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**Report on the collaborative study to evaluate performance of candidate
WHO Reference Reagents in the sIPV rat potency assay**

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Summary

This study focuses on evaluating the suitability of the 17/160 International Standard (IS) for Sabin Inactivated Polio Vaccine (sIPV) in a *in vivo* rat potency assay, a key step as global routine immunisation shifts from oral polio vaccines (OPV) to inactivated polio vaccines (IPV). Switching to sIPV involves using Sabin strains rather than wild-type strains in order to develop safer vaccines. The primary aim of the study was to determine if the 17/160 IS could be used as a reliable reference for evaluating sIPV in animal models.

The study involved five laboratories, each conducting rat potency assays using three reference standards: the 12/104 WHO IS for conventional IPV (cIPV), 17/160 sIPV IS, and 17/130, a reference reagent for sIPV. The laboratories followed their in-house protocols to measure neutralising antibody titres against the three Sabin S19 poliovirus strains and the MHRA performed real-time stability checks on the 17/160 and 17/130 references.

Results showed that there was considerable variability between assays and laboratories, as expected for *in vivo* biological testing, with geometric coefficients of variation (GCV) ranging from 20% to 80% in line to previous similar collaborative studies with cIPV. Despite this variability, findings were broadly consistent with established differences in immunogenicity between sIPV and cIPV across types and showed good correlation with *in vitro* potency estimates. Relative potencies of the two sIPV references similarly showed consistency when Sabin D-Antigen units (SDU) were considered. However, the known disparity in antigen unit composition among sIPV products from different manufacturers - reflected by the differences observed between the two sIPV references evaluated here - supports the proposal to use both 17/160 and 17/130 as reference reagents for the sIPV rat potency assay.

The findings confirm that both 17/160 and 17/130 are suitable references for *in vivo* potency testing of sIPV, supporting their use in the evaluation of new sIPV candidates as the global switch from OPV to IPV continues. The study underscores the importance of establishing reliable standards for evaluating the immunogenicity and potency of new vaccines, particularly as the use of sIPV expands.

Introduction

With most regions of the world now designated polio-free, the use of inactivated polio vaccines (IPV) for routine immunisation will increase dramatically (1). Laboratory containment of all wild-type polioviruses will soon be a requirement as the world switches from oral polio vaccines (OPV) to IPV use; containment for wild-type 2 viruses has been implemented since August 2016 (2). In order to produce IPV more safely, several manufacturers are producing IPV using Sabin poliovirus strains rather than the usual wild-type strains that have been used for conventional IPV (cIPV) for more than 50 years. Licensed Sabin IPV (sIPV) products are already being manufactured in China, Japan and Korea and as many as 17 new manufacturers are producing or plan to produce sIPV in the near future. In addition, there are four sIPV products that have been WHO pre-qualified for global use so the establishment of suitable reference standards and methodologies for the quality assessment of sIPV products is urgently needed (3–6).

Prior to initiating clinical trials, the immunogenic properties of a candidate IPV should be studied in a suitable animal model (7). According to the European Pharmacopoeia guidelines, this evaluation involves administering the vaccine intramuscularly into the hind limb, typically

using no fewer than 3 dilutions of the candidate vaccine. Sera taken 21 days post inoculation is assessed for neutralising antibodies against all 3 poliovirus types (8). Proof-of-concept nonclinical studies play a critical role in early vaccine development. These studies should include the comparative assessment of the immunogenicity of the candidate IPV versus that of a licensed reference IPV. The data obtained from such studies are not only instrumental in confirming the vaccine's potential efficacy but may also assist in the selection of D-antigen content to be evaluated in dose-finding studies in humans. A multitude of animal models have been evaluated for use in the assessment of neutralising antibody titres produced by IPV vaccines. These have included chicks, guinea pigs, monkeys and rats. Due to sub-optimal testing conditions and lack of sensitivity in the other animal models, the rat potency test has become the test of choice. This is due to it producing the highest titres, giving a good dose related titre response, being consistent across different strains, and showing minimal variability between laboratories. Moreover, it has demonstrated a strong correlation with human potency and may therefore serve as a useful tool for IPV dose determination (9–11).

In addition to serving as a basis for immunogenicity analysis, *in vivo* testing provides valuable information for vaccine characterisation. This is particularly important when significant modifications are introduced to the manufacturing process, as they help to establish comparability between the original and altered production methods (12). Moreover, as the use of sIPV expands with global routine immunisation programs, continuous post-marketing surveillance will be vital. This surveillance ensures long-term safety and efficacy of the vaccine and supports early identification of any potential issues related to vaccine performance or adverse effects in the general population (1).

At the ECBS meeting in October 2016, this study was endorsed alongside the proposal for the 1st WHO International Standard for *in vitro* potency testing of sIPV. This report describes the results of a collaborative study conducted, with the aim to establish a suitable standard for the *in vivo* potency testing of sIPV. This will be used to evaluate sIPV products and support the harmonisation of vaccine development and production.

Aim of the study

The aim of the study was to assess whether the current sIPV International Standard (IS), 17/160 (13), would be a suitable reference for the evaluation of inactivated poliovirus vaccines in a rat potency assay.

Materials and Methods

Study samples

Two international standards and one reference reagent were provided to all participants. These were supplied alongside three (types 1, 2 and 3) Sabin S19 strains for use in the neutralisation assay under Biosafety Level 2 conditions, as these strains are exempt from GAPIV containment. (14). All samples were shipped on dry ice and storage at $\geq -70^{\circ}\text{C}$ was recommended. Instruction for use documents were provided which included Material Safety Data Sheets. The study samples and viruses are listed below. Established potencies for all samples are displayed in Table 4.

WHO IS for cIPV, 12/104

12/104 was established in 2013 with an assigned D-Ag potency of 277, 65 and 248 D-Ag/ml for poliovirus type 1, 2 and 3 respectively (15).

WHO IS for sIPV, 17/160

17/160 was established in 2018 with an assigned potency using the WHO IS for cIPV 12/104 as reference of 239, 136 and 237 of D-Ag/ml for poliovirus type 1, 2 and 3 respectively. It was assigned specific Sabin D-Ag units (SDU) of 100, 100, and 100 SDU/ml for poliovirus type 1, 2 and 3, respectively (13).

Sabin IPV vaccine sample, 17/130

17/130 is a reference reagent for sIPV. It was analysed alongside 17/160 with an assigned potency using the WHO IS for cIPV 12/104 as reference of 50, 608 and 450 D-Ag/ml for poliovirus type 1, 2 and 3 respectively (13).

Product summary details for each of the two filled sIPV candidate samples are shown in Table 1. There are currently 4,764 and 1,749 vials of 17/130 and 17/160, respectively, and they are both stored at -70°C.

Design of the Collaborative Study

Participants

Five laboratories were invited to participate in this study (see Appendix 1). They were all able to receive the samples and returned data within the requested timeframe.

Study plan

Participants were requested to perform their own in-house SOPs (details in Tables 2 and 3) with a few stipulations:

- Perform the *in vivo* rat potency test using all three references (12/104, 17/160 and 17/130) at the same time.
- Perform three independent assays.
- Use the following for the sample dilutions;
17/160 and 17/130 should be pre-diluted 1 in 2 and 12/104 should be pre-diluted 1 in 5 before making any dilutions. This is considered the 'Neat' material (as below).
- Sample dilutions to include - Neat, 1/2, 1/4 and 1/16.
- Bleed rats after 21 days.
- Determine the neutralisation titre of each of the rat sera (with a minimum of two-wells per dilution) against all three Sabin S19 challenge viruses that were provided. We requested that the participants each use their own in-house SOP for the neutralisation method.
- Grow and titrate the sabin S19-PV strains using the instructions that were provided (see Appendix 3).
- Use 100 TCID₅₀ / well of virus challenge.

- Include the WHO IS 82/585 (16) as a positive serum control and use in-house positive and negative rat serum in their assays.
- Use freshly opened ampoules of samples for each assay.

Participants were requested to report their results electronically using standard forms provided by the study coordinator. Results including raw neutralisation data and calculated serum titres were submitted by the participants.

Statistical Methods

All assay data were analysed at the MHRA using the approach described below. Study samples used as reference standards for the analysis were the existing IS preparations 17/130 and 17/160.

Potency estimates relative to the sample selected as reference standard were calculated by probit analysis. To ensure the data were suitable for this, thresholds were established to determine if an individual rat's response was positive or negative, according to the method described in the European Pharmacopoeia (Ph Eur) potency test for IPV (8), by determining the mid-point on a \log_2 scale between the geometric mean titres for the reference sample at the highest and lowest dilutions tested. This must be defined for each laboratory data set separately, using responses from the reference sample in a minimum of three assays.

Validity criteria applied to the analysis of individual assays required no significant deviations from linearity or parallelism ($p > 0.05$) and confidence limits on the relative potency estimate being not less than 25% or more than 400% of the estimated relative potency. Where a sample gave assay responses $<40\%$ at all dilutions tested, or $>60\%$ at all dilutions tested, the sample was excluded from further analysis due insufficient coverage of the response range. All assay potency calculations were performed using CombiStats (17), and R software (18) was used for further summary calculations and to produce graphs.

Results from all valid assays were combined as unweighted geometric mean (GM) potencies. Variability between assays has been expressed using geometric coefficients of variation ($GCV = \{10^s - 1\} \times 100\%$ where s is the standard deviation of the \log_{10} transformed potencies).

Stability Studies

In the initial report for the endorsement of 17/160 as the 1st IS for sIPV in 2018 (13), both long term and accelerated degradation studies were conducted for 17/160 and 17/130 reference reagents. Therefore, for this study, real-time stability checks were performed at the MHRA to continue monitoring the constancy of the references.

Results of the Collaborative Study

Study data returned

Data were received from 5 laboratories, each performing 3 assays for each of the three serotypes using the method described in the European Pharmacopoeia (Ph Eur) potency test for IPV (8). The data from one of the collaborating laboratories (#5) showed little response in

the assay, and upon further investigation this was found to be largely due to the male animals. Using only female animal data allowed for sufficient proportions of responses to permit valid analysis, so only that part of the data for laboratory 5 was used.

Assay Validity

The pharmacopoeial method used for analysis relies on the potency of reference and test samples having similar potencies, as common dilutions of both samples are used for testing and cut-off of values are calculated from data obtained for the reference sample. Thus, the assay validity rates shown in Table 6 are to be expected given the relative potencies of the samples tested and the often high invalidity rate of such an *in vivo* assay. For example, in the case of 17/160 relative to 17/130, where an approximate five-fold difference in Type 2 potency is present, a validity rate of only 20% is obtained in the analysis performed. In other cases the validity rate can be as high as 87%, as was observed for 12/104 relative to 17/130 for Type 3 potency.

For situations where a high invalidity rate is observed it may be possible to modify the cut-off calculation in individual laboratories to account for this issue. This approach was investigated for Type 2 calculations relative to 17/160 by scaling the existing cut-off values by the existing relative potency estimate for 17/160 relative to 17/130. Based on these data, a factor of 0.22 was applied and the analysis outcomes are shown in Appendix 2, Table 2c. An increase in the number of valid relative potency estimates is observed for laboratories 1 and 4 (compared to Table 2b) illustrating the benefit of this approach for those participants.

Monovalent challenge vs sabin S19 strains

All participants were provided with all three serotypes of Sabin S19 strains to be used as the challenge virus. These viruses have been temporarily exempt from GAPIV containment requirements and can be used safely at Biosafety level 2, therefore were more appropriate for an international study. Before being sent as part of the study pack, the MHRA performed analysis of the current in-house monovalent Sabin strains compared to the Sabin S19 strains to decide if they were a suitable replacement for the challenge virus. Results shown in Figure 1a and 1b indicated the S19 strains produced similar relative potencies to that of the monovalent Sabin strains for all three references tested.

Relative potencies, intra-laboratory and inter-laboratory variability

Estimates of relative potency are shown in Appendix 2, with an overall summary in Table 6. Figure 2 shows the relative potency estimates, where valid results were obtained.

Intra-laboratory variability was quantified using the ratio of maximum to minimum relative potency estimates for laboratories where $n \geq 2$ valid estimates were obtained, as shown in Table 5. From the 36 values shown, 35 (97%) were < 4 and 27 (75%) were < 2 , indicating a level of intra-laboratory variability consistent with expectations for this assay.

Inter-laboratory variability was quantified as both %GCV and the percentage of estimates within 25-400% of the overall study GM (Table 6). Excluding Type 1 potencies for 17/130 and 17/160 relative to each other, values of %GCV ranged from 20% to 80% with a median of 63%, and all estimates (100%) were within 25-400% of the study GM, again indicating a level of variability consistent with expectations for this assay. Higher variability in Type 1 relative

potencies for 17/130 and 17/160 was observed to be due to laboratory 5 giving discrepant results when compared to the other laboratories.

Stability Studies

Results of the analysis of real-time stability at the storage temperature (-70°C) are shown in Figure 3a and b. The results indicate the potency of both 17/160 and 17/130 has been maintained over the last 7 years for all three types indicating good long-term stability.

Discussion

The transition from oral polio vaccines (OPV) to inactivated polio vaccines (IPV) in global immunisation strategies necessitates the development of robust and standardized evaluation methods to ensure the safety and efficacy of IPV products. This collaborative study was conducted to assess the suitability of the current WHO International Standard for sIPV, 17/160, as a reference material in the *in vivo* rat potency assay, a critical step in the quality control of sIPV production.

The increasing adoption of sIPV, particularly in countries such as China, Japan, and Korea, and the expansion of global manufacturing efforts underscore the importance of having a stable, consistent, and widely accepted international standard. The study design adhered closely to the European Pharmacopoeia guidelines for *in vivo* potency testing using rats, which is in line with WHO recommendations set out in Annex 3, TRS No. 993 Appendix 2 (7), providing a well-established framework for assessing the immunogenicity of IPV formulations through the measurement of neutralising antibody titres.

Results from the five participating laboratories demonstrated generally good assay validity. Although several data sets were excluded due to non-linearity, non-parallelism, and out-of-specification confidence limits, these exclusions reflect the inherent variability of *in vivo* models and the rigorous adherence to predefined validity criteria. Importantly, the rat model, despite its limitations, remains a valuable tool for comparative immunogenicity assessments, especially during early stages of vaccine development and manufacturing changes.

The use of the Sabin S19 strains as challenge viruses proved appropriate, with results confirming their comparability to the monovalent Sabin strains previously used. Their ability to be handled at Biosafety Level 2 also supports broader international application in collaborative studies and routine testing, facilitating wider participation and improving global consistency.

The inter-laboratory variability observed in this study ranged from 20% to 80% GCVs, which is consistent with the 37.2% to 70.4% range reported in a previous, comparable EDQM-coordinated study assessing the suitability of the rat assay for cIPV. This level of between-laboratory agreement is within expectations for *in vivo* assays (9). Overall, the moderate variability observed is acceptable and underscores the importance of continued standardisation, harmonised procedures, and ongoing training across participating laboratories to further minimise residual discrepancies.

Comparisons between cIPV and sIPV samples have established that the D-antigen content per virion can differ significantly. It has been demonstrated that the rat immunogenicity of sIPV1 is approximately ~3-fold higher, and that of sIPV2 approximately ~10-fold lower, than the corresponding cIPV types 1 and 2 respectively. The specific immunogenicity of sIPV3 and cIPV3 was comparable (19). The results obtained from this study appear to consistently mirror these findings. 12/104 relative to 17/160 had a lower type 1, higher type 2 and fairly comparable type 3 median values. The comparability between 17/130 and 12/104 is striking for all three types (taking into consideration the D-Antigen content) as 12/104 is the established cIPV IS and its utility has been demonstrated for vaccine assessment in multiple rat assays. Comparisons between 17/130 and 17/160 were also consistent when Sabin D-antigen units were considered. There was lower type 1, higher type 2 and comparable type 3 median values for 17/130 relative to 17/160. This also suggests that potencies of Sabin vaccines *in vitro* and *in vivo* are comparable.

The pharmacopoeial method used to analyse these data assumes that the reference and test vaccines have comparable potencies, because the cut-off values are derived from the performance of the reference preparation. However, this assumption may not always hold for sIPV. Unlike cIPV, sIPV vaccines from different manufacturers have been shown to contain differing amounts of type 1, 2 and 3 antigens, meaning that antigen composition can vary substantially between products. This variability was evident in the comparison of references 17/160 and 17/130 in this and the previous WHO study (13). Therefore, both 17/160 and 17/130 are being proposed as reference reagents for the sIPV rat potency test, to better accommodate inter-manufacturer variability in sIPV vaccine composition.

The results from this study support the application of both 17/160 and 17/130 as appropriate reference reagents for potency assays, capable of serving as a benchmark for evaluating both current and future sIPV products. It is, however, critical that the chosen reference standard should closely match the antigen content of the test vaccine to ensure that the assay meets the required validity criteria. The long-term stability analysis of both 17/160 and 17/130 confirmed that their potencies have been maintained over seven years of storage at -70°C. This demonstrates their suitability for use in global IPV standardisation efforts, providing confidence to manufacturers and regulatory bodies in their reliability as reference materials.

In conclusion, the findings from this collaborative study validate the use of WHO IS 17/160 and 17/130 reference reagent as suitable reference reagents for sIPV in rat potency assays. They demonstrated good stability, appropriate immunogenicity profiles, and acceptable variability in assay performance across multiple laboratories. As the global shift towards IPV continues, having such robust references will be crucial in supporting vaccine quality and regulatory harmonisation efforts worldwide.

Recommendations

It is proposed that:

- The WHO International Standard for sIPV, 17/160, is additionally a WHO Reference Reagent for the *in vivo* potency assay.

- At the same time, 17/130 is also suitable as a WHO Reference Reagent for the *in vivo* potency assay.

They both demonstrated consistent dose-dependent responses across laboratories and poliovirus types and yielded relative potency estimates in line with previously assigned D-antigen values established via *in vitro* methods.

Sabin D-antigen units (SDU/mL) were assigned to 17/160 and 17/130 in an earlier WHO collaborative study (13) and this study provides *in vivo* confirmation of the utility of these reference preparations in potency testing using the rat model. These results support their ongoing use as reference materials for evaluating sIPV products.

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Tables

Table 1: Product summary of candidate materials

NIBSC Code	17/130	17/160
Presentation	0.5 ml screw cap tubes	0.5 ml screw cap tubes
No. of containers	4764	1749
Mean fill volume	0.5ml	0.5ml
Date of fill	May 2017	Sep 2017
Storage temperature	-70°C	-70°C
Microbial contamination	None detected	None detected

Table 2: Details of immunogenicity assays used by participants

Laboratory	Weight range of rats (g)	Volume of inoculation (ml)	Route of inoculation	Time of terminal bleed (days)
1	180-220	0.5	I.M	21
2	160-225	0.5	I.M	21
3	175-200	0.5	I.M	21
4	170-200	0.5	I.M	21
5	175-250	0.5	I.M	20-22

Table 3: Details of neutralisation assays used by participants

Lab	Challenge virus strain	Challenge virus dose (TCID ₅₀)	Serum-virus incubation time (h/°C)	No. of replicates	Serum dilution range	Types of cells	Post-inoculation times (days/°C)
1	Sabin S19 Types 1,2 and 3	100	3/35	2	1:2 – 1:4096	Hep-2	7/35
2	Sabin S19 Types 1,2 and 3	100	3/35	2	1:2 – 1:4096	Hep-2C	6/35
3	Sabin S19 Types 1,2 and 3	100	3/37	2	1:2 – 1:4096	Hep-2	7/35.5
4	Sabin S19 Types 1,2 and 3	100	3/35	2	Neat – 1:2048	Hep-2C	8/35
5	Sabin S19 Types 1,2 and 3	100	3/36	3	1:8 – 1:1024	Hep-2C	5-6/36

Table 4: Details of sample potencies

Samples	Units D-Antigen units / Sabin D Antigen units	Serotype		
		1	2	3
cIPV 12/104	D _{Ag} /ml	277	65	248
sIPV 17/160	D _{Ag} /ml	239	136	237
	S _{DU} /ml	100	100	100
sIPV 17/130	D _{Ag} /ml	50	608	450
	S _{DU} /ml	26	417	197

Table 5: Intra-laboratory variation – ratio of maximum to minimum relative potency estimates for laboratories where $n \geq 2$ valid estimates obtained

Serotype	Lab	12/104 relative to 17/130	17/160 relative to 17/130	12/104 relative to 17/160	17/130 relative to 17/160
1	1	3.12	1.19	n=1	n=0
1	2	1.75	1.78	1.97	1.32
1	3	1.25	n=1	n=1	n=1
1	4	n=0	n=0	1.13	n=0
1	5F	2.30	1.29	n=1	1.29
2	1	3.35	1.21	1.15	n=0
2	2	n=1	n=0	n=1	n=1
2	3	2.20	n=1	1.61	1.75
2	4	n=1	n=0	1.04	n=0
2	5F	2.01	n=0	n=1	n=0
3	1	1.70	1.14	1.38	n=1
3	2	1.60	1.08	2.62	1.97
3	3	1.03	1.35	1.41	1.35
3	4	3.81	1.27	4.06	1.28
3	5F	1.69	n=1	3.78	n=1

Table 6: Summary of relative potencies

Comparison	Summary Statistics	Type 1	Type 2	Type 3
12/104 rel. to 17/130	GM	0.99	0.79	0.96
	Min	0.44	0.36	0.44
	Max	1.81	1.99	2.98
	n	9	9	13
	GCV	65%	78%	63%
	% within 25-400%	100%	100%	100%
	% valid	60%	60%	87%
17/160 rel. to 17/130	GM	2.95	0.20	0.69
	Min	0.56	0.16	0.46
	Max	6.19	0.28	0.91
	n	8	4	10
	GCV	162%	27%	21%
	% within 25-400%	75%	100%	100%
	% valid	53%	27%	67%
12/104 rel. to 17/160	GM	0.23	4.68	1.44
	Min	0.13	2.85	0.67
	Max	0.74	9.90	4.21
	n	7	8	13
	GCV	79%	62%	73%
	% within 25-400%	100%	100%	100%
	% valid	47%	53%	87%
17/130 rel. to 17/160	GM	0.36	7.19	1.53
	Min	0.07	4.94	1.09
	Max	1.78	8.74	2.46
	n	5	3	8
	GCV	307%	39%	28%
	% within 25-400%	60%	100%	100%
	% valid	33%	20%	53%

Table 7a: Sample ED50 values, using 17/130 as a reference

Lab	Serotype	17/160			17/130			12/104		
		Assay 1	Assay 2	Assay 3	Assay 1	Assay 2	Assay 3	Assay 1	Assay 2	Assay 3
1	1	NSR	0.082	0.054	0.155	0.426	0.222	0.068	NSR	0.434
2	1	0.139	0.050	0.095	0.532	0.310	0.583	0.343	0.292	0.337
3	1	NSR	0.036	0.082	0.306	0.316	0.335	0.167	0.250	0.388
4	1	NSR	0.036	0.036	0.371	0.259	0.270	0.073	0.189	0.089
5F	1	0.455	NSR	-	0.268	0.550	0.751	0.616	NSR	0.751
1	2	0.203	1.132	0.941	0.118	0.234	0.170	0.735	0.120	0.258
2	2	NSR	NSR	NSR	0.173	0.109	0.136	NSR	0.091	0.167
3	2	1.216	NSR	NSR	0.297	0.088	0.150	0.076	0.150	0.119
4	2	1.414	-	NSR	NSR	0.282	0.410	0.741	0.614	0.822
5F	2	-	-	1.414	0.203	0.091	0.519	0.561	NSR	0.711
1	3	0.475	0.308	0.177	0.250	0.201	0.183	0.273	0.444	0.263
2	3	1.153	0.458	0.507	0.570	0.282	0.760	0.375	0.303	0.532
3	3	0.422	0.259	0.345	0.286	0.236	0.325	0.342	0.284	0.376
4	3	0.271	0.160	0.197	0.218	0.132	0.154	0.082	0.169	0.112
5F	3	0.625	NSR	1.414	0.282	0.536	NSR	0.536	0.561	0.352

NSR = No Significant Regression

- = Insufficient positive or negative data

Table 7b: Sample ED50 values, using 17/160 as a reference

Lab	Serotype	17/160			17/130			12/104		
		Assay 1	Assay 2	Assay 3	Assay 1	Assay 2	Assay 3	Assay 1	Assay 2	Assay 3
1	1	0.050	0.082	0.223	NSR	-	NSR	1.433	-	1.216
2	1	0.304	0.124	0.171	1.128	0.717	0.793	1.049	1.043	0.667
3	1	0.086	0.092	0.154	-	NSR	NSR	NSR	NSR	NSR
4	1	0.181	0.204	0.187	NSR	-	NSR	1.201	0.829	0.861
5F	1	0.455	NSR	-	0.268	0.550	0.751	0.616	NSR	0.751
1	2	0.195	0.258	0.171	0.047	0.047	0.047	0.065	0.047	0.072
2	2	0.903	0.439	0.532	0.064	NSR	0.063	NSR	0.035	0.063
3	2	0.532	0.434	0.404	0.106	0.063	0.065	0.039	0.078	0.035
4	2	0.155	0.266	0.157	-	-	NSR	0.054	0.095	0.063
5F	2	0.282	NSR	NSR	0.054	0.086	0.116	0.095	0.119	0.333
1	3	NSR	0.308	0.086	0.218	0.183	0.154	0.207	0.313	0.263
2	3	0.614	0.343	0.381	0.452	0.138	0.710	0.201	0.215	0.337
3	3	0.422	0.259	0.345	0.286	0.236	0.325	0.342	0.284	0.376
4	3	0.339	0.223	0.268	0.257	0.181	0.183	0.091	0.199	0.145
5F	3	0.247	NSR	NSR	NSR	0.467	0.485	0.400	0.346	0.352

NSR = No Significant Regression

- = Insufficient positive or negative data

Figures

Figure 1a: Potencies of 12/104 and 17/130 relative to 17/160 comparing the monovalent Sabin strains versus Sabin S19 challenge strains (Geometric mean with 2 Standard Error bars).

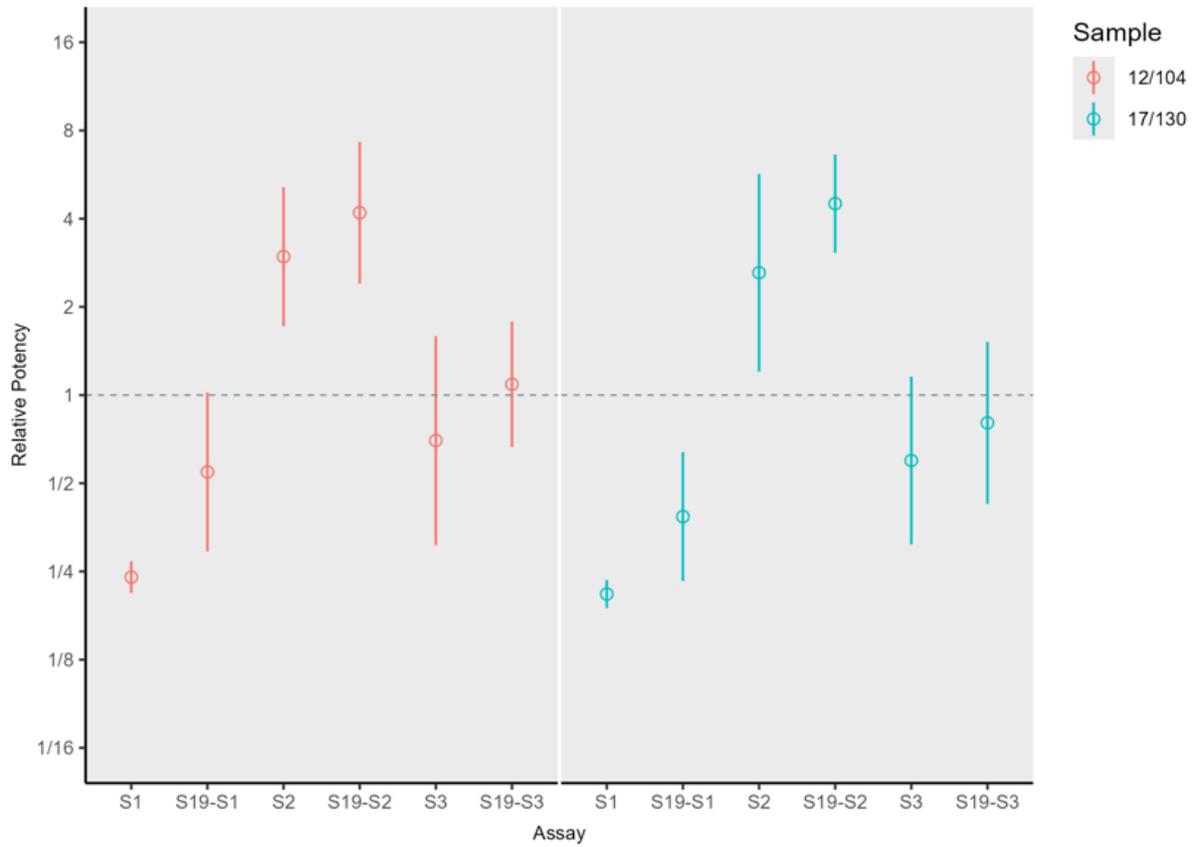


Figure 1b: Potencies of 12/104 and 17/160 relative to 17/130 comparing the monovalent Sabin strains versus Sabin S19 challenge strains (Geometric mean with 2 Standard Error bars).

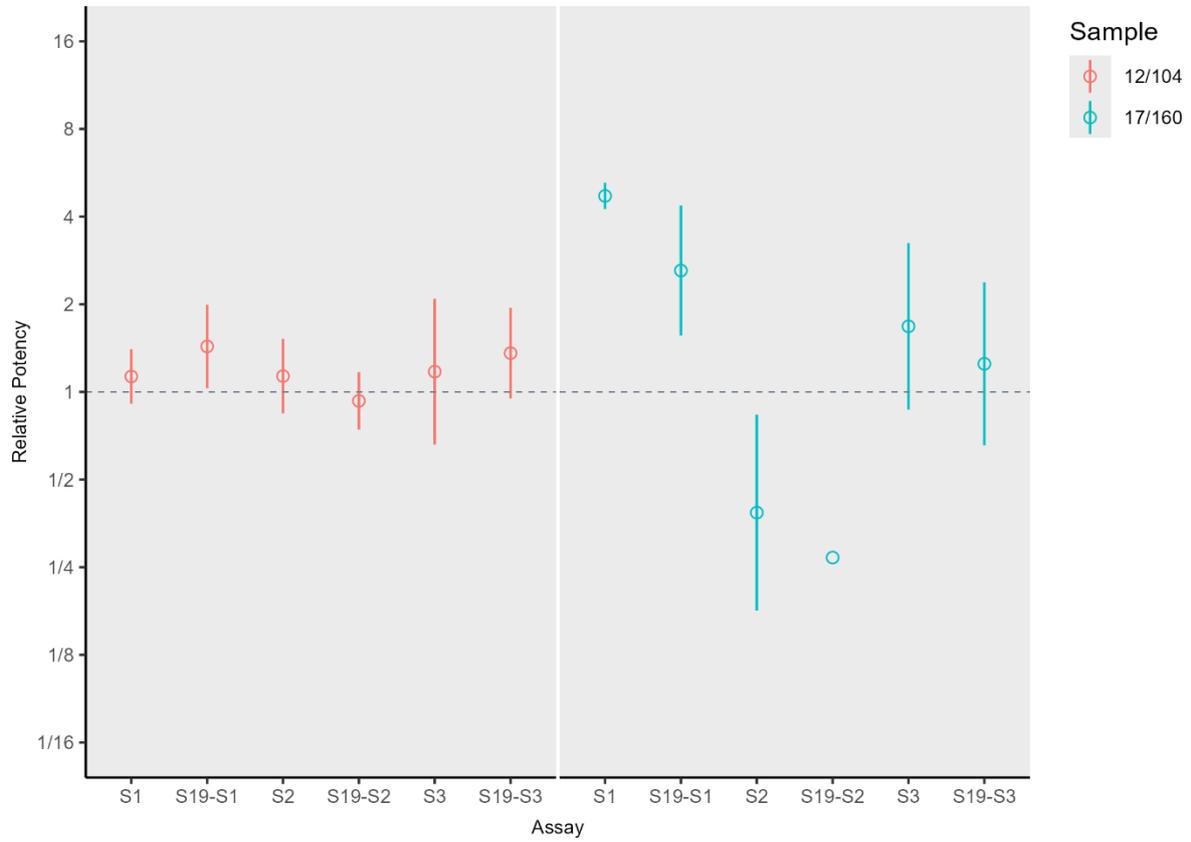


Figure 2: Summary of relative potencies of all serotypes

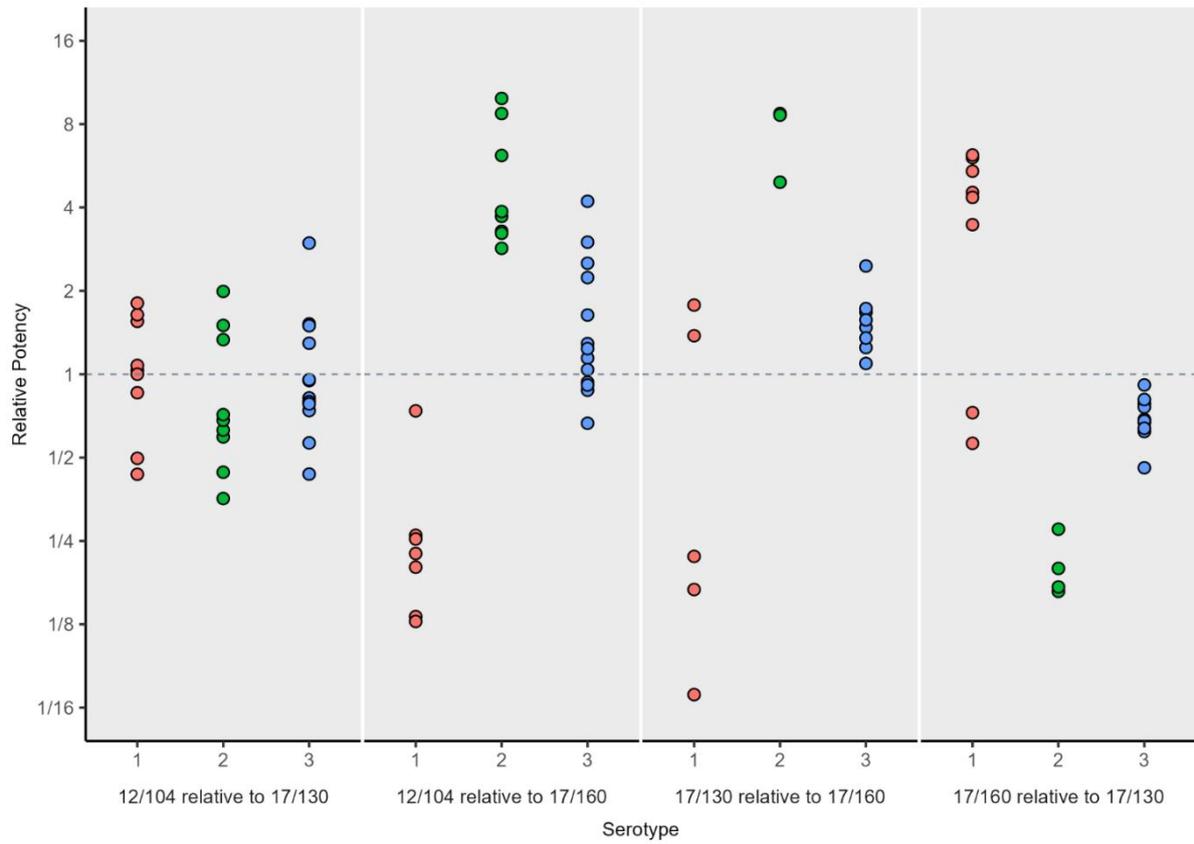


Figure 3a: Real time stability data of 17/160

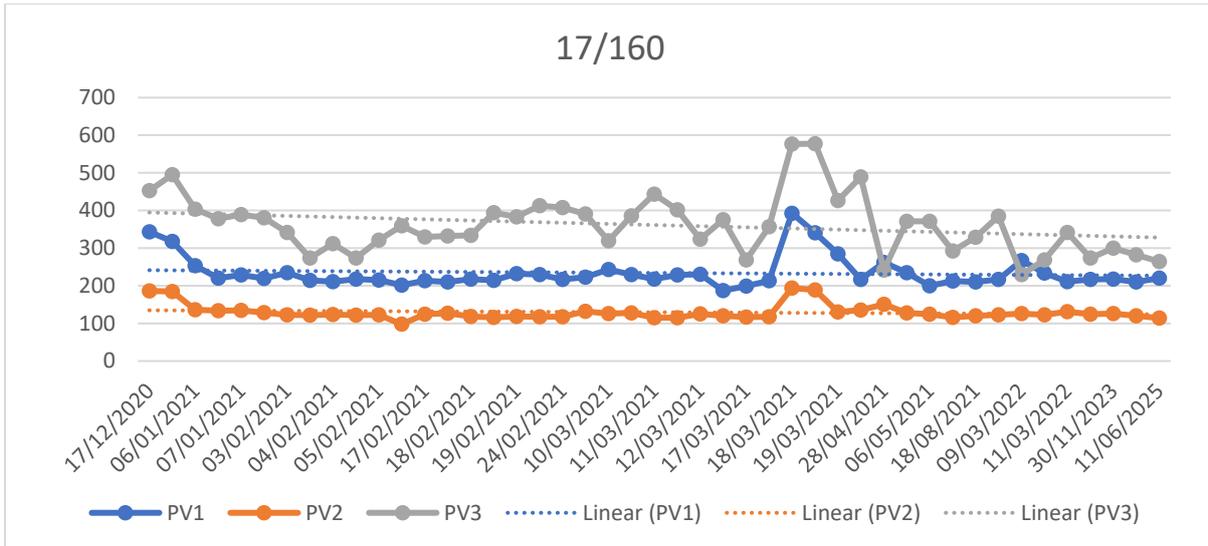
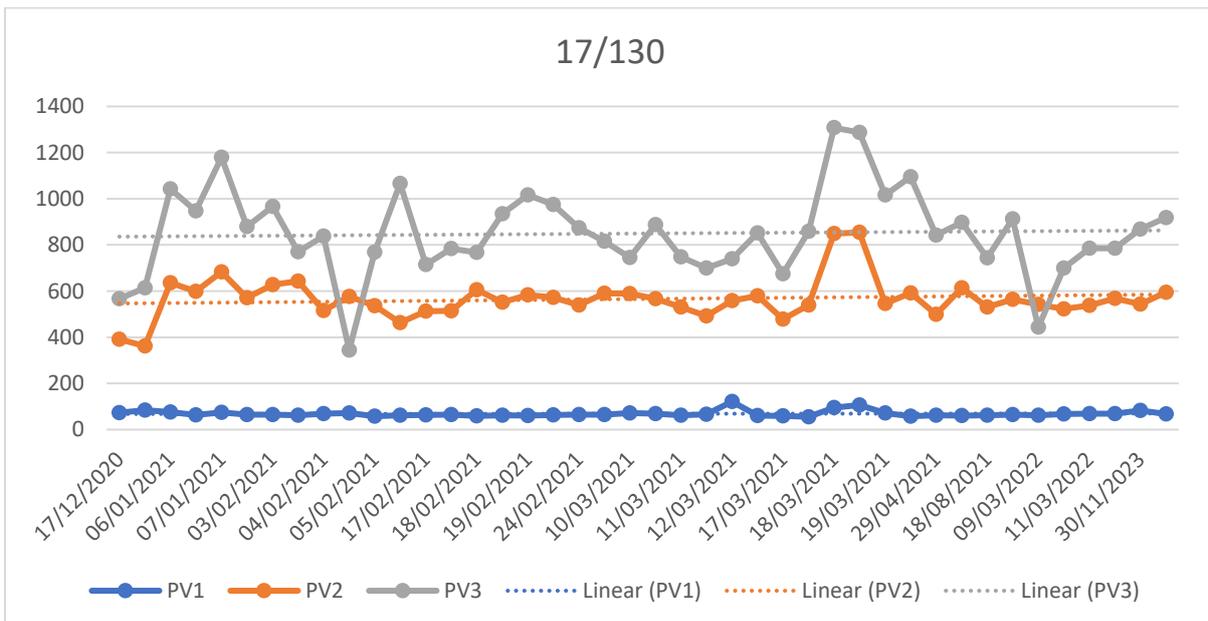


Figure 3b: Real time stability data of 17/130



Appendix 1. Collaborative study participants

Name	Laboratory	Country
Dan Yu Jun Li	Sinovac Biotech Co., Ltd.	China
Jie Song Yao Yufeng	Institute of Medical Biology, Chinese Academy of Medical Science (IMBCAMS)	China
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Mohammad Feraz Ahsan Gurbaksh Singh, VK Srinivas	Bharat Biotech International Ltd	India
Elaine Pegg	MHRA	UK

Appendix 2

Table 1a: Serotype 1 potency estimates, using 17/130 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/130	17/160 rel. to 17/130
1	1	17/160	10 / 10	10 / 10	10 / 10	10 / 10	1.55	RR
		17/130	10 / 10	10 / 10	7 / 10	1 / 10		
		12/104	10 / 10	9 / 10	8 / 10	5 / 10		
	2	17/160	10 / 10	10 / 10	10 / 10	3 / 10	NP	5.41
		17/130	10 / 10	5 / 10	2 / 10	0 / 10		
		12/104	7 / 10	5 / 10	3 / 10	3 / 10		
	3	17/160	10 / 10	10 / 10	10 / 10	6 / 10	0.50	4.53
		17/130	10 / 10	9 / 10	4 / 10	1 / 10		
		12/104	7 / 10	5 / 10	5 / 10	0 / 10		
2	1	17/160	8 / 10	8 / 9	5 / 9	3 / 9	1.64	3.47
		17/130	9 / 10	3 / 10	2 / 10	0 / 10		
		12/104	9 / 10	4 / 10	5 / 10	1 / 10		
	2	17/160	10 / 10	10 / 10	9 / 10	6 / 10	1.03	6.06
		17/130	10 / 10	5 / 10	4 / 10	1 / 10		
		12/104	7 / 10	7 / 10	7 / 10	0 / 10		
	3	17/160	10 / 10	10 / 10	10 / 10	2 / 10	1.81	6.19
		17/130	7 / 10	3 / 10	4 / 10	0 / 10		
		12/104	9 / 10	5 / 10	3 / 10	2 / 10		
3	1	17/160	10 / 10	10 / 10	9 / 10	8 / 10	NP	RR
		17/130	10 / 10	9 / 10	3 / 10	0 / 10		
		12/104	10 / 10	6 / 10	6 / 10	3 / 10		
	2	17/160	10 / 10	10 / 10	10 / 10	9 / 10	1.08	RR
		17/130	10 / 10	7 / 10	4 / 10	0 / 10		
		12/104	8 / 10	6 / 10	7 / 10	1 / 10		

	3	17/160 17/130 12/104	10 / 10 9 / 10 8 / 10	10 / 10 7 / 10 5 / 10	10 / 10 4 / 10 3 / 10	3 / 10 0 / 10 2 / 10	0.86	4.35
4	1	17/160 17/130 12/104	10 / 10 9 / 10 10 / 10	9 / 10 6 / 10 9 / 10	10 / 10 1 / 10 10 / 10	9 / 10 2 / 10 4 / 10	NL	RR
	2	17/160 17/130 12/104	10 / 10 9 / 10 10 / 10	10 / 10 7 / 10 10 / 10	10 / 10 5 / 10 9 / 10	9 / 10 1 / 10 0 / 10	NP	RR
	3	17/160 17/130 12/104	10 / 10 10 / 10 10 / 10	10 / 10 8 / 10 9 / 10	10 / 10 5 / 10 10 / 10	9 / 10 0 / 10 3 / 10	NL	RR
5F	1	17/160 17/130 12/104	5 / 5 5 / 5 5 / 5	2 / 5 5 / 5 1 / 5	1 / 5 2 / 5 0 / 5	0 / 5 0 / 5 0 / 5	0.44	0.56
	2	17/160 17/130 12/104	2 / 5 4 / 5 1 / 5	3 / 5 3 / 5 1 / 5	0 / 5 0 / 5 0 / 5	0 / 5 0 / 5 0 / 5	RR	0.73
	3	17/160 17/130 12/104	1 / 5 3 / 5 3 / 5	0 / 5 2 / 5 2 / 5	0 / 5 0 / 5 0 / 5	0 / 5 0 / 5 0 / 5	1.00	RR

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 1b: Serotype 1 potency estimates, using 17/160 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/160	17/130 rel. to 17/160
1	1	17/160	10 / 10	10 / 10	9 / 10	6 / 10	CI	RR
		17/130	2 / 10	1 / 10	1 / 10	0 / 10		
		12/104	4 / 10	2 / 10	2 / 10	0 / 10		
	2	17/160	10 / 10	10 / 10	10 / 10	3 / 10	RR	RR
		17/130	1 / 10	0 / 10	0 / 10	0 / 10		
		12/104	1 / 10	0 / 10	0 / 10	0 / 10		
	3	17/160	10 / 10	8 / 10	7 / 10	0 / 10	0.20	RR
		17/130	2 / 10	2 / 10	1 / 10	0 / 10		
		12/104	5 / 10	0 / 10	1 / 10	0 / 10		
2	1	17/160	7 / 10	7 / 9	4 / 9	1 / 9	CI	CI
		17/130	5 / 10	1 / 10	1 / 10	0 / 10		
		12/104	4 / 10	4 / 10	1 / 10	0 / 10		
	2	17/160	10 / 10	10 / 10	6 / 10	3 / 10	0.13	0.17
		17/130	8 / 10	1 / 10	1 / 10	0 / 10		
		12/104	4 / 10	4 / 10	2 / 10	0 / 10		
	3	17/160	10 / 10	9 / 10	9 / 10	0 / 10	0.26	0.22
		17/130	6 / 10	3 / 10	1 / 10	0 / 10		
		12/104	8 / 10	3 / 10	0 / 10	0 / 10		
3	1	17/160	10 / 10	10 / 10	8 / 10	4 / 10	RR	0.07
		17/130	5 / 10	0 / 10	0 / 10	0 / 10		
		12/104	3 / 10	2 / 10	0 / 10	0 / 10		
	2	17/160	10 / 10	10 / 10	9 / 10	3 / 10	RR	RR
		17/130	3 / 10	2 / 10	0 / 10	0 / 10		
		12/104	2 / 10	0 / 10	2 / 10	0 / 10		
	3	17/160	10 / 10	9 / 10	8 / 10	1 / 10	0.13	RR
		17/130	1 / 10	1 / 10	1 / 10	0 / 10		
		12/104	4 / 10	1 / 10	1 / 10	0 / 10		
		17/160	10 / 10	6 / 10	7 / 10	2 / 10		

4	1	17/130	2 / 10	0 / 10	0 / 10	1 / 10	CI	RR	
		12/104	4 / 10	3 / 10	3 / 10	0 / 10			
	2	17/160	10 / 10	7 / 10	5 / 10	2 / 10	0.25	RR	
		17/130	0 / 10	0 / 10	0 / 10	0 / 10			
	3	12/104	6 / 10	2 / 10	3 / 10	0 / 10	0.23	RR	
		17/160	10 / 10	10 / 10	5 / 10	1 / 10			
17/130	12/104	17/160	5 / 5	2 / 5	1 / 5	0 / 5	0.74	1.78	
		17/130	5 / 5	5 / 5	2 / 5	0 / 5			
5F	2	12/104	5 / 5	1 / 5	0 / 5	0 / 5	CI	1.38	
		17/160	2 / 5	3 / 5	0 / 5	0 / 5			
	3	17/130	4 / 5	3 / 5	0 / 5	0 / 5	RR(Ref)	RR(Ref)	
		12/104	2 / 5	1 / 5	0 / 5	0 / 5			
	17/160	17/130	17/160	1 / 5	0 / 5	0 / 5	0 / 5	RR(Ref)	RR(Ref)
			17/130	3 / 5	2 / 5	0 / 5	0 / 5		
12/104	3 / 5	2 / 5	2 / 5	0 / 5	0 / 5	RR(Ref)	RR(Ref)		
		3 / 5	2 / 5	0 / 5	0 / 5				

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 2a: Serotype 2 potency estimates, using 17/130 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/130	17/160 rel. to 17/130
1	1	17/160	6 / 10	3 / 10	3 / 10	0 / 10	0.59	0.16
		17/130	10 / 10	9 / 10	9 / 10	2 / 10		
		12/104	10 / 10	9 / 10	5 / 10	1 / 10		
	2	17/160	4 / 10	2 / 10	0 / 10	0 / 10	1.99	0.17
		17/130	9 / 10	7 / 10	6 / 10	1 / 10		
		12/104	10 / 10	9 / 10	7 / 10	3 / 10		
	3	17/160	5 / 10	3 / 10	1 / 10	0 / 10	0.63	0.20
		17/130	10 / 10	10 / 10	6 / 10	1 / 10		
		12/104	9 / 10	8 / 10	6 / 10	0 / 10		
2	1	17/160	2 / 10	0 / 10	1 / 10	0 / 10	CI	RR
		17/130	5 / 6	8 / 10	5 / 10	3 / 10		
		12/104	9 / 10	7 / 10	8 / 10	5 / 10		
	2	17/160	3 / 10	1 / 10	0 / 10	0 / 10	1.33	RR
		17/130	10 / 10	7 / 10	7 / 10	4 / 10		
		12/104	10 / 10	8 / 10	8 / 10	4 / 10		
	3	17/160	2 / 10	2 / 10	0 / 10	0 / 10	NL	RR
		17/130	9 / 10	10 / 10	9 / 10	1 / 10		
		12/104	10 / 10	8 / 10	8 / 10	1 / 10		
3	1	17/160	5 / 10	0 / 10	1 / 10	0 / 10	NP	0.28
		17/130	10 / 10	8 / 10	4 / 10	0 / 10		
		12/104	10 / 10	9 / 10	7 / 10	5 / 10		
	2	17/160	3 / 10	2 / 10	1 / 10	0 / 10	0.68	RR
		17/130	10 / 10	9 / 10	8 / 10	4 / 10		
		12/104	9 / 10	10 / 10	8 / 10	1 / 10		
	3	17/160	3 / 10	1 / 10	0 / 10	0 / 10	1.50	RR
		17/130	9 / 10	9 / 10	7 / 10	2 / 10		
		12/104	10 / 10	10 / 10	8 / 10	2 / 10		
		17/160	0 / 10	3 / 10	0 / 10	0 / 10		

4	1	17/130	5 / 10	7 / 10	7 / 10	3 / 10	CI	RR
		12/104	6 / 10	3 / 10	4 / 10	1 / 10		
	2	17/160	1 / 10	0 / 10	0 / 10	0 / 10	0.44	RR
		17/130	8 / 10	7 / 10	5 / 10	1 / 10		
	3	12/104	6 / 10	6 / 10	1 / 10	0 / 10	NL	RR
		17/160	1 / 10	0 / 10	0 / 10	1 / 10		
		17/130	9 / 10	2 / 10	4 / 10	3 / 10		
		12/104	5 / 10	5 / 10	2 / 10	1 / 10		
5F	1	17/160	2 / 5	0 / 5	0 / 5	0 / 5	0.36	CI
		17/130	5 / 5	4 / 5	4 / 5	0 / 5		
		12/104	3 / 5	4 / 5	0 / 5	0 / 5		
	2	17/160	1 / 5	0 / 5	0 / 5	0 / 5	CI	RR
		17/130	5 / 5	4 / 5	4 / 5	2 / 5		
		12/104	3 / 5	1 / 5	1 / 5	0 / 5		
3	17/160	0 / 5	1 / 5	0 / 5	0 / 5	0.71	RR	
	17/130	5 / 5	1 / 5	1 / 5	0 / 5			
	12/104	4 / 5	1 / 5	0 / 5	0 / 5			

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 2b: Serotype 2 potency estimates, using 17/160 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/160	17/130 rel. to 17/160
1	1	17/160	9 / 10	8 / 10	7 / 10	1 / 10	3.28	RR
		17/130	10 / 10	10 / 10	10 / 10	7 / 10		
		12/104	10 / 10	10 / 10	9 / 10	5 / 10		
	2	17/160	9 / 10	8 / 10	6 / 10	0 / 10	RR	RR
		17/130	10 / 10	10 / 10	10 / 10	7 / 10		
		12/104	10 / 10	10 / 10	10 / 10	7 / 10		
	3	17/160	10 / 10	8 / 10	6 / 10	2 / 10	2.85	RR
		17/130	10 / 10	10 / 10	10 / 10	7 / 10		
		12/104	10 / 10	10 / 10	10 / 10	4 / 10		
2	1	17/160	4 / 10	5 / 10	2 / 10	0 / 10	RR	CI
		17/130	6 / 6	10 / 10	9 / 10	5 / 10		
		12/104	9 / 10	10 / 10	9 / 10	8 / 10		
	2	17/160	8 / 10	8 / 10	1 / 10	0 / 10	RR	RR
		17/130	10 / 10	9 / 10	8 / 10	7 / 10		
		12/104	10 / 10	10 / 10	9 / 10	7 / 10		
	3	17/160	9 / 10	3 / 10	2 / 10	0 / 10	8.74	8.74
		17/130	10 / 10	10 / 10	10 / 10	5 / 10		
		12/104	10 / 10	10 / 10	10 / 10	5 / 10		
3	1	17/160	9 / 10	3 / 10	2 / 10	0 / 10	9.90	4.94
		17/130	10 / 10	9 / 10	10 / 10	2 / 10		
		12/104	10 / 10	9 / 10	10 / 10	6 / 10		
	2	17/160	7 / 10	5 / 10	5 / 10	0 / 10	6.16	8.62
		17/130	10 / 10	10 / 10	10 / 10	5 / 10		
		12/104	10 / 10	10 / 10	9 / 10	4 / 10		
	3	17/160	10 / 10	4 / 10	2 / 10	1 / 10	RR	NL
		17/130	10 / 10	10 / 10	9 / 10	5 / 10		
		12/104	10 / 10	10 / 10	8 / 10	7 / 10		
		17/160	10 / 10	8 / 10	7 / 10	2 / 10		

4	1	17/130 12/104	10 / 10 10 / 10	10 / 10 10 / 10	10 / 10 10 / 10	10 / 10 6 / 10	3.72	RR
	2	17/160 17/130 12/104	8 / 10 10 / 10 10 / 10	5 / 10 10 / 10 10 / 10	6 / 10 8 / 10 10 / 10	2 / 10 10 / 10 2 / 10	NP	RR
	3	17/160 17/130 12/104	9 / 10 9 / 10 10 / 10	7 / 10 9 / 10 10 / 10	8 / 10 9 / 10 10 / 10	2 / 10 10 / 10 5 / 10	3.87	RR
5F	1	17/160 17/130 12/104	5 / 5 5 / 5 5 / 5	3 / 5 5 / 5 5 / 5	3 / 5 5 / 5 5 / 5	0 / 5 3 / 5 1 / 5	3.23	CI
	2	17/160 17/130 12/104	3 / 5 5 / 5 5 / 5	1 / 5 5 / 5 5 / 5	2 / 5 4 / 5 4 / 5	0 / 5 2 / 5 1 / 5	CI	CI
	3	17/160 17/130 12/104	1 / 5 5 / 5 4 / 5	1 / 5 4 / 5 4 / 5	0 / 5 3 / 5 2 / 5	0 / 5 2 / 5 0 / 5	CI	CI

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 2c: Serotype 2 potency estimates, using 17/160 as the reference with alternative cut-off calculation

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/160	17/130 rel. to 17/160

1	1	17/160	6 / 10	7 / 10	3 / 10	0 / 10	2.66	4.25
		17/130	10 / 10	9 / 10	9 / 10	2 / 10		
		12/104	10 / 10	10 / 10	5 / 10	1 / 10		
	2	17/160	5 / 10	7 / 10	1 / 10	0 / 10	5.68	NL
		17/130	9 / 10	10 / 10	10 / 10	1 / 10		
		12/104	10 / 10	10 / 10	7 / 10	3 / 10		
	3	17/160	6 / 10	5 / 10	2 / 10	0 / 10	3.50	6.92
		17/130	10 / 10	10 / 10	9 / 10	3 / 10		
		12/104	9 / 10	9 / 10	7 / 10	1 / 10		
2	1	17/160	2 / 10	1 / 10	1 / 10	0 / 10	CI	CI
		17/130	6 / 6	10 / 10	8 / 10	4 / 10		
		12/104	9 / 10	9 / 10	9 / 10	6 / 10		
	2	17/160	5 / 10	5 / 10	1 / 10	0 / 10	CI	CI
		17/130	10 / 10	8 / 10	7 / 10	4 / 10		
		12/104	10 / 10	8 / 10	8 / 10	6 / 10		
	3	17/160	5 / 10	2 / 10	1 / 10	0 / 10	10.43	9.47
		17/130	10 / 10	10 / 10	10 / 10	2 / 10		
		12/104	10 / 10	9 / 10	9 / 10	3 / 10		
3	1	17/160	7 / 10	0 / 10	1 / 10	0 / 10	CI	NL
		17/130	10 / 10	9 / 10	4 / 10	0 / 10		
		12/104	10 / 10	9 / 10	9 / 10	5 / 10		
	2	17/160	3 / 10	5 / 10	2 / 10	0 / 10	NP	CI
		17/130	10 / 10	10 / 10	9 / 10	4 / 10		
		12/104	10 / 10	10 / 10	8 / 10	1 / 10		
	3	17/160	4 / 10	3 / 10	0 / 10	0 / 10	CI	8.39
		17/130	10 / 10	9 / 10	8 / 10	2 / 10		
		12/104	10 / 10	10 / 10	8 / 10	6 / 10		
4	1	17/160	4 / 10	3 / 10	0 / 10	1 / 10	NL	CI
		17/130	8 / 10	8 / 10	8 / 10	5 / 10		
		12/104	10 / 10	9 / 10	9 / 10	2 / 10		
		17/160	4 / 10	0 / 10	0 / 10	0 / 10		

	2	17/130	10 / 10	9 / 10	6 / 10	5 / 10	CI	CI
		12/104	8 / 10	8 / 10	4 / 10	1 / 10		
	3	17/160	2 / 10	0 / 10	0 / 10	1 / 10	NL	NSR
		17/130	9 / 10	3 / 10	5 / 10	3 / 10		
	1	12/104	9 / 10	7 / 10	5 / 10	1 / 10	CI	CI
		17/160	2 / 5	2 / 5	0 / 5	0 / 5		
5F	2	17/130	5 / 5	4 / 5	5 / 5	1 / 5	CI	CI
		12/104	4 / 5	5 / 5	1 / 5	0 / 5		
	3	17/160	1 / 5	0 / 5	1 / 5	0 / 5	CI	CI
		17/130	5 / 5	4 / 5	4 / 5	2 / 5		
	1	12/104	4 / 5	2 / 5	1 / 5	0 / 5	CI	CI
		17/160	0 / 5	1 / 5	0 / 5	0 / 5		
	2	17/130	5 / 5	2 / 5	1 / 5	0 / 5	CI	CI
		12/104	4 / 5	2 / 5	0 / 5	0 / 5		
	3	17/160	0 / 5	1 / 5	0 / 5	0 / 5	CI	CI
		17/130	5 / 5	2 / 5	1 / 5	0 / 5		
	1	12/104	4 / 5	2 / 5	0 / 5	0 / 5	CI	CI
		17/160	0 / 5	1 / 5	0 / 5	0 / 5		

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 3a: Serotype 3 potency estimates, using 17/130 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/130	17/160 rel. to 17/130
1	1	17/160	9 / 10	2 / 10	3 / 10	3 / 10	NP	NL
		17/130	10 / 10	10 / 10	5 / 10	0 / 10		
		12/104	9 / 10	7 / 10	3 / 10	2 / 10		

	2	17/160	8 / 10	9 / 10	4 / 10	0 / 10	0.44	0.69
		17/130	10 / 10	8 / 10	6 / 10	1 / 10		
		12/104	6 / 10	7 / 10	3 / 10	1 / 10		
	3	17/160	8 / 10	8 / 10	5 / 10	3 / 10	0.74	0.78
		17/130	10 / 10	8 / 10	7 / 10	1 / 10		
		12/104	10 / 10	7 / 10	6 / 10	0 / 10		
2	1	17/160	4 / 10	3 / 9	1 / 9	0 / 10	1.52	0.67
		17/130	9 / 10	3 / 10	1 / 10	0 / 10		
		12/104	10 / 10	7 / 10	2 / 10	0 / 10		
	2	17/160	9 / 10	4 / 10	3 / 10	0 / 10	0.95	0.62
		17/130	10 / 10	5 / 10	5 / 10	1 / 10		
		12/104	8 / 10	8 / 10	5 / 10	0 / 10		
3	17/160	6 / 10	6 / 10	3 / 10	1 / 10	1.50	NP	
	17/130	8 / 10	1 / 10	0 / 9	0 / 10			
	12/104	9 / 10	3 / 10	2 / 10	0 / 10			
3	1	17/160	9 / 10	4 / 10	4 / 10	0 / 10	0.82	0.68
		17/130	10 / 10	7 / 10	3 / 10	1 / 10		
		12/104	9 / 10	5 / 10	4 / 10	1 / 10		
	2	17/160	10 / 10	6 / 10	5 / 10	1 / 10	0.79	0.91
		17/130	10 / 10	6 / 10	6 / 10	1 / 10		
		12/104	7 / 10	9 / 10	4 / 10	1 / 10		
3	17/160	8 / 10	6 / 10	4 / 10	1 / 10	0.80	NP	
	17/130	10 / 10	8 / 10	3 / 10	0 / 10			
	12/104	9 / 10	5 / 10	3 / 10	1 / 10			
4	1	17/160	10 / 10	5 / 10	4 / 10	2 / 10	2.98	0.76
		17/130	10 / 10	7 / 10	6 / 10	1 / 10		
		12/104	10 / 10	10 / 10	10 / 10	3 / 10		
	2	17/160	10 / 10	10 / 10	5 / 10	2 / 10	0.78	0.81
		17/130	10 / 10	10 / 10	7 / 10	2 / 10		
		12/104	10 / 10	9 / 10	7 / 10	1 / 10		
		17/160	9 / 10	7 / 10	6 / 10	2 / 10		

	3	17/130 12/104	10 / 10 10 / 10	9 / 10 10 / 10	8 / 10 7 / 10	1 / 10 3 / 10	1.29	0.64
5F	1	17/160	4 / 5	2 / 5	0 / 5	0 / 5	0.56	0.46
		17/130 12/104	5 / 5 5 / 5	3 / 5 2 / 5	3 / 5 0 / 5	0 / 5 0 / 5		
	2	17/160	1 / 5	2 / 5	0 / 5	0 / 5	0.96	NP
		17/130 12/104	5 / 5 3 / 5	2 / 5 4 / 5	0 / 5 0 / 5	0 / 5 0 / 5		
	3	17/160	0 / 5	1 / 5	0 / 5	0 / 5	NP	RR
		17/130 12/104	2 / 5 5 / 5	0 / 5 4 / 5	2 / 5 1 / 5	0 / 5 0 / 5		

RR = Insufficient coverage of response range for test sample

RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Table 3b: Serotype 3 potency estimates, using 17/160 as the reference

Lab	Assay	Sample	Dilutions				Potency Estimates	
			Neat	1/2	1/4	1/16	12/104 rel. to 17/160	17/130 rel. to 17/160
1	1	17/160	9 / 10	4 / 10	5 / 10	4 / 10	1.29	NP
		17/130	10 / 10	10 / 10	7 / 10	0 / 10		
		12/104	10 / 10	8 / 10	4 / 10	2 / 10		
	2	17/160	8 / 10	9 / 10	4 / 10	0 / 10	0.93	1.68
		17/130	10 / 10	8 / 10	7 / 10	1 / 10		
		12/104	8 / 10	7 / 10	4 / 10	1 / 10		
	3	17/160	9 / 10	8 / 10	8 / 10	4 / 10	NP	NP
		17/130	10 / 10	9 / 10	8 / 10	1 / 10		
		12/104	10 / 10	7 / 10	6 / 10	0 / 10		
2	1	17/160	6 / 10	4 / 9	3 / 9	0 / 10	3.00	1.25
		17/130	10 / 10	3 / 10	3 / 10	0 / 10		
		12/104	10 / 10	8 / 10	6 / 10	1 / 10		
	2	17/160	9 / 10	4 / 10	5 / 10	1 / 10	1.64	2.46
		17/130	10 / 10	7 / 10	7 / 10	3 / 10		
		12/104	9 / 10	8 / 10	6 / 10	1 / 10		
	3	17/160	8 / 10	6 / 10	3 / 10	1 / 10	1.14	NP
		17/130	8 / 10	2 / 10	0 / 9	0 / 10		
		12/104	9 / 10	5 / 10	3 / 10	2 / 10		
3	1	17/160	9 / 10	4 / 10	4 / 10	0 / 10	1.24	1.48
		17/130	10 / 10	7 / 10	3 / 10	1 / 10		
		12/104	9 / 10	5 / 10	4 / 10	1 / 10		
	2	17/160	10 / 10	6 / 10	5 / 10	1 / 10	0.88	1.09
		17/130	10 / 10	6 / 10	6 / 10	1 / 10		
		12/104	7 / 10	9 / 10	4 / 10	1 / 10		
	3	17/160	8 / 10	6 / 10	4 / 10	1 / 10	0.91	NP
		17/130	10 / 10	8 / 10	3 / 10	0 / 10		
		12/104	9 / 10	5 / 10	3 / 10	1 / 10		
		17/160	9 / 10	4 / 10	4 / 10	2 / 10		

4	1	17/130	10 / 10	5 / 10	6 / 10	1 / 10	4.21	1.35
		12/104	9 / 10	10 / 10	9 / 10	3 / 10		
	2	17/160	10 / 10	10 / 10	3 / 10	1 / 10	1.04	NL
		17/130	8 / 10	10 / 10	5 / 10	2 / 10		
	3	12/104	9 / 10	9 / 10	6 / 10	1 / 10	2.23	1.73
		17/160	8 / 10	6 / 10	5 / 10	2 / 10		
5F	1	17/160	5 / 5	3 / 5	2 / 5	1 / 5	0.67	CI
		17/130	5 / 5	4 / 5	3 / 5	4 / 5		
		12/104	5 / 5	3 / 5	1 / 5	0 / 5		
	2	17/160	2 / 5	3 / 5	0 / 5	0 / 5	CI	1.57
		17/130	5 / 5	3 / 5	0 / 5	0 / 5		
		12/104	4 / 5	5 / 5	1 / 5	0 / 5		
3	17/160	2 / 5	2 / 5	0 / 5	0 / 5	2.52	CI	
	17/130	3 / 5	3 / 5	2 / 5	0 / 5			
	12/104	5 / 5	4 / 5	1 / 5	0 / 5			

RR = Insufficient coverage of response range for test sample

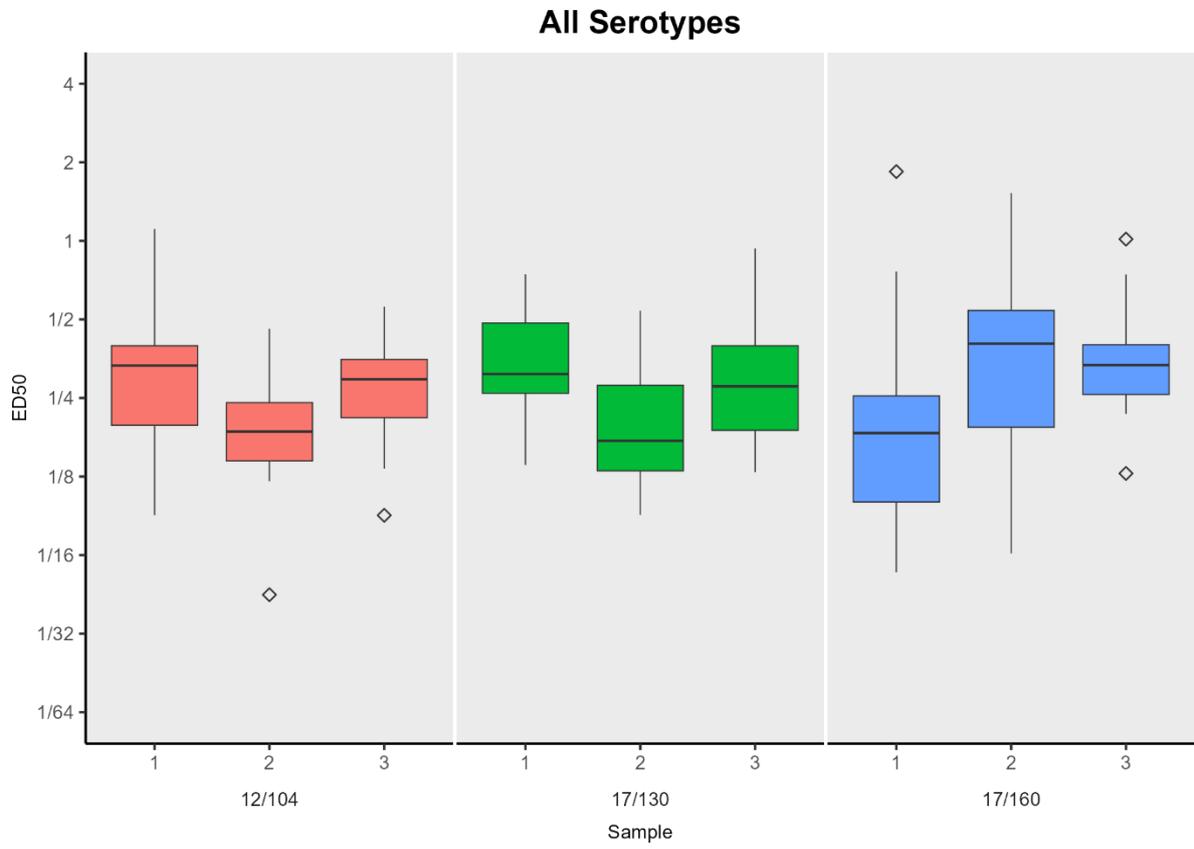
RR(Ref) = Insufficient coverage of response range for reference sample

NL = Non-Linear

NP = Non-Parallel

CI = Assay fails precision requirement (based on 95% confidence interval)

Figure 1. ED50 Values of Samples



Appendix 3

Preparation of sabin S19-PV stocks

Sabin S19-PV stocks should be prepared following a standard virus propagation protocol (see WHO Polio Manual for guidance) with the following requirements:

- Use the content of one ampoule to infect confluent HEp-2c cells in two 75 cm² flasks.
- Incubate the flasks, containing the inoculated culture, at 33°C.
- Examine the inoculated culture daily, using an inverted microscope, for the appearance of cytopathic effect (CPE).
- When 75 to 100% of cells show CPE (3+ to 4+ CPE), transfer the flask to <-20°C. Freeze and thaw the contents of the flask, shaking the flask when it is semi-thawed to ensure that all cells are disrupted. Repeat two additional times.
- Spin for 20 minutes at 1500g in a refrigerated centrifuge after ensuring that centrifuge caps are securely in place and centrifuge buckets are sealed.
- Label each storage vial with the name of the cell line, the name of the virus preparation and the date.
- Working in a BSC, transfer aliquots of the supernatant into labelled storage vials (0.1 ml supernatant per vial to give a total of approximately 500 aliquots).
- Store the aliquots of the virus preparation, which should be used subsequently as the laboratory reference standards, in a <-80°C freezer designated for storage of infectious materials.
- Few aliquots should be used for quality control of each stock virus. It might be required that few aliquots are sent to NIBSC for further characterization.
- The titre of each laboratory reference preparation should be determined and assigned following standard procedures (see Polio Manual for guidance). Three independent titration determinations should be used for this purpose.
- A master stock should be prepared initially and then working stocks can be prepared from it but the sabin S-19 PV strains should never be subjected to more than two passages from the original stocks.

Appendix 4

Summary of comments from participants

The final report was sent to all contributors involved in the study for their comments and feedback. Participants replied supporting the report together with its recommendations and there were no comments for amendments.

Appendix 5

Proposed instruction for use

**WHO 1st IS for Sabin Inactivated Polio Vaccines *in vitro* and
WHO Reference Reagent for Sabin Inactivated Polio Vaccines *in vivo*
NIBSC code 17/160**

“This material is not for *in vitro* diagnostic use”

1. INTRODUCTION

The WHO International Standard (IS) for Sabin Inactivated Polio Vaccine (sIPV) was established by the WHO Expert Committee on Biological Standardisation (ECBS) in October 2018. It was shown to be suitable for the determination of antigenic content of sIPV by *in vitro* assays. The preparation is a liquid trivalent blend of formaldehyde-inactivated monovalent pools of Sabin poliovirus type 1, 2 and 3. The material has been prepared by a manufacturer and has been tested for sterility and absence of adventitious agents. Due to the antigenic differences between IPV made with wild-type or Sabin strains the sIPV IS has been assigned a new antigen unit, Sabin D-Antigen Unit (SDU). This is independent of the D-Antigen units used for conventional IPV products, for which the IS 12/104 is required.

The WHO Reference Reagent for sIPV for the *in vivo* potency assay was also established by ECBS in April 2026. It was shown to be suitable for use as a control in the rat potency assay.

2. UNITAGE

Units assigned for WHO IS, 17/160, for the *in vitro* potency assay:

100 SDU/ml for poliovirus type 1

100 SDU/ml for poliovirus type 2

100 SDU/ml for poliovirus type 3

(SDU/ml: Sabin D-Antigen Unit per millilitre)

There are no units assigned for 17/160 as a WHO Reference Reagent for *in vivo* potency testing.

3. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

This preparation has been processed under clean controlled conditions but cannot be guaranteed sterile. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

4. DIRECTIONS FOR OPENING THE DIN AMPOULE

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

5. USE OF AMPOULED MATERIAL

The WHO Universal Reagents for the D-Antigen potency testing of Inactivated Polio Vaccines should be used as the capture and detection antibodies in a D-Antigen ELISA to measure vaccine potency. These should help standardize testing methods for manufacturers and national control laboratories allowing consistency and harmonisation. We would recommend these to be used in conjunction with the CBER method as detailed in Appendix 2.

Unopened ampoules should be stored at $\leq -20^{\circ}\text{C}$. Ampoules should be reconstituted on the day of the assay by adding exactly 0.5 ml of pure sterile distilled water. To remove the reagent from the ampoule it is necessary to use some form of transfer pipette rather than a volumetric pipette. The contents of the ampoules should not be assumed to be sterile.

This material is supplied for use in its final form and must not be further diluted other than as required for individual assay procedures. Each ampoule/vial is intended to be used only once. The vial should be opened as directed in section 4.

6. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC with assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Users should determine the stability of the material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

7. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the title of the preparation, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

8. PRODUCT LIABILITY AND LOSS

- 8.1 Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials ([http://www.nibsc.org/About Us/Terms and Conditions.aspx](http://www.nibsc.org/About%20Us/Terms%20and%20Conditions.aspx)) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference.
- 8.2 Unless the context otherwise requires, the definitions in the Conditions shall apply.
- 8.3 Nothing in this document or the Conditions shall limit or exclude NIBSC's liability for fraud or fraudulent misrepresentation, death or personal injury caused by its negligence, or the negligence of its employees.
- 8.4 Subject to clause 0:
- 8.4.1 NIBSC shall under no circumstances whatsoever be liable to the Recipient, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of data, loss of profit, loss of business or goodwill, or any indirect or consequential loss or damage suffered or incurred by the Recipient arising in relation to the supply of the Materials or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or by any other person; and
- 8.4.2 NIBSC's total liability to the Recipient in respect of all other losses arising under or in connection with the Contract, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed 100% of the fees paid to NIBSC for the Materials.
- 8.5 The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

MATERIAL SAFETY SHEET

WHO 1st IS for Sabin Inactivated Polio Vaccines <i>in vitro</i> and WHO Reference Reagent for Sabin Inactivated Polio Vaccines <i>in vivo</i> NIBSC code 17/160 Physical properties (at room temperature)			
Physical appearance	Liquid		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	<i>Contains inactivated Sabin Poliovirus types 1, 2 and 3</i>		
Handling:	See caution, section 3		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation:	Seek medical advice		
Ingestion:	Seek medical advice		
Contact with eyes:	Wash with copious amounts of water. Seek medical advice.		
Contact with skin:	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with a virucidal agent. Rinse area with a virucidal agent followed by water.			
Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			

Proposed instruction for use

WHO Reference Reagent for Sabin Inactivated Polio Vaccines *in vivo* NIBSC codes 17/130

“This material is not for *in vitro* diagnostic use”

1. INTRODUCTION

The WHO International Standard (IS) for Sabin Inactivated Polio Vaccine (sIPV), 17/160, was established by the WHO Expert Committee on Biological Standardisation (ECBS) in October 2018. It was shown to be suitable for the determination of antigenic content of sIPV by *in vitro* assays. 17/130 was tested alongside this reference in the collaborative study and shown to be equally suitable. The preparation is a liquid trivalent blend of formaldehyde-inactivated monovalent pools of Sabin poliovirus type 1, 2 and 3. The material has been prepared by a manufacturer and has been tested for sterility and absence of adventitious agents. Due to the antigenic differences between IPV made with wild-type or Sabin strains the sIPV IS has been assigned a new antigen unit, Sabin D-Antigen Unit (SDU). This is independent of the D-Antigen units used for conventional IPV products, for which the IS 12/104 is required.

The WHO Reference Reagent for sIPV, 17/130, was established for the *in vivo* potency assay by ECBS in April 2026. It was shown to be suitable for use as a control in the rat potency assay.

2. UNITAGE

Units assigned for 17/130 for the *in vitro* potency assay:

26 SDU/ml for poliovirus type 1

417 SDU/ml for poliovirus type 2

197 SDU/ml for poliovirus type 3

(SDU/ml: Sabin D-Antigen Unit per millilitre)

There are no units assigned for 17/130 as a WHO Reference Reagent for *in vivo* potency testing.

3. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

This preparation has been processed under clean controlled conditions but cannot be guaranteed sterile. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

4. DIRECTIONS FOR OPENING THE DIN AMPOULE

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

5. USE OF AMPOULED MATERIAL

The WHO Universal Reagents for the D-Antigen potency testing of Inactivated Polio Vaccines should be used as the capture and detection antibodies in a D-Antigen ELISA to measure vaccine potency. These should help standardize testing methods for manufacturers and national control laboratories allowing consistency and harmonisation. We would recommend these to be used in conjunction with the CBER method as detailed in Appendix 2.

Unopened ampoules should be stored at $\leq -20^{\circ}\text{C}$. Ampoules should be reconstituted on the day of the assay by adding exactly 0.5 ml of pure sterile distilled water. To remove the reagent from the ampoule it is necessary to use some form of transfer pipette rather than a volumetric pipette. The contents of the ampoules should not be assumed to be sterile.

This material is supplied for use in its final form and must not be further diluted other than as required for individual assay procedures. Each ampoule/vial is intended to be used only once. The vial should be opened as directed in section 4.

6. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC with assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. Users should determine the stability of the material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

7. CITATION

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8. PRODUCT LIABILITY AND LOSS

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- 8.2 Unless the context otherwise requires, the definitions in the Conditions shall apply.
- 8.3 Nothing in this document or the Conditions shall limit or exclude NIBSC's liability for fraud or fraudulent misrepresentation, death or personal injury caused by its negligence, or the negligence of its employees.
- 8.4 Subject to clause 0:
- 8.4.1 NIBSC shall under no circumstances whatsoever be liable to the Recipient, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of data, loss of profit, loss of business or goodwill, or any indirect or consequential loss or damage suffered or incurred by the Recipient arising in relation to the supply of the Materials or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or by any other person; and
- 8.4.2 NIBSC's total liability to the Recipient in respect of all other losses arising under or in connection with the Contract, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed 100% of the fees paid to NIBSC for the Materials.
- 8.5 The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

MATERIAL SAFETY SHEET

WHO Reference Reagent for Sabin Inactivated Polio Vaccines <i>in vivo</i> NIBSC code 17/130 Physical properties (at room temperature)			
Physical appearance	Liquid		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	<i>Contains inactivated Sabin Poliovirus types 1, 2 and 3</i>		
Handling:	See caution, section 3		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation:	Seek medical advice		
Ingestion:	Seek medical advice		
Contact with eyes:	Wash with copious amounts of water. Seek medical advice.		
Contact with skin:	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with a virucidal agent. Rinse area with a virucidal agent followed by water.			
Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			