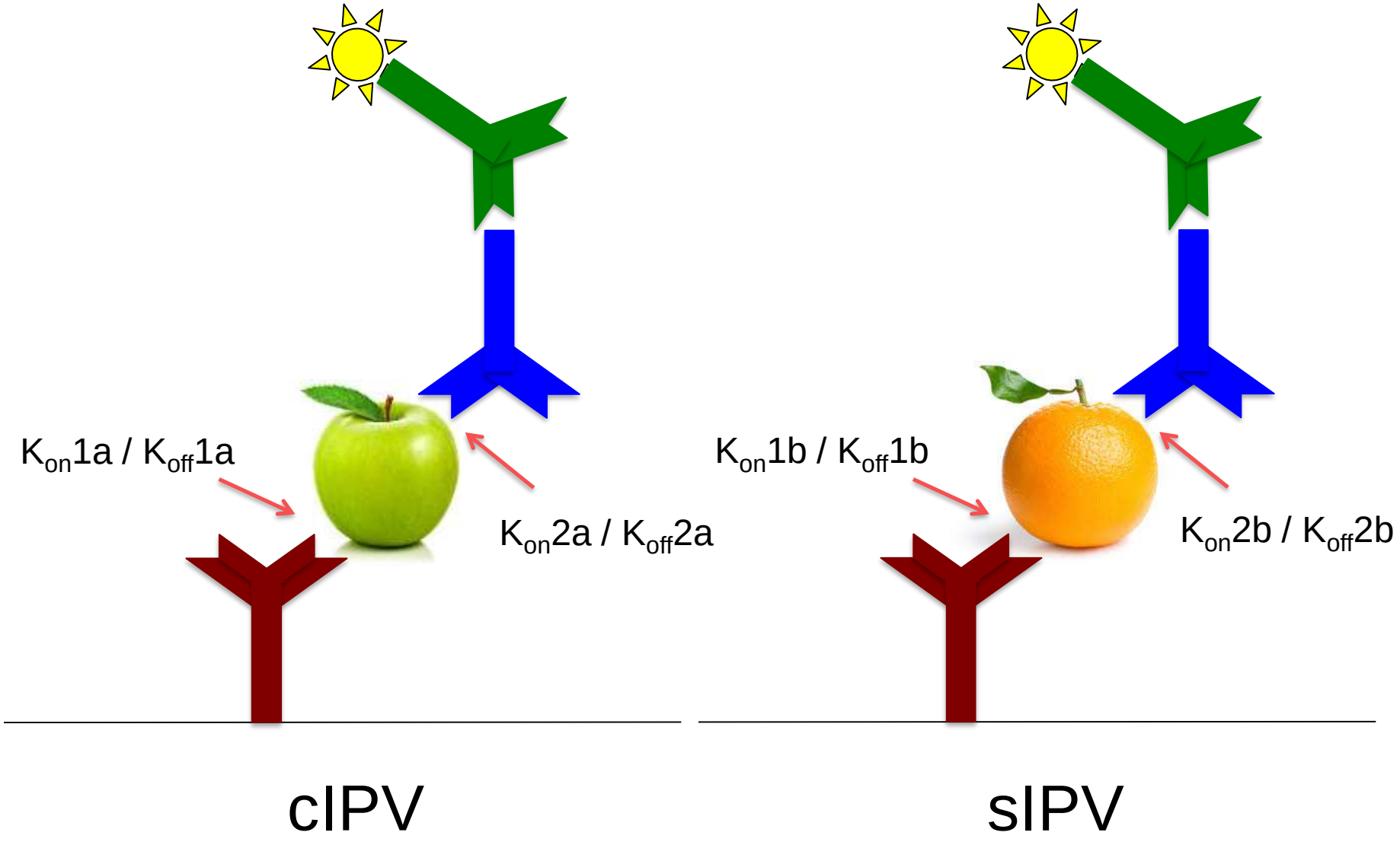
Cross-calibration of cIPV and sIPV





cIPV and sIPV have different immunochemical profiles, both qualitatively a quantitatively



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Courtesy: Kostya Chumako

2ND COLLABORATIVE STUDY FOR THE ESTABLISHMENT OF 1ST INTERNATIONAL STANDARD (IS) FOR sIPV

Aim of this study

Assess the suitability of two candidate materials to serve as a WHO International Standard for sIPV

Candidate Materials

Two concentrated sIPV trivalent bulks kindly donated by 2 producers. Filled at NIBSC in 0.5ml plastic screw cap tubes and stored at -70°C.

NIBSC codes 17/130 and 17/160.

Participants

Fourteen laboratories were invited to participate in this study. Thirteen accepted, seven from Manufacturers and six from National Control Laboratories. Twelve participants returned data in requested timeframe.

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2ND COLLABORATIVE STUDY FOR THE ESTABLISHMENT OF 1ST IS FOR sIPV

Study Samples

Two candidate samples: 17/130 and 17/160 – tested in Duplicate

Current cIPV IS for IPV 12/104 and cIPV 08-143

sIPV vaccine samples: 17/131 and 17/161

Low monitor sIPV vaccine sample: 17/134.

Design of study

Study participants are required to evaluate the D-Ag content of a set of 7 blinded sIPV samples using their in-house validated ELISA method and a common method (NIBSC DU ELISA). NIBSC Reagents Poly and monoclonal Ab were provided.

Perform 3 independent assays per serotype and determine the D- antigen content of each study sample against the cIPV IS 12/104.

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sIPV IS Collaborative Study – Summary

- Assay validity can be improved using common method or existing method with sIPV standard
- High between-lab variability in potency values for study samples was observed with in-house methods when using a non-homologous reference. Potencies of current sIPV products not comparable
- Between-lab variability in potency values for study samples decreased when a common method was used in all labs.
- Between-lab variability in potency values of study samples decreased when a homologous reference (cIPV or sIPV) was used
- **Utility of sIPV as reference standard is identified from this study.**

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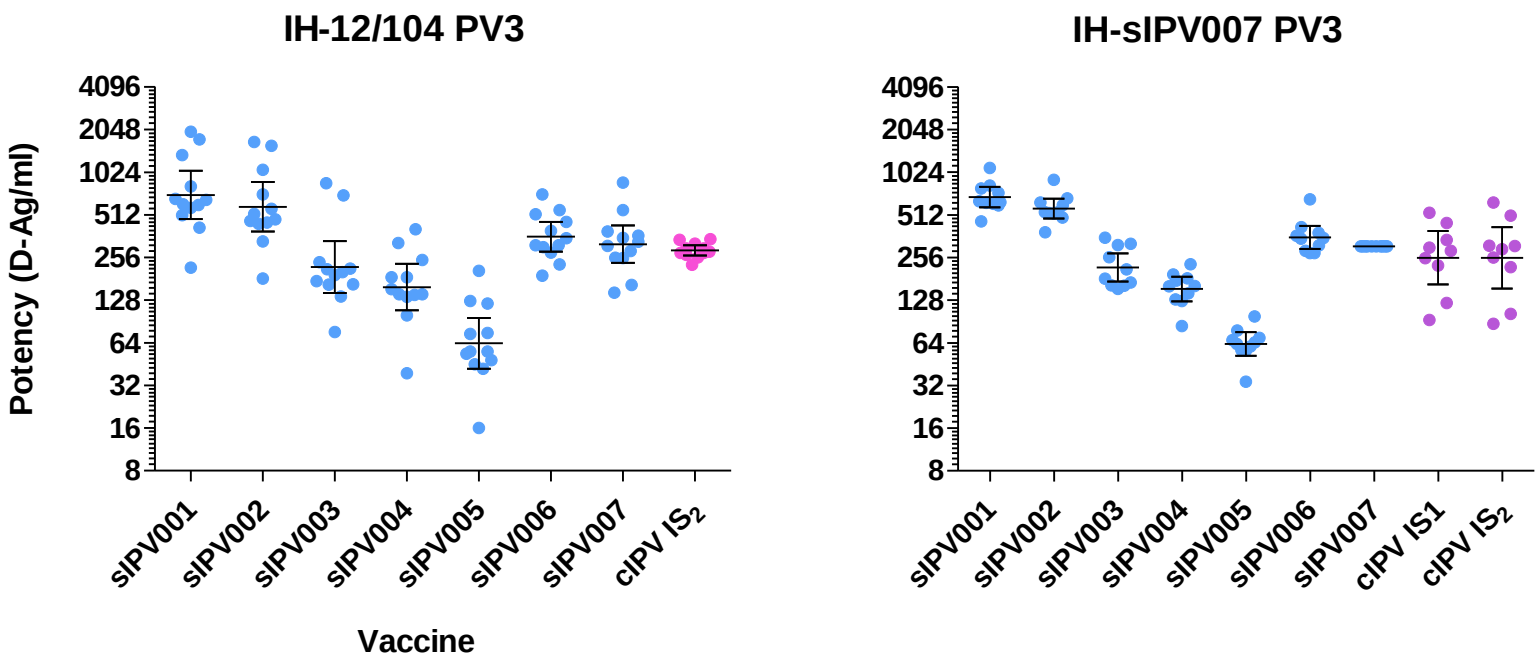
Potency results cIPV vs sIPV reference – PV3




Global Workshop to Establish an International Standard and Reagents to Evaluate Sabin Inactivated Polio Vaccine Potency
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Conclusions of sIPV potency work

- sIPV and cIPV antigen content measure in ELISA cannot be cross-calibrated
- D-antigen units established for cIPV cannot be used to measure potency of sIPV
- New Sabin D-antigen units (sDU) were proposed
- The first International Standard for sIPV was assigned 100:100:100 sDU for each serotype
- The proposal submitted to 2018 ECBS

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WHO UPDATE ON sIPV IS

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)

Laura Crawt[1,3], Eleanor Atkinson[2], Alison Tedcastle[1], Elaine Pegg[1], Study participants (see Appendix 2), Philip Minor[1], Gillian Cooper[1,3], Peter Rigsby[2], Javier Martin[1,3]

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E-mail: Laura.Crawt@nibsc.org; Gill.Cooper@nibsc.org; Javier.Martin@nibsc.org

NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **8 October 2018** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted

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UPDATE ON sIPV IS

World Health Organization

WHO/BS/2019.2354
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Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated)

Proposed amendments (2019) to Annex 3 of WHO Technical Report Series, No. 993

NOTE:

This draft document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein which will then be considered by the WHO Expert Committee on Biological Standardization (ECBS). The distribution of this document is intended to provide information on a number of proposed amendments to the WHO Recommendations to assure the quality, safety and efficacy of poliomyelitis vaccines (inactivated) and is intended to improve the transparency of the consultation process.

The text in its present form does not necessarily represent the final conclusions of the ECBS. Written comments proposing modifications to this text MUST be received by 6 September 2019 using the Comment Form available separately and should be addressed to the Department of Essential Medicines and Health Products (EMP), World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

Comments may also be submitted electronically to the Responsible Officer: Dr Tiequn Zhou at zhout@who.int.

Replace section A.1.3 "International reference materials" with the following text:

A.1.3 International reference materials

A WHO International Standard is available for use in in vitro assays to measure the D-antigen content of wIPV. However, several studies have revealed differences in the reactivity of antibody reagents used in various ELISA methods used by manufacturers and control laboratories to wIPV and sIPV products. This has resulted in high inter-laboratory variability in the potency results obtained for IPV products when using a heterologous reference – that is, a wild-type reference for sIPV or a Sabin reference for wIPV. For this reason, a new WHO International Standard specific to sIPV products was established in 2018 and a new sIPV antigen unit – the Sabin D-Antigen Unit (SDU) – was defined *(13)*. This new unit is unrelated to the D-antigen unit (DU) used to express the potency of wIPV. Both wIPV and sIPV International Standards are intended for use in calibrating secondary reference preparations of IPV which are then used in potency tests to calculate the D-antigen content of wIPV and sIPV, expressed in DU or SDU respectively. At present there is no standardized ELISA assay available as a resource for laboratories, and efforts are under way to develop standardized reagents and protocols. International standards and reference reagents for the control of in vivo potency assays in rats are also currently being evaluated.

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Moving Forward with sIPV IS

- Use of International Standard in sIPV D-Ag potency testing
- Identify trends of variation in the D-Ag content and way forward for harmonization
- Evaluate need for common reagents versus in-house reagents
- Integrate use of IS for rat immunogenicity potency testing for sIPV

Development of Universal Reagents for Sabin IPV

- Non-GMP scale up polio capsid specific monoclonal antibody reagents. Following scale-up, the antibodies are long-term stored, and available distribution by NIBSC.
- Four poliovirus human monoclonal antibodies (serotype 1,2 and 3 specific and cross reactive), characterized, and scaled up, aliquoted, lyophilized and stored at -20^{0}C in NIBSC.
- Three poliovirus serotype specific mouse antibodies (developed at NIBSC) also being scaled

Mab	Serotype	Amount Available
Human (total 4 Mabs)	Type 1,2,3 specific Mabs Cross reactive Mab	$\geq$ 2 grams of purified Mab- Completed, aliquoted, lyophilized,
Mouse (total 3 Mabs)	Type 1,2,3 specific Mabs	Target ~2 grams, One Mab Completed, aliquoted, lyophilized,

- WHO Collaborative study with mouse and human antibody reagents completed
  - More than 20 labs globally agreed to participate in the study,
  - Initiated in 2021 with complete set of data received from 16 labs globally in early 2022

Development of a Panel of Human mAbs

S.No	Antibody	Type 1		Type 2		Type 3		Donor ID
		Sabin 1	Mahoney	Sabin 2	MEF1	Sabin 3	Saukett	
1	6D11	x	x	x	x	364,900	1,158,500	P3
2	6D2	x	x	x	x	14,400	7200	P3
3	7A1	x	x	x	x	91200	444800	P3
4	7E.2	x	x	x	x	459800	1459600	P3
5	8A12	182500	364900	x	x	x	x	P3
6	1E.4	45600	91200	x	200	57500	17800	P1
7	12F8	57500	1150500	57500	229900	200	100	P4
8	6A1	1600	2250	>144800	72400	>144800	>144800	P4
9	6B8	50	100	12800	18100	400	100	P4
10	10D1	>144800	>144800	1600	3200			P4
11	7B1	x	x	102400	>144800	18100	18100	P4
12	6G7	x	x	x	x	>144800	>144800	P4
13	2B3	x	x	x	50	4550	>144800	P3
14	1E.10	x	x	x	x	>144800	>144800	P3
15	8E.1	x	x	x	x	>144800	>144800	P4
16	2F11	1131	289631	x	x	x	x	P3
17	3C10	x	x	x	x	1158524	289631	P3
18	2F7	289631	289631	36204	72408	x	x	P3
19	9F10	>72408	12800	400	1131			P6
20	10D2			1600	12800	36204	18102	P6
21	6B4					>72408	>72408	P6
22	8F2					>72408	>72408	P6
23	6B5					>72408	36204	P6
24	9H2	>72408	>72408	>72408	>72408	36204	1600	P6
25	8H4	>72408	>72408	>72408	>72408			P6
26	7E.5	>72408	>72408	>72408	>72408			P6
27	7C9			3200	9051	>72408	18102	P6
28	7D3					>72408	>72408	P6
29	8D3					>72408	>72408	P6
30	3G9	400		3200	283	6400		P2

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Human Mabs for cryoEM studies

Antibody	Type 1	Type 2	Type 3
9H2	>72408	>72408	36204
LX 2D6	289631		
1B8		23170	
6B5			72408
6A1	2250	72400	144800
LX 10C6	√	√	√
7E5	>72408	>72408	
10D2		12800	36204
5E12	2317048		
5H2	√	25600	
1G1			289631
LX 2E1			√

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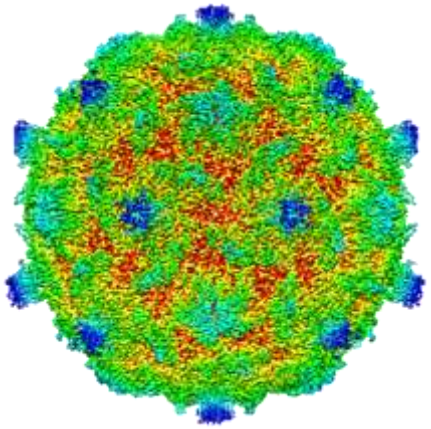
HuMab-Fab & Poliovirus Complexes

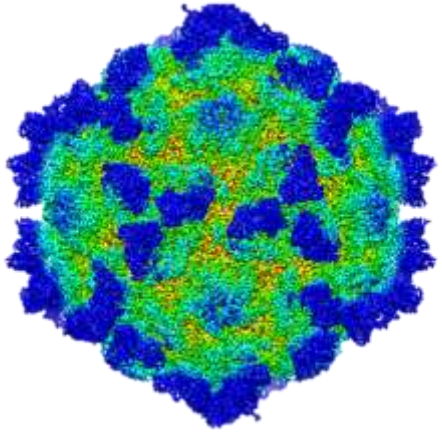
PV3-6A1 2.8 Å

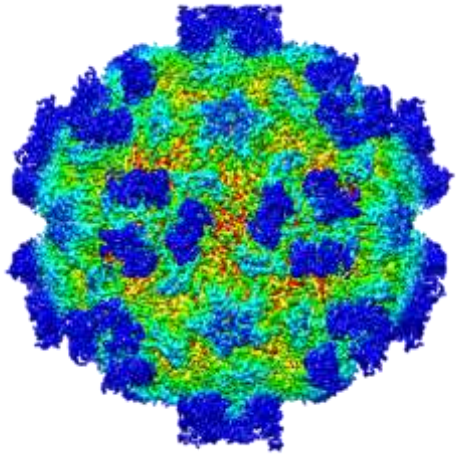
IPV2-1B8 2.6 Å

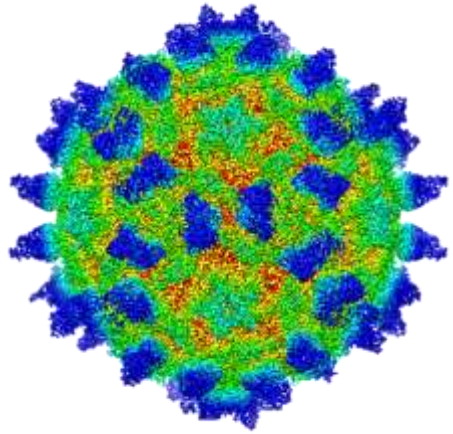
IPV1-2D6 3.8 Å

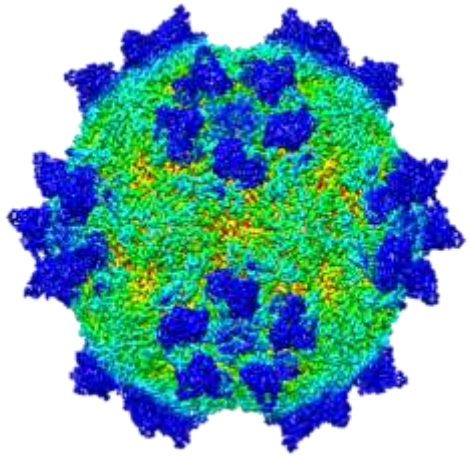
IPV3-1G1 2.4 Å

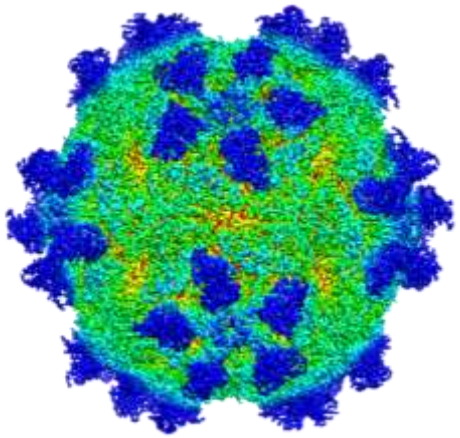


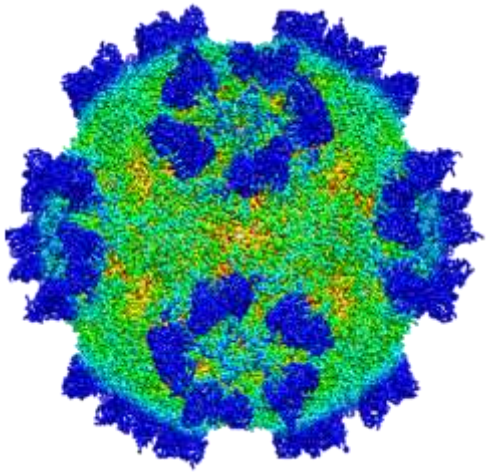


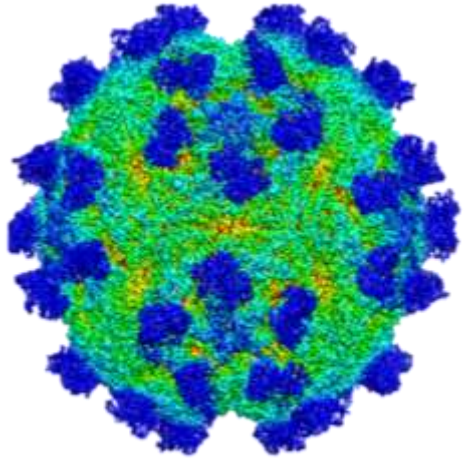


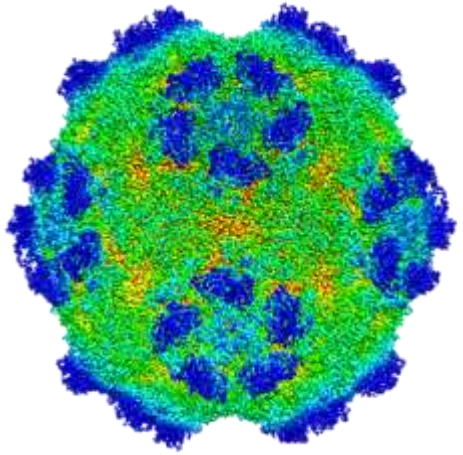












PV3-10D2 3.6 Å

IPV3- 2E1 3.0 Å

IPV1-5E12 2.8 Å

IPV3-6B5 2.9 Å

IPV2-9H2 2.5 Å

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HuMab stock at NIBSC for use as 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

Collaborative Study report on use of HuMab as universal reagents

World Health Organization

WHO/BS/2022.xxxx
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

Alison Tedcastle[1,5], Elaine Pegg[1], Peter Rigsby[2], Diana Kouiavskaia[3], sIPV HuMAb Study Group (see Appendix 1), Kutub Mahmood[4], Konstantin Chumakov[3], and Javier Martin[1, 5]

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[5]Study Coordinators
E-mail: Alison.Tedcastle@nibsc.org, Elaine.Pegg@nibsc.org, Javier.Martin@nibsc.org

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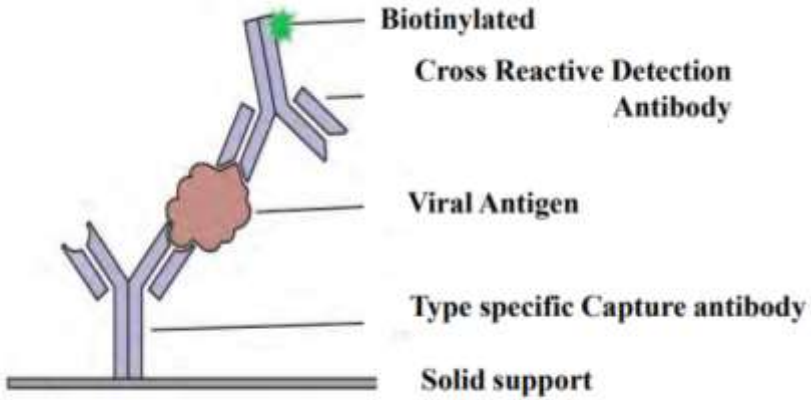
Comparative study with two sets of critical reagents

HuMAb and CBER method



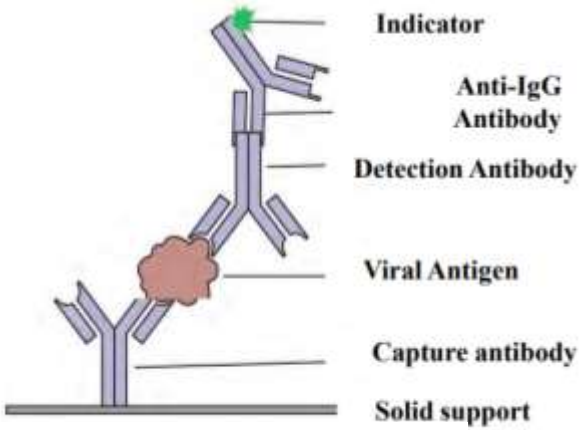
Capture HuMAbs;

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



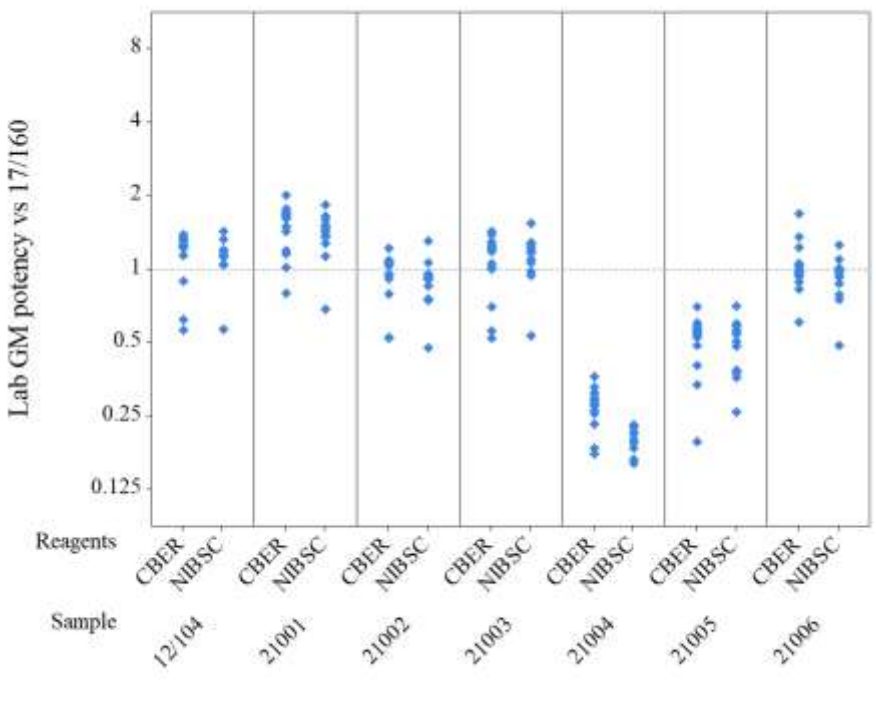
Capture mMAbs;

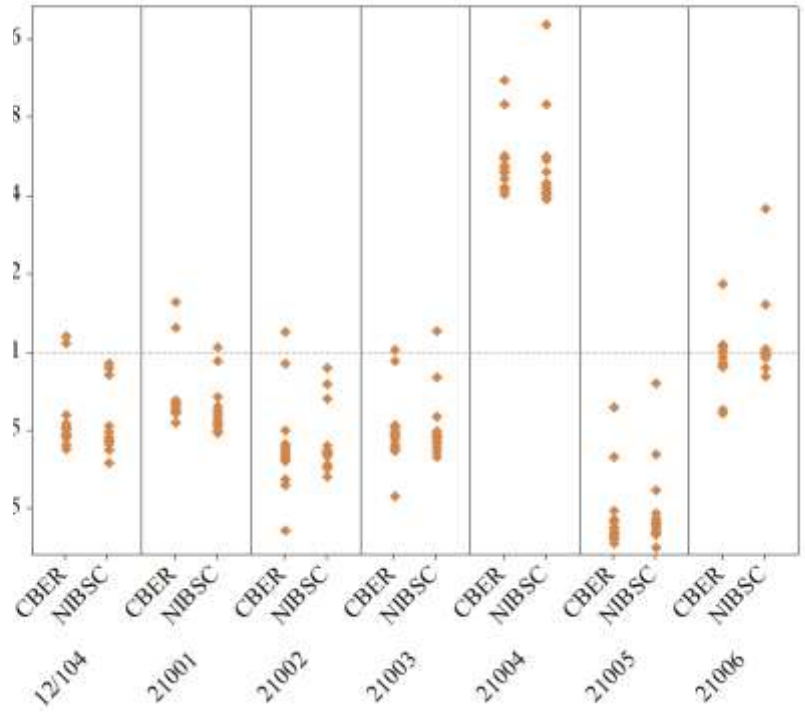
Type 1	13/118
Type 2	13/120
Type 3	13/122

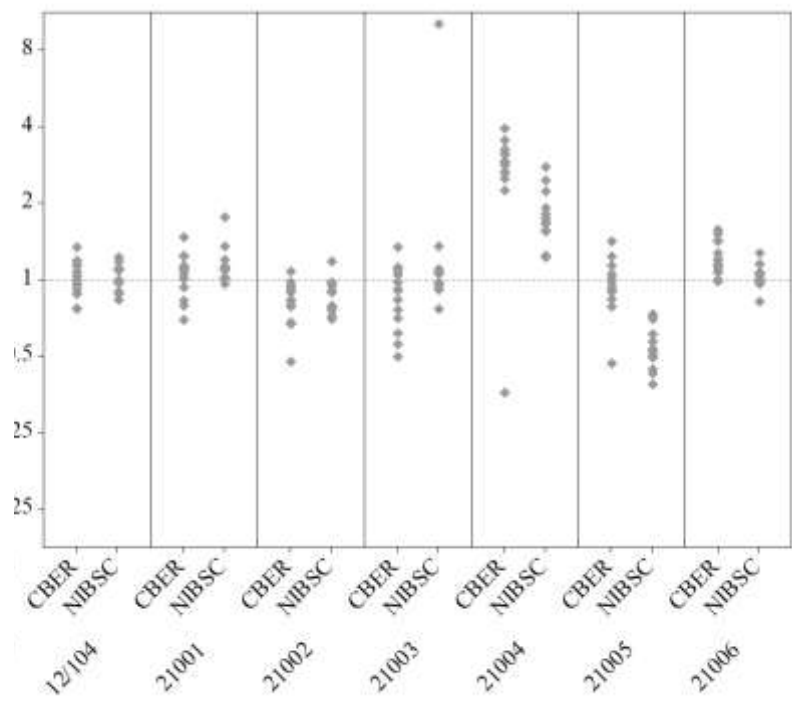
Detection mMAb;

Type 1	234
Type 2	1050
Type 3	520

Geometric Mean potencies of samples tested with murine Mab (NIBSC) and HuMab (CBER) as universal reagents







Conclusion: Both murine Mabs (NIBSC) and HuMabs (CBER) can be used as universal reagents

PATH

Acknowledgements

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