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Report on the WHO collaborative study to establish the 4th International Standard (replacement) for inactivated polio vaccine

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein. Written comments on the proposal(s) MUST be received in English by **25 September 2026** and should be addressed to:

Biologicals Norms and Standards and Transplantation
Product Standards, Specifications and Nomenclature
Department of Medicines and Health Products Policies and Standards
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Comments should be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int

The distribution of this document is intended to provide information to a broad audience of potential stakeholders and to improve the transparency of the consultation process. Following consideration of all comments received, the proposal(s) will then be considered by the WHO Expert Committee on Biological Standardization (ECBS) prior to a final decision being made and published in the WHO Technical Report Series.

Summary

The 3rd WHO International Standard (IS) for Inactivated Poliovirus Vaccine (IPV) (12/104), established in 2013, has been widely used for calibration of reference reagents and standardization of D-antigen potency assays. As stocks of the current standard are now depleted, a replacement IS is required to ensure continuity of IPV potency testing worldwide.

A collaborative study was conducted during 2025–2026 to evaluate two candidate replacement preparations, 08-143 and 11/188, which had previously been assessed during the study that established the 3rd WHO IS. Ten laboratories, comprising vaccine manufacturers and National Control Laboratories, participated in the evaluation using both routine in-house ELISA methods and a common NIBSC ELISA method.

Both candidates demonstrated satisfactory performance and were shown to be suitable replacement standards. Potency estimates were consistent with those obtained during the original collaborative study, and inter-laboratory agreement was generally good. Real-time stability monitoring demonstrated that both preparations have maintained their potency following approximately 13 years of storage at −70°C, confirming their long-term stability.

Based on the results of the collaborative study, candidate 08-143 demonstrated robust performance, good agreement between laboratories and excellent long-term stability. It is therefore proposed that candidate 08-143 be established as the 4th WHO International Standard for Inactivated Poliovirus Vaccine.

1. Introduction

With most regions of the world now certified or designated as polio-free following sustained progress under the Global Polio Eradication Initiative (GPEI), the role of inactivated poliovirus vaccine (IPV) in routine immunization programmes has increased substantially (1). In particular, the phased withdrawal of oral poliovirus vaccine (OPV), implemented to reduce the risk of vaccine-derived polioviruses and vaccine-associated paralytic poliomyelitis, has resulted in a significant expansion in global demand for IPV (2). Consequently, the availability of robust and internationally harmonized standards for the evaluation and control of IPV potency remains essential to ensure the quality, consistency, and comparability of vaccine products worldwide.

The potency of IPV is determined *in vitro* using a validated enzyme-linked immunosorbent assay (ELISA) with appropriate reference preparations and is expressed in D-antigen units. To support global standardization of potency testing, the 3rd WHO International Standard (IS) for IPV (12/104) was established by the WHO Expert Committee on Biological Standardization (ECBS) in 2013 (3). The standard was evaluated in an international collaborative study involving manufacturers, national control laboratories, and other participating laboratories, and was shown to be suitable for the determination of antigenic content by *in vitro* assays and for the assessment of immunogenicity *in vivo*. Following its establishment, the 3rd IS has been extensively used by vaccine manufacturers and control laboratories for the calibration of secondary and working reference preparations, thereby facilitating harmonization of assay performance and supporting consistency in IPV potency determination across laboratories and over time.

Since the establishment of the 3rd IS, the material has been widely adopted and used internationally. Owing to the sustained and increasing demand associated with expanded IPV use globally, stocks of the current standard are now depleted. The replacement of the existing standard is therefore considered a matter of urgency to ensure continuity in the calibration of reference reagents and the ongoing comparability of IPV potency measurements.

The international collaborative study undertaken during the establishment of the 3rd IS included the evaluation of two additional candidate preparations, coded 08-143 and 11/188, alongside the material eventually established as 12/104 (3). Data generated during that study demonstrated that both additional candidates exhibited comparable performance characteristics and were equally suitable for use as replacement international standards. These materials have remained in long-term storage under appropriate conditions pending any future requirement for replacement of the current IS.

At its meeting in April 2023, the WHO Expert Committee on Biological Standardization reviewed the status of remaining stocks of the 3rd IS and endorsed the proposal to proceed with the establishment of a replacement WHO International Standard for IPV using one of the previously evaluated candidate materials. This approach was considered scientifically justified given the extensive characterization of the candidate preparations during the original collaborative study and the demonstrated equivalence of their performance relative to the current standard.

1.1. Aim of the study

A new study was conducted during 2025-2026 with an aim to establish a new IS for IPV. The study included two candidate samples that had been established as possible replacement reagents in 2013 3rd IS collaborative study (3). The primary aim of the study was to characterise the D-

Antigen content of the two potential candidate IPVs and decide a recommendation for the replacement of the 3rd IS for IPV, 12/104.

2. Bulk material

The candidate samples are concentrated trivalent bulks from commercially available IPVs produced and kindly donated by European manufacturers.

3. Processing of the candidate WHO IBRS

Candidate 11/188 was filled into 3ml DIN ampoules as described in the previous report (3). Candidate 08-143 was filled into 1ml DIN ampoules by the manufacturer and shipped to NIBSC. Detailed information on manufacture, processing and characterization of these candidate preparations is provided in the original collaborative study report (3).

Product summaries for both samples are shown in Table 1.

A total of six samples were provided to the participants. The samples were shipped on dry ice and storage at ≤ -70°C was recommended. Instruction for use documents were provided which included Material Safety Data Sheets.

Conventional IPV vaccine samples IPV24001 and IPV24002
08-143 reference is equivalent to Sample A used in previous collaborative studies which was analysed in 2013 alongside 12/104 with an assigned D-Ag potency of 381, 82, and 288 D-Ag/ml for poliovirus type 1, 2 and 3 respectively (3).

Conventional IPV vaccine samples IPV24003 and IPV24004
11/188 is a candidate replacement WHO IS for cIPV with an assigned potency of 224, 55 and 205 D-Ag/ml for poliovirus type 1, 2 and 3 respectively (3).

WHO IS for IPV, 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 (3)

European Pharmocopoeia (Ph.Eur.) Biological Reference Preparation batch no. 3 (BRP-3)
BRP-3 was established in 2016 with an assigned D-Ag potency of 320, 78 and 288 D-Ag/ml for poliovirus type 1, 2 and 3 respectively (4).

4. Stability studies

In the report for the establishment of the 3rd IS for IPV, both long term and accelerated degradation studies were conducted for candidates 08-143 and 11/188 alongside 12/104. Therefore, for this

study, real time stability checks were performed at the MHRA to continue to monitor the constancy of the references.

5. Collaborative study

5.1. Participants

Sixteen laboratories were invited to participate in this study. Ten of them accepted, five from vaccine manufacturers and five from National Control Laboratories (see Appendix 1). All ten participants were able to receive the study samples and returned data within the requested timeframe.

5.2. Study Plan

Laboratories are referred to by a code number, allocated at random, and not reflecting the order of listing in Appendix 1.

Participants were requested to:

- Determine the D-Antigen content of a panel of 4 coded trivalent IPV samples (IPV24001 to IPV24004) using standards 12/104 and BRP-3. This was completed using their own routine in-house method and a common method (NIBSC method as detailed in Appendix 3).
- Participants should also include an in-house vaccine sample that has previously been tested (if available).
- Perform three independent assays per serotype per method to determine the D-Antigen content of the 3 poliovirus serotypes for each study sample.
- The D-Ag content of the 4 coded samples should be estimated using IPV IS 12/104 as the reference.
- Test all study samples at the same time for each of the three independent determinations. Use freshly opened samples for the preparation of dilutions used on any day.

Participants were requested to report their results electronically using standard forms provided by the coordinator. Results including raw OD data, potency calculations and statistical validity criteria were to be sent using the Raw Data and Results Forms. Information on the method and assay reagents used was also requested using the Method Form.

5.3. Statistical Methods

Potency estimates relative to IS 12/104 were calculated by parallel line analysis using a minimum of three dilutions in a linear section of the assay response range. All calculations were performed using the software program Combistats (5). Results from assays that were reported as ‘invalid’ at a 1% significance level for non-parallelism or non-linearity were excluded. As an additional check, the ratio of the coded duplicate samples 24001 and 24002, and 24003 and 24004, was calculated for each plate, and any plate with a ratio greater than 1.2 was excluded from further summary calculations.

Results from all valid assays were combined to generate unweighted geometric mean (GM) potencies for each laboratory and these laboratory means were used to calculate overall unweighted geometric mean potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\}\times100\%$ where s is the standard deviation of the $\log_{10}$ transformed potencies). Grubb's test was applied to the log transformed laboratory GM estimates in order to identify any outliers in the results obtained for each of the study samples. Due to outliers and anomalous results, Huber's robust mean was also calculated using the R package 'WRS2' (6).

6. Results

6.1. Study Data Returned

Data were received from 10 laboratories, each performing 3 assays for each of the three serotypes. All laboratories performed their own in-house method and 8 out of the 10 laboratories also performed a common NIBSC ELISA method.

For the majority of laboratories, the re-analysis of assay raw data at NIBSC/MHRA was in close agreement to the results reported by the participants, and the reanalysed assays were used for subsequent analysis.

6.2. Assay Validity

An acceptance value for the ratio of the coded duplicate samples was derived using data returned for samples 24001 and 24002, and 24003 and 24004 from all laboratories, as their potencies relative to 12/104 are expected to be equivalent. Values of m= max(RP_{24001}/RP_{24002}, RP_{24002}/RP_{24001}) or m=max(RP_{24003}/RP_{24004}, RP_{24004}/RP_{24003}), were calculated for each plate and an upper equivalence bound for m was set as the 90th percentile of those values, using all values obtained across the study. This gave an acceptance value of <1.2. It should be noted that this value was intended for use in the analysis of data from this study only and should not be interpreted as suitable for routine use in the assessment of assay validity and may be overly stringent or lenient in the case of some laboratories.

Some assay results were also excluded due to non-linearity, or due to non-parallelism as described in section 5.3. Across all samples and methods, 2%, 5% and 5% of results were excluded due to non-linearity, and 5%, 7% and 7% of results were excluded due to non-parallelism, for types 1, 2 and 3 respectively. Individual assay results and any assay exclusions are shown in Appendix 2, Tables 1 to 3.

6.3. Relative potencies, intra-laboratory and inter-laboratory variability

Potency estimates relative to the current IS, 12/104, in DU/ml are summarised in Tables 3-8 and as scatterplots in Figures 1-3. The estimated content of samples 24001, 24002, 24003, 24004, and BRP-3 have been determined as both the geometric mean (GM) and robust GM of laboratory estimates. Samples 24001 and 24002, and samples 24003 and 24004 were also combined to give an overall potency estimate for the candidate preparations.

The intra-laboratory (between assay) variability is shown in Table 9 as average %GCVs across study samples 24001-24004 for each laboratory. The majority of laboratories achieved between assay repeatability better than 20% (86%), and in 69% of cases better than 10%. The pooled GCV was noted to be higher for the NIBSC method than for the in-house method, and the NIBSC method showed a few higher individual values which might be due to single anomalous results. Intra-laboratory GCVs were also low for sample BRP-3, with 83% better than 20% across all serotypes and methods.

Overall, the agreement between laboratories is very good for all three serotypes when the in-house method is used, with all inter-laboratory GCVs below 15% for coded duplicate samples 24001/24002 and 24003/24004, and sample BRP-3. There were also no outliers detected for any of the samples when using the in-house method. Variability was slightly higher for assays using the NIBSC common method across all three serotypes, with inter-laboratory GCVs between 15% and 20% for the coded duplicate samples. In some cases, a higher GCV was due to outlying data points, as seen with sample BRP-3 for Type 1 (110.64% inter-laboratory GCV), and the equivalent figures excluding outliers are shown in Tables 4, 6 and 8.

6.4. Potencies relative to 12/104

The potency estimates for 08-143 (24001 and 24002 combined) ranged from 298-422 DU/ml for Type 1, 70-91 DU/ml for Type 2, and 214-308 DU/ml for Type 3, when using in-house methods, with a robust GM potency of 359 DU/ml, 81 DU/ml, and 265 DU/ml respectively. Laboratories showed better agreement when using in-house methods compared to the NIBSC method, with in-house inter-laboratory GCVs of 10.68%, 8.31% and 10.41% for Type 1, 2 and 3 respectively, compared with 16.30%, 16.70% and 12.77% for the NIBSC method.

The potency estimates for 11/188 (24003 and 24004 combined) ranged from 191-270 DU/ml for Type 1, 44-68 DU/ml for Type 2, and 161-245 DU/ml for Type 3, when using in-house methods, with a robust GM potency of 234 DU/ml, 55 DU/ml, and 198 DU/ml respectively. Similarly to 08-143, laboratories showed better agreement for 11/188 when using in-house methods compared to the NIBSC method, with in-house inter-laboratory GCVs of 10.53%, 13.61% and 12.18% for Type 1, 2 and 3 respectively, compared with 18.26%, 19.96% and 17.14% for the NIBSC method.

6.5. Vaccine test samples

In order to assess continuity, six laboratories also tested a vaccine batch sample using the laboratory in-house method, and the potencies relative to the current IS, 12/104, the candidate IS, 08-143, the candidate IS, 11/188, and laboratory in-house references, are summarised in Table 10, with the percentage differences between the samples relative to 12/104 and the in-house references shown in Tables 11 and 12 respectively. Overall, estimates for the vaccine samples were comparable when calculated relative to 08-143 or 11/188 compared to samples relative to 12/104, for all three serotypes, with the majority of laboratories showing a percentage difference of <10% (71%). The results were also similar when comparing results relative to 08-143 or 11/188 against results relative to in-house references with 52% of laboratories showing a percentage difference of <10%, and in 71% of cases better than 15%.

6.6. Stability

Results of the analysis of real-time stability at the storage temperature (-70°C) are shown in Figures 4 and 5. The results indicate the potency of both 08-143 and 11/188 has been maintained over the last 13 years for all three types indicating good long-term stability.

7. Discussion, Conclusions and Proposals

The continued availability of an International Standard (IS) for inactivated poliovirus vaccine (IPV) is essential to support the global standardization of D-antigen quantification and to ensure the consistency, comparability, and quality control of IPV products worldwide. Following depletion of stocks of the 3rd WHO IS for IPV (12/104), the present collaborative study was undertaken to evaluate two previously characterised candidate replacement materials, 08-143 and 11/188, and to determine their suitability for establishment as a replacement international standard.

Overall, the results demonstrated that both candidate preparations performed satisfactorily when evaluated against the current IS, 12/104. Potency estimates obtained using both in-house and common NIBSC ELISA methods showed good agreement between laboratories, with inter-laboratory variability generally below 15% when in-house methods were employed. Such levels of variability are comparable to those observed during the original collaborative study conducted for establishment of the 3rd IS and are considered acceptable for complex biological assays of this nature.

The duplicate coded samples for each candidate preparation (24001/24002 representing 08-143 and 24003/24004 representing 11/188) yielded highly consistent potency estimates, confirming the integrity and homogeneity of the study materials. Furthermore, no significant differences were observed between the duplicate samples, supporting their suitability as reference preparations. The application of both conventional geometric mean and robust statistical approaches produced similar potency estimates, indicating that the overall conclusions were not unduly influenced by occasional outlying results.

Both candidate preparations demonstrated performance characteristics consistent with those observed during the original international collaborative study conducted in 2013 (3). The potency estimates obtained in the current study were in close agreement with the values assigned previously, confirming the long-term stability and preservation of antigenic integrity of the materials after approximately thirteen years of storage at −70°C. This observation is supported by the real-time stability monitoring data, which showed no evidence of significant loss of potency for any of the three poliovirus serotypes in either candidate preparation over the monitoring period.

The evaluation of vaccine test samples demonstrated good continuity between the current WHO International Standard (12/104), the candidate replacement materials (08-143 and 11/188), and laboratories' existing in-house reference preparations. Potency estimates obtained relative to either candidate standard were generally comparable to those calculated relative to 12/104 across all three poliovirus serotypes, with the majority of results differing by less than 10%. Similarly, comparisons with laboratory in-house references showed good agreement, with over half of estimates within 10% and the majority within 15%. These findings indicate that adoption of either candidate material would not be expected to introduce systematic changes in routine D-antigen

potency determination and support the continuity of assay calibration following replacement of the current WHO International Standard.

A notable strength of the present study is that the candidate materials were not newly manufactured preparations but were previously evaluated extensively during the establishment of the 3rd WHO IS. Consequently, their performance characteristics, commutability, and suitability for use as international standards have now been demonstrated independently in two separate international collaborative studies conducted more than a decade apart. This provides an unusually strong evidence base supporting their use as replacement standards and minimises the uncertainty often associated with the introduction of newly prepared reference materials.

Although both candidates were shown to be suitable replacement materials, candidate 08-143 demonstrated several advantages. Firstly, it exhibited lower inter-laboratory variability than 11/188 across all three poliovirus serotypes when assessed using participants' routine in-house methods. Secondly, the higher D-antigen content of 08-143 provides a broader working range for calibration purposes and may facilitate preparation of secondary and working reference standards by users. Finally, 08-143 consistently produced potency estimates that were highly reproducible across participating laboratories and assay formats.

Taken together, the results demonstrate that both 08-143 and 11/188 are suitable candidates for replacement of the 3rd WHO IS for IPV. However, based on the overall performance characteristics observed in the present study, candidate 08-143 is considered the preferred material for establishment as the 4th WHO International Standard for IPV.

Establishment of 08-143 as the replacement WHO IS will ensure continuity in the calibration of secondary standards and maintenance of globally harmonised IPV potency measurements, thereby supporting vaccine quality control and regulatory oversight during the ongoing transition to increased IPV use worldwide.

7.1. Recommendations

It is proposed that candidate 08-143 should be established as the 4th IS for IPV. The assigned potency for this should be:
359 D-Ag/ml for poliovirus type 1
81 D-Ag/ml for poliovirus type 2
265 D-Ag/ml for poliovirus type 3

These will be stored at NIBSC and entered into the catalogue for customers to order. An example of the Instructions For Use and the safety data sheet for the reference reagents can be found in Appendix 4.

8. Comments Received from Collaborative Study Participants

One participant suggested minor editorial changes and some requested additional information. Where appropriate, these have been addressed in the revised report.

Another participant questioned the use of an older material for the 4th IS given the previous issue with the 2nd IS for IPV, 91/574. We explained that the issues with 91/574 were actually due to partial degradation of the product during shipment not the age of the material. And that a similar product made from the same material is still fit for purpose over 30 years later. The real time stability of the proposed IS will be continuously monitored and this candidate has now been shown to be suitable in two separate collaborative studies.

9. Acknowledgements

We would like to thank all study participants, particularly manufacturers that contributed with candidate and study materials. We would also like to thank our colleagues in the Standards Processing Department and Dispatch teams that provided invaluable support.

10. References

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11. Tables and Figures

Table 1: Product information

NIBSC Code	08-143	11/188
Presentation	1ml DIN ampoules	3ml DIN ampoules
No. of containers	9335	1609
Mean fill volume	0.6ml	1.0ml
Date of fill	July 2009	November 2011
Storage temperature	-70°C	-70°C
Microbial Contamination	None detected	None detected

Table 2: Summary of methods used by participants for their in-house assay

Lab	Coating antibody	Detection antibody	Conjugated antibody	No. of replicates / dilutions	Substrate
1	Mab (IgG)	-	Mab (IgG) conjugated to HRP	2/4	OPD
2	Rabbit polyclonal Ab	Type specific MAb	Sheep anti-mouse HRP	2/7	TMB
3	Sheep polyclonal serum	Mouse MAb	Anti-mouse IgG HRP	2/4	OPD
4	Bovine polyclonal serum	Rabbit polyclonal serum	Goat anti-rabbit IgG HRP	3/4	ABTS
5	Bovine polyclonal serum	Mouse MAb	Anti-mouse IgG HRP	2/7	TMB
6	Bovine antibody	Rabbit antibody	Goat anti-rabbit IgG HRP	2/6	ABTS
7	Bovine polyclonal serum	Mouse MAb	Sheep anti-mouse IgG HRP	3/5	TMB
8	Bovine polyclonal serum	Rabbit antibody	Goat anti-rabbit IgG HRP	2/7	TMB
9	Anti-polio serum	Mouse MAb	Sheep anti-mouse IgG HRP	2/7	TMB
10	Bovine polyclonal serum	Mouse MAb	Sheep anti-mouse IgG HRP	2/4	TMB

Table 3: Laboratory geometric mean potency estimates relative to current IS, 12/104, using in-house methods (DU/ml) – Type 1

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	330	n/a	2	333	n/a	1	331	3.75	3	206	n/a	2	218	n/a	2	212	5.65	4	319	n/a	1
02	339	n/a	2	332	n/a	2	335	3.26	4	230	n/a	2	231	n/a	2	231	3.12	4	292	n/a	2
03	438	6.78	3	406	8.48	3	422	8.08	6	267	n/a	2	263	n/a	2	265	21.25	4	349	n/a	2
04	293	n/a	2	308	n/a	1	298	10.73	3	194	n/a	2	188	n/a	2	191	10.02	4	278	n/a	2
05	385	n/a	2	384	n/a	2	384	2.08	4	223	n/a	2	240	n/a	2	231	4.51	4	279	1.49	3
06	388	31.55	3	397	30.62	3	393	27.44	6	266	n/a	2	274	n/a	2	270	27.26	4	399	28.63	5
07	375	1.76	3	392	3.82	3	383	3.62	6	228	5.77	3	228	4.12	3	228	4.46	6	303	7.01	6
08	340	3.49	3	339	2.17	3	340	2.60	6	245	2.52	3	246	6.72	3	245	4.52	6	321	4.30	6
09	353	10.32	3	361	10.24	3	357	9.25	6	233	9.01	3	236	11.47	3	235	9.20	6	336	3.97	6
10	340	n/a	2	359	6.26	3	351	5.85	5	235	n/a	2	233	0.72	3	234	2.58	5	334	n/a	2
Overall	356	11.67	10	360	9.64	10	358	10.68	10	231	10.50	10	235	10.84	10	233	10.53	10	319	11.66	10
Robust GM	356			360			359			232			236			234			316		

N = number of estimates used in calculations; n/a = not calculated as N < 3

Table 4: Laboratory geometric mean potency estimates relative to current IS, 12/104, using common NIBSC method (DU/ml) – Type 1

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	341	2.54	3	333	6.70	3	337	4.67	6	227	n/a	2	226	3.55	3	226	3.45	5	332	8.33	3
02		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
03	438	6.78	3	406*	8.48	3	422	8.08	6	267	n/a	2	263	n/a	2	265	21.25	4	349	n/a	2
04	336	6.47	3	352	n/a	2	342	5.32	5	228	2.59	3	247	7.25	3	237	6.64	6	319	7.55	3
05	474	n/a	1	502*	n/a	1	488	n/a	2	315	n/a	1	341	61.11	3	335	47.93	4	509*	49.68	4
06	319	22.78	3	342	12.56	3	330	16.71	6	251	n/a	2	258	n/a	2	255	14.49	4	298	15.97	3
07		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
08	310	n/a	1	320	n/a	1	315	n/a	2	193	n/a	1	197	n/a	1	195	n/a	2	2709*	n/a	1
09	338	n/a	2	336	8.63	3	337	6.25	5	236	n/a	2	240	6.53	3	239	5.79	5	319	5.89	3
10	330	n/a	2	343	n/a	1	334	3.15	3	202	2.39	3	205	3.48	3	204	2.80	6	337	3.98	3
Overall	357	16.84	8	363	16.05	8	359	16.30	8	237	16.75	8	244	18.62	8	241	18.26	8	448	110.64	8
Overall**	357	16.84	8	338	3.28	6	359	16.30	8	237	16.75	8	244	18.62	8	241	18.26	8	325	5.63	6
Robust GM	337			345			338			235			239			237			337		

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; *=outlier by Grubbs test; ** = results excluding outliers

Table 5: Laboratory geometric mean potency estimates relative to current IS, 12/104, using in-house methods (DU/ml) – Type 2

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	69	n/a	1	71	n/a	1	70	n/a	2	43	n/a	2	45	n/a	2	44	22.91	4	68	6.62	3
02	89	n/a	2	85	n/a	2	87	3.31	4	59	n/a	2	57	n/a	2	58	5.07	4	73	n/a	2
03	88	n/a	2	97	n/a	1	91	22.03	3	71	n/a	1	67	n/a	2	68	16.57	3	94	n/a	1
04	80	4.82	3	79	4.42	3	79	4.20	6	53	4.66	3	55	7.31	3	54	5.64	6	77	4.00	3
05	76	n/a	2	76	n/a	2	76	3.69	4	53	n/a	2	52	n/a	2	52	2.75	4	69	2.92	4
06	79	1.51	3	81	2.34	3	80	2.04	6	55	n/a	2	56	n/a	2	55	3.87	4	87	1.51	4
07	76	1.76	3	78	2.93	3	77	2.60	6	53	n/a	2	52	n/a	2	53	2.16	4	70	4.50	5
08	91	8.21	3	87	4.37	3	89	6.23	6	66	2.52	3	63	0.83	3	64	3.43	6	88	3.89	6
09	83	9.76	3	83	5.62	3	83	7.07	6	57	3.98	3	57	4.42	3	57	3.80	6	82	7.60	4
10	79	10.26	3	75	n/a	2	77	8.32	5	48	9.71	3	49	10.73	3	48	9.28	6	87	n/a	2
Overall	81	8.78	10	81	9.03	10	81	8.31	10	55	15.65	10	55	12.06	10	55	13.61	10	79	12.36	10
Robust GM		81			80			81			55			55			55			79	

N = number of estimates used in calculations; n/a = not calculated as N < 3

Table 6: Laboratory geometric mean potency estimates relative to current IS, 12/104, using common NIBSC method (DU/ml) – Type 2

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	73	4.93	3	82	n/a	1	75	6.94	4	53	5.65	3	56	n/a	1	54	5.21	4	75	6.96	3
02		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
03	88	n/a	2	97	n/a	1	91	22.03	3	71	n/a	1	67	n/a	2	68	16.57	3	94	n/a	1
04	75	n/a	2	72	n/a	2	74	8.29	4	61	13.94	3	59	n/a	2	60	10.14	5	81	n/a	2
05	115	n/a	2	108	n/a	2	111	36.75	4	113*	n/a	2	78	59.34	3	91*	78.63	5	116	46.05	5
06	82	n/a	1	82	n/a	2	82	1.44	3	60	n/a	2	63	n/a	2	61	4.26	4	86	n/a	1
07		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
08	64	n/a	2	70	n/a	2	67	9.36	4	50	n/a	2	51	n/a	2	51	7.56	4		NP	
09	78	0.94	3	83	6.19	3	80	5.27	6	59	5.45	3	59	6.88	3	59	5.53	6	83	3.55	3
10	77	2.06	3	80	6.88	3	79	4.88	6	56	7.90	3	55	8.04	3	55	7.23	6	76	3.17	3
Overall	80	18.63	8	83	15.40	8	81	16.70	8	63	29.19	8	61	14.40	8	61	19.96	8	86	16.35	7
Overall**	80	18.63	8	83	15.40	8	81	16.70	8	58	11.49	7	61	14.40	8	58	10.23	7	86	16.35	7
Robust GM		79			82			80			60			60			59			84	

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; NP = non-parallel to reference sample; *=outlier by Grubbs test; ** = results excluding outliers

Table 7: Laboratory geometric mean potency estimates relative to current IS, 12/104, using in-house methods (DU/ml) – Type 3

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	280	n/a	2	263	3.92	3	270	11.29	5	187	n/a	2	198	17.46	3	193	14.81	5	273	5.55	3
02	284	3.63	3	273	3.59	3	278	3.99	6	197	n/a	2	201	n/a	2	199	1.68	4	284	3.04	3
03		NL/NP		291	n/a	2	291	n/a	2	263	n/a	1	228	n/a	1	245	n/a	2		NP	
04	241	3.65	3	258	5.36	3	249	5.62	6	193	2.02	3	186	6.87	3	189	4.89	6	261	3.97	3
05	258	n/a	2	272	n/a	1	263	4.49	3	187	n/a	1	196	n/a	2	193	8.47	3	214	13.70	4
06	305	27.08	3	311	26.68	3	308	23.76	6	225	26.11	3	234	23.63	3	230	22.13	6	333	26.31	6
07	247	1.31	3	258	2.82	3	253	3.24	6	188	n/a	2	198	n/a	2	193	3.92	4	229	3.74	5
08	268	1.57	3	268	0.67	3	268	1.09	6	218	4.64	3	212	1.41	3	215	3.44	6	266	3.27	5
09	210	31.84	3	219	34.71	3	214	29.44	6	197	2.66	3	200	0.20	3	199	1.83	6	253	7.96	5
10	253	n/a	2	244	n/a	1	250	7.19	3		NP		161	n/a	1	161	n/a	1	222	n/a	2
Overall	259	11.49	9	265	9.93	10	263	10.41	10	205	12.22	9	200	10.99	10	201	12.18	10	257	14.60	9
Robust GM	261			265			265			200			201			198			255		

N = number of estimates used in calculations; n/a = not calculated as N < 3; NP = non-parallel to reference sample; NL = sample non-linear

Table 8: Laboratory geometric mean potency estimates relative to current IS, 12/104, using common NIBSC method (DU/ml) – Type 3

Lab	24001			24002			24001/24002			24003			24004			24003/24004			BRP-3		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	259	6.20	3	278	n/a	2	266	6.33	5	204	n/a	2	194	8.85	3	198	6.79	5	282	4.28	2
02		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
03		NL/NP		291	n/a	2	291	n/a	2	263	n/a	1	228	n/a	1	245	n/a	2		NP	
04	240	n/a	2	289	7.97	3	268	13.09	5	202	6.89	3	206	7.04	3	204	6.34	6	247	9.54	3
05	369	33.75	3	348	29.75	3	359	28.28	6	367*	78.35	3	271	59.67	3	315*	64.71	6	379*	38.76	6
06	277	n/a	2	273	n/a	2	275	3.88	4	207	n/a	2	229	n/a	2	218	8.98	4	256	1.14	2
07		n/t			n/t			n/t			n/t			n/t			n/t			n/t	
08	224	n/a	1	254	n/a	1	239	n/a	2	184	n/a	1	204	n/a	1	193	n/a	2		NP	
09	272	7.79	3	263	n/a	2	269	6.49	5	226	n/a	2	213	n/a	2	220	9.59	4	230	9.05	3
10	298	1.86	3	311	10.68	3	304	7.14	6	213	8.98	3	207	6.30	3	210	7.10	6	239	9.87	3
Overall	274	17.58	7	287	10.51	8	282	12.77	8	228	24.46	8	218	10.96	8	223	17.14	8	268	20.08	6
Overall**	274	17.58	7	287	10.51	8	282	12.77	8	213	11.96	7	218	10.96	8	212	8.25	7	250	8.09	5
Robust GM		269			284			278			215			215			215			257	

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; NP = non-parallel to reference sample; NL = sample non-linear; *=outlier by Grubbs test; ** = results excluding outliers

Table 9: Intra-laboratory (between assay) variability for study samples (coded 24001-24004): Average %GCV

Method	Lab	Type 1	Type 2	Type 3
In-house	01	n/a	n/a	10.69
In-house	02	n/a	n/a	3.61
In-house	03	7.63	n/a	n/a
In-house	04	n/a	5.30	4.47
In-house	05	n/a	n/a	n/a
In-house	06	31.08	1.92	25.88
In-house	07	3.86	2.35	2.06
In-house	08	3.72	3.98	2.07
In-house	09	10.26	5.95	17.36
In-house	10	3.49	10.23	n/a
Pooled (in-house method)		10.01	4.95	9.45
NIBSC	01	4.26	5.29	7.53
NIBSC	02	n/a	n/a	n/a
NIBSC	03	7.63	n/a	n/a
NIBSC	04	5.44	13.94	7.30
NIBSC	05	61.11	59.34	50.38
NIBSC	06	17.67	n/a	n/a
NIBSC	07	n/a	n/a	n/a
NIBSC	08	n/a	n/a	n/a
NIBSC	09	7.58	4.86	7.79
NIBSC	10	2.94	6.22	6.96
Pooled (NIBSC method)		15.23	17.93	15.99

Table 10: Laboratory geometric mean potency estimates for vaccine batch samples relative to current IS, 12/104, the proposed IS 08-143, and 11/188, and in-house references (DU/ml)

Method	Lab	Type 1				Type 2				Type 3			
		Vs 12/104	Vs 08-143	Vs 11/188	Vs IH Ref	Vs 12/104	Vs 08-143	Vs 11/188	Vs IH Ref	Vs 12/104	Vs 08-143	Vs 11/188	Vs IH Ref
In-house	01	66	73	73	85	18	20	18	18	46	46	46	57
In-house	05	71	66	72	71	21	22	22	NP	44	44	43	42
In-house	06	72	64	66	58	15	15	15	13	62	51	51	52
In-house	08	78	82	74	72	20	18	17	16	74	73	68	62
In-house	09	77	78	77	76	17	16	16	17	70	86	70	71
In-house	10	103	105	104	NP	39	41	45	93	98	108	NP	89

NP=non-parallel to reference sample

Table 11: Percentage difference between vaccine batch samples relative to 12/104 and vaccine batch samples relative to 08-143 and 11/188 and (percentage differences <10 are highlighted in orange)

Method	Lab	Type 1		Type 2		Type 3	
		Vs 08-143	Vs 11/188	Vs 08-143	Vs 11/188	Vs 08-143	Vs 11/188
In-house	01	10.07	10.07	10.53	0.00	0.00	0.00
In-house	05	7.30	1.40	4.65	4.65	0.00	2.30
In-house	06	11.76	8.70	0.00	0.00	19.47	19.47
In-house	08	5.00	5.26	10.53	16.22	1.36	8.45
In-house	09	1.29	0.00	6.06	6.06	20.51	0.00
In-house	10	1.92	0.97	5.00	14.29	9.71	-

Table 12: Percentage difference between vaccine batch samples relative to in-house references and vaccine batch samples relative to 12/104, 08-143 and 11/188 (percentage differences <10 are highlighted in orange)

Method	Lab	Type 1		Type 2		Type 3	
		Vs 08-143	Vs 11/188	Vs 08-143	Vs 11/188	Vs 08-143	Vs 11/188
In-house	01	15.19	15.19	10.53	0.00	21.36	21.36
In-house	05	7.30	1.40	-	-	4.65	2.35
In-house	06	9.84	12.90	14.29	14.29	1.94	1.94
In-house	08	12.99	2.74	11.76	6.06	16.30	9.23
In-house	09	2.60	1.31	6.06	6.06	19.11	1.42
In-house	10	-	-	77.61	69.57	19.29	-

Figure 1: Laboratory geometric mean estimates in DU/ml – Type 1 (Outlier for BRP-3, NIBSC method, out of range of plot)

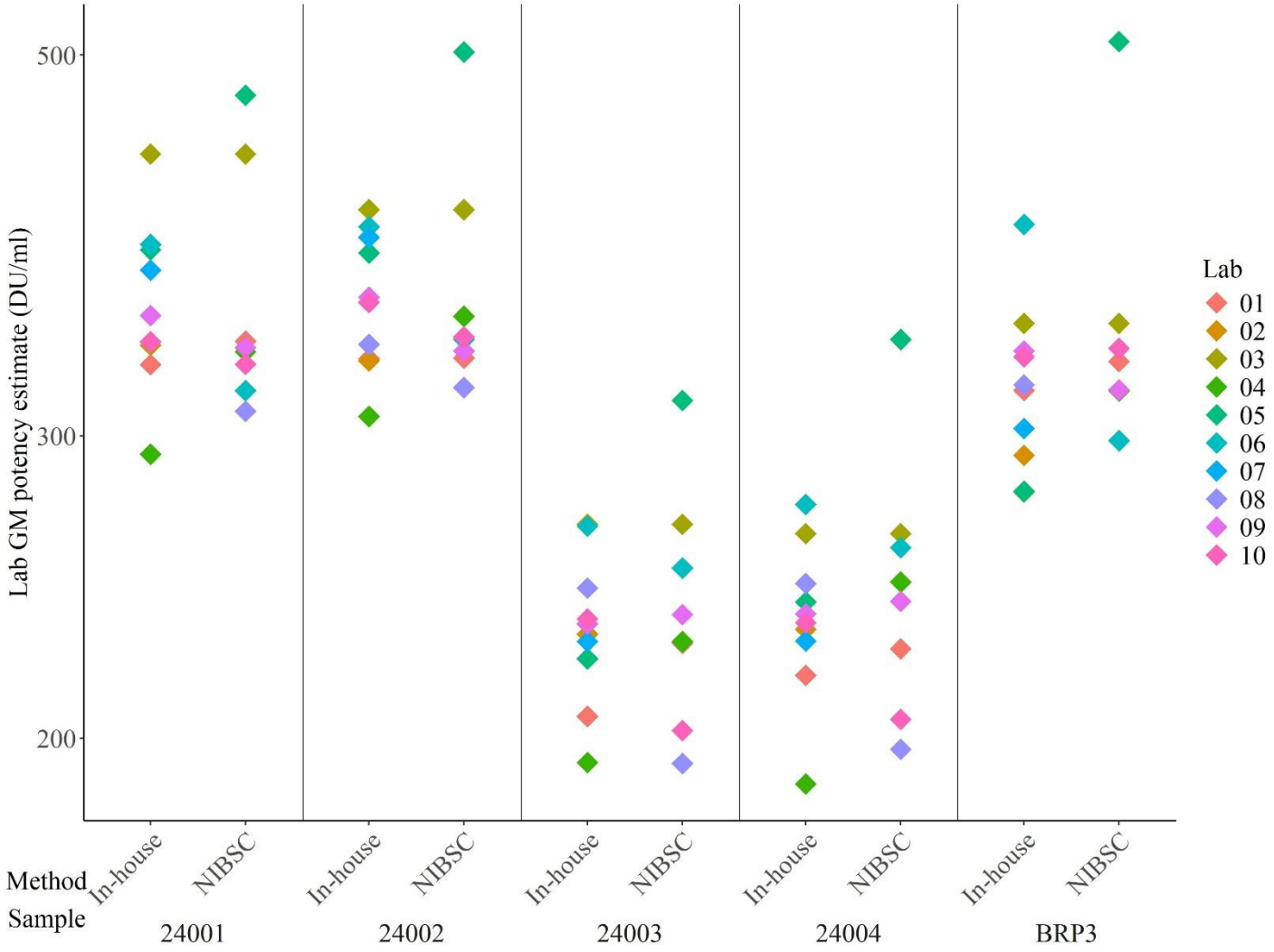


Figure 2: Laboratory geometric mean estimates in DU/ml – Type 2

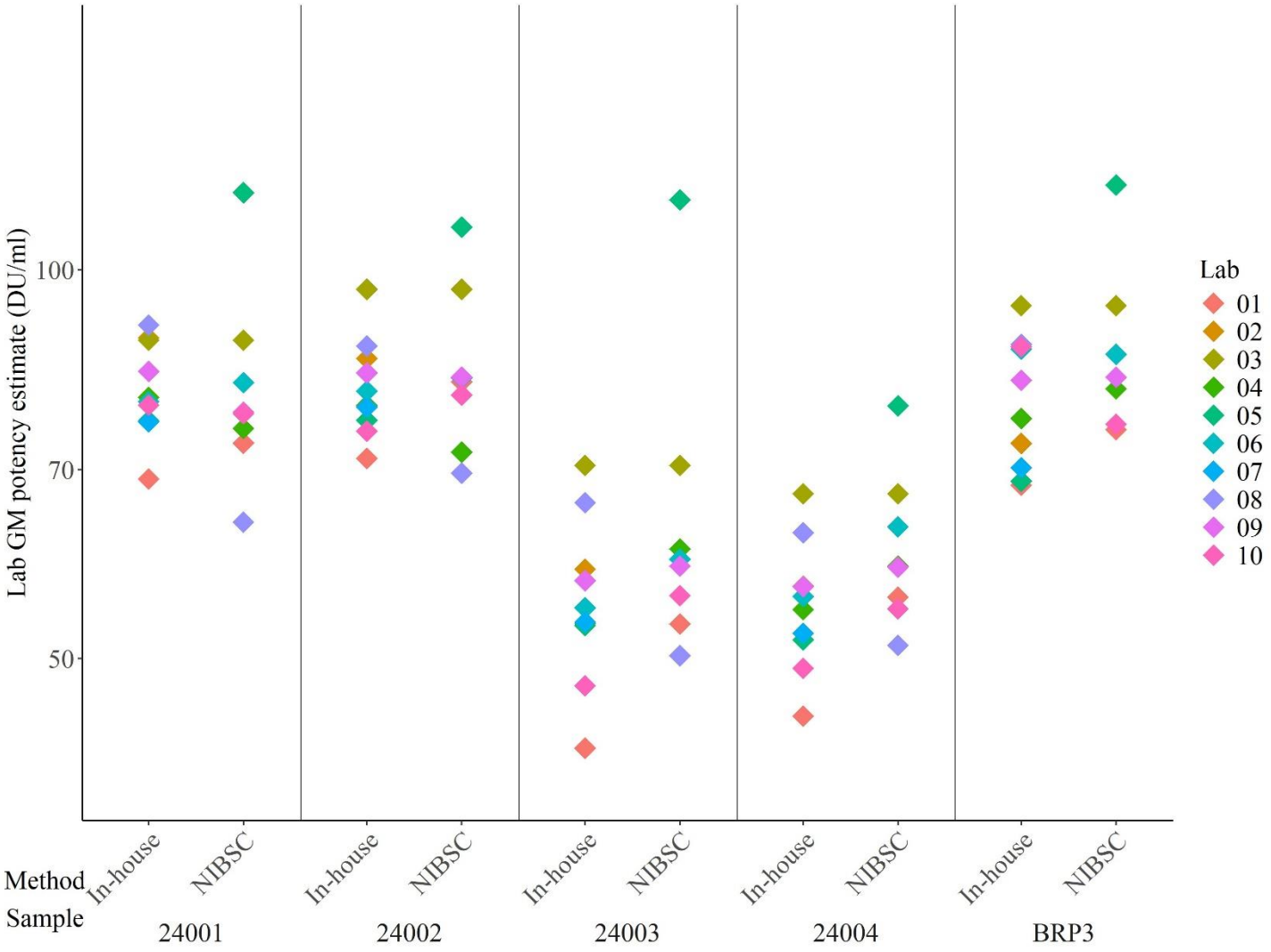


Figure 3: Laboratory geometric mean estimates in DU/ml – Type 3

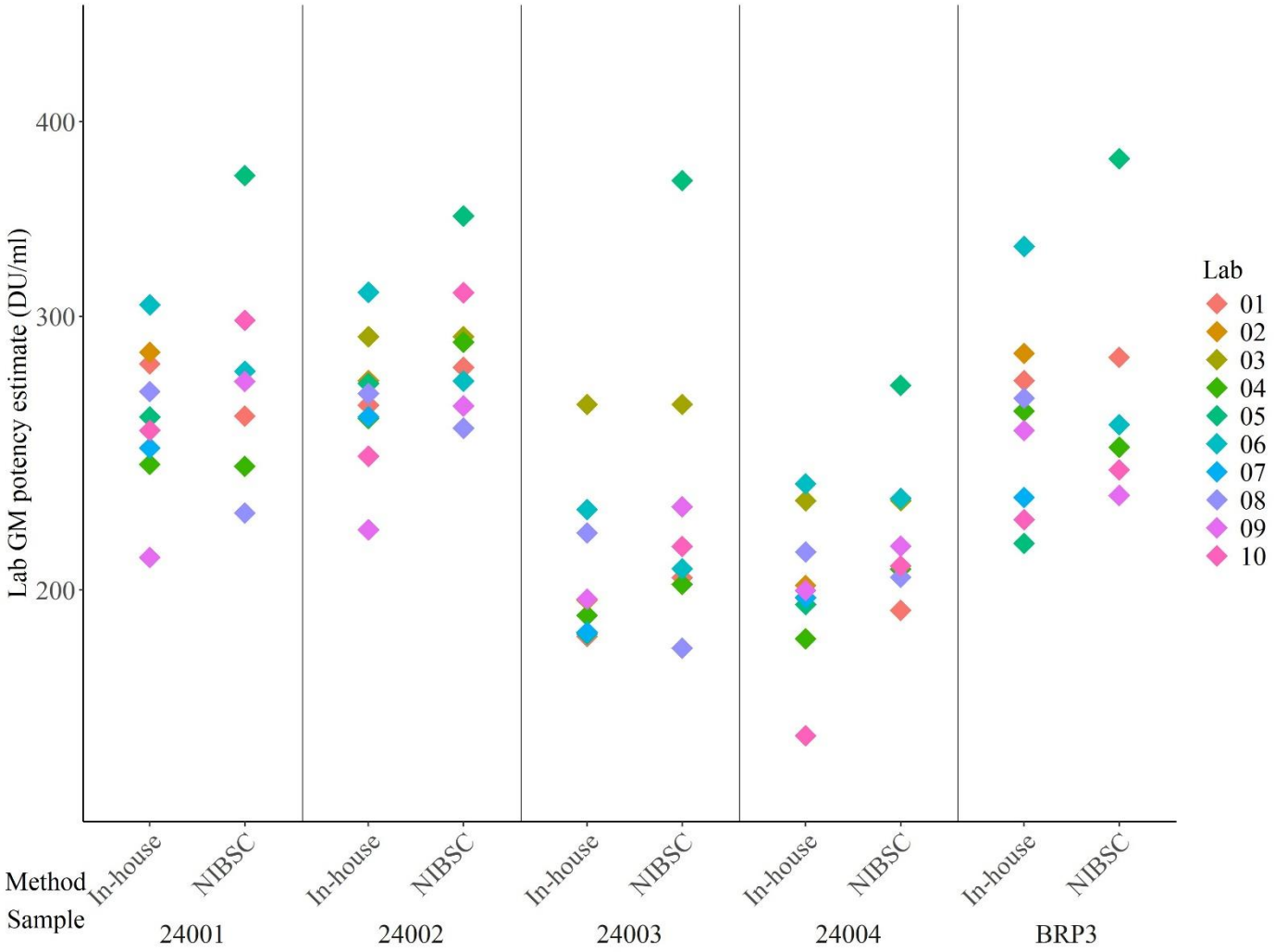


Figure 4: Real time stability of candidate 08-143

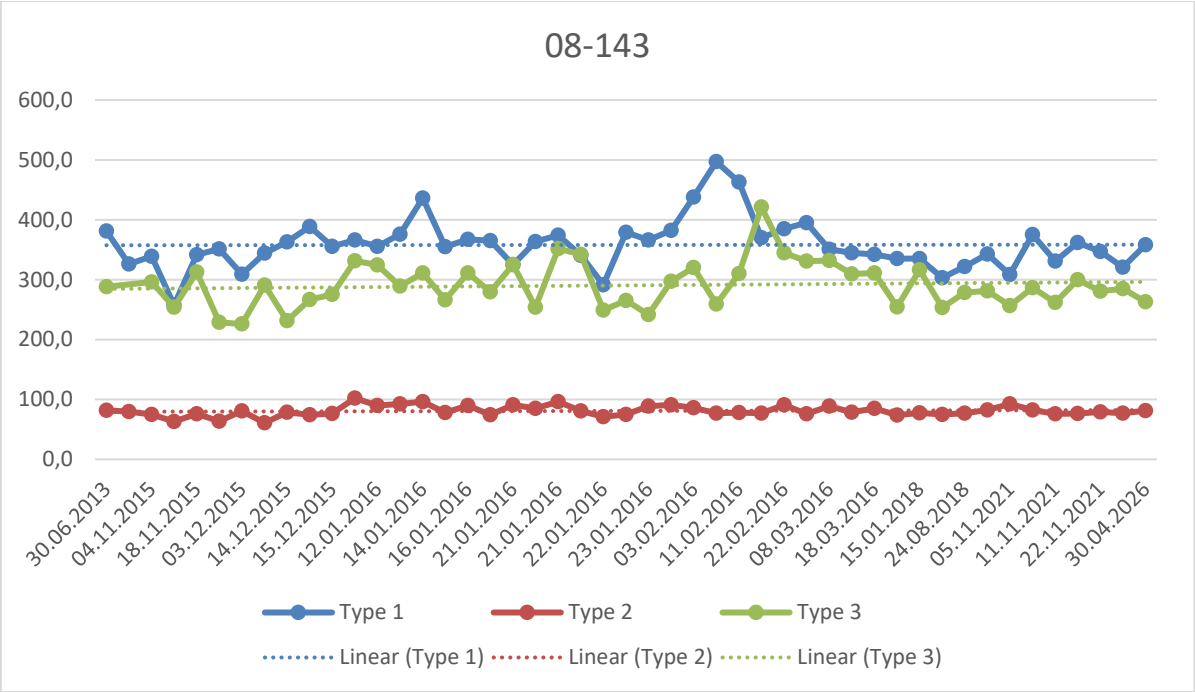
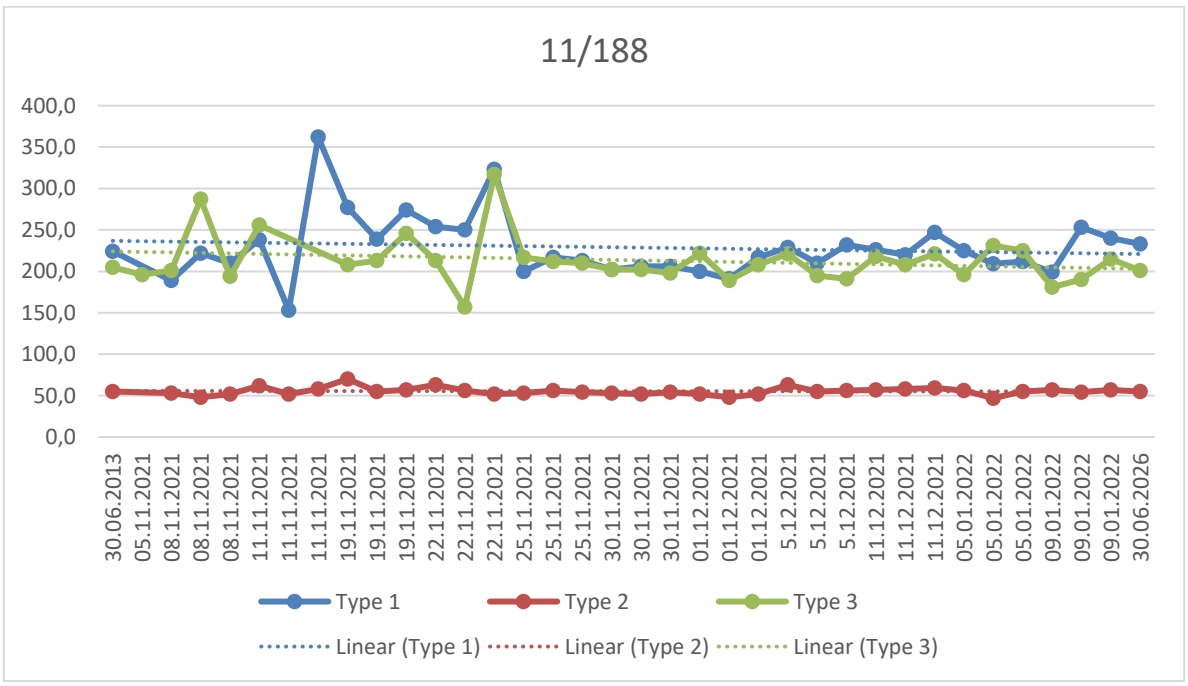


Figure 5: Real time stability of candidate 11/188



12. Appendices

Appendix 1: Collaborative study participants

Name	Laboratory	Country
Elisabeth Davis Tong Wu	Health Canada	Canada
Liu Yueyue	NIFDC	China
Jun Li Dan Yu	Sinovac Biotech Co., Ltd.	China
Sabine Gaillard	Sanofi Pasteur	France
Laura Stephens	NIBSC	UK
Fabiola Opitz	Paul-Ehrlich-Institut (PEI)	Germany
Maya Ramdas	Panacea Biotec	India
Satyaprasad Manney	Serum Institute of India	India
Sandra van de Weijer	RIVM	The Netherlands
Mallikarjuna Panchakshari	Biological E	India

Appendix 2

Table 1: Individual assay relative potency estimates, relative to current IS 12/104 - Type 1

Lab	Assay	Plate	In-house method					NIBSC method				
			24001	24002	24003	24004	BRP3	24001	24002	24003	24004	BRP3
01	1	1	318	NP	198	210	NL	348	353	235	233	360
01	2	1	CD	CD	CD	CD	CD	331	311	220	227	307
01	3	1	342	333	215	226	319	343	337	NP	217	330
02	1	1	337	320	236	238	302	-	-	-	-	-
02	2	1	CD	CD	CD	CD	CD	-	-	-	-	-
02	3	1	340	344	224	225	283	-	-	-	-	-
03	1	1	418	435	NP	NL/NP	NP	418	435	NP	NL/NP	NP
03	2	1	472	415	308	317	370	472	415	308	317	370
03	3	1	426	372	230	219	329	426	372	230	219	329
04	1	1	323	NP	210	205	312	352	349	225	265	302
04	2	1	266	308	179	173	248	313	NP	234	230	310
04	3	1	CD	CD	CD	CD	CD	345	356	224	246	346
05	1	1	186	191	118	-	146	CD	CD	CD	-	CD
05	1	2	-	-	-	NL	141	-	-	-	202	298
05	2	1	385	374	223	-	275	474	502	315	-	531
05	2	2	-	-	-	241	NL/NP	-	-	-	382	536
05	3	1	385	393	222	-	283	CD	CD	CD	-	CD
05	3	2	-	-	-	239	279	-	-	-	515	793
06	1	1	337	348	-	-	341	262	299	NP	NP	257
06	1	2	-	-	CD	CD	CD	-	-	-	-	-
06	2	1	532	540	-	-	529	315	358	231	225	299

06	2	2	-	-	329	336	521	-	-	-	-	-
06	3	1	326	333	-	-	325	394	373	274	297	345
06	3	2	-	-	215	223	329	-	-	-	-	-
07	1	1	370	401	-	-	314	-	-	-	-	-
07	1	2	-	-	234	237	330	-	-	-	-	-
07	2	1	382	399	-	-	311	-	-	-	-	-
07	2	2	-	-	236	229	303	-	-	-	-	-
07	3	1	-	-	214	218	271	-	-	-	-	-
07	3	2	372	375	-	-	293	-	-	-	-	-
08	1	1	329	348	-	-	301	310	320	193	197	2709
08	1	2	-	-	238	239	314	-	-	-	-	-
08	2	1	340	334	-	-	330	CD	CD	CD	CD	CD
08	2	2	-	-	247	236	317	-	-	-	-	-
08	3	1	353	336	-	-	330	CD	CD	CD	CD	CD
08	3	2	-	-	250	265	337	-	-	-	-	-
09	1	1	389	392	-	-	343	330	363	NP	245	305
09	1	2	-	-	257	268	327	-	-	-	-	-
09	2	1	320	324	-	-	359	NP	308	225	224	313
09	2	2	-	-	225	221	332	-	-	-	-	-
09	3	1	352	371	-	-	335	345	341	247	253	340
09	3	2	-	-	219	223	322	-	-	-	-	-
10	1	1	352	341	243	233	351	323	343	208	197	351
10	2	1	NL	353	227	235	317	NL	NP	200	210	325
10	3	1	329	384	NP	232	NL	338	NP	199	208	336

NL: Non-linear; NP: Non-parallel; CD: Coded duplicate sample ratio >1.2

Table 2: Individual assay relative potency estimates, relative to current IS 12/104 - Type 2

Lab	Assay	Plate	In-house method					NIBSC method				
			24001	24002	24003	24004	BRP3	24001	24002	24003	24004	BRP3
01	1	1	69	71	51	54	71	77	82	56	NP	80
01	2	1	NL/NP	NL/NP	36	38	63	70	NP	50	NP	70
01	3	1	NL/NP	NL/NP	NL/NP	NL/NP	70	74	NP	53	56	76
02	1	1	86	85	57	54	67	-	-	-	-	-
02	2	1	CD	CD	CD	CD	CD	-	-	-	-	-
02	3	1	91	86	60	60	80	-	-	-	-	-
03	1	1	73	NL	NL	58	NP	73	NL	NL	58	NP
03	2	1	107	97	71	78	94	107	97	71	78	94
03	3	1	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD
04	1	1	75	75	53	51	74	NL	67	64	NL	NL
04	2	1	82	79	51	55	77	79	NP	67	61	81
04	3	1	82	81	56	58	79	72	78	52	57	81
05	1	1	39	38	29	-	38	89	81	61	-	86
05	1	2	-	-	-	32	41	-	-	-	48	72
05	2	1	76	73	52	-	68	CD	CD	CD	-	CD
05	2	2	-	-	-	51	69	-	-	-	85	124
05	3	1	77	80	54	-	71	148	143	209	-	160
05	3	2	-	-	-	52	66	-	-	-	120	172
06	1	1	79	82	-	-	85	CD	CD	CD	CD	CD
06	1	2	-	-	CD	CD	CD	-	-	-	-	-
06	2	1	78	79	-	-	NL	NP	84	58	64	NP
06	2	2	-	-	53	54	86	-	-	-	-	-

06	3	1	80	81	-	-	88	82	81	61	62	86
06	3	2	-	-	57	57	88	-	-	-	-	-
07	1	1	75	81	-	-	74	-	-	-	-	-
07	1	2	-	-	CD	CD	CD	-	-	-	-	-
07	2	1	76	78	-	-	72	-	-	-	-	-
07	2	2	-	-	52	52	69	-	-	-	-	-
07	3	1	-	-	55	52	66	-	-	-	-	-
07	3	2	78	76	-	-	71	-	-	-	-	-
08	1	1	83	86	-	-	83	70	69	53	55	NP
08	1	2	-	-	65	62	84	-	-	-	-	-
08	2	1	93	92	-	-	89	58	70	48	48	NP
08	2	2	-	-	68	63	89	-	-	-	-	-
08	3	1	96	84	-	-	89	CD	CD	CD	CD	CD
08	3	2	-	-	65	62	91	-	-	-	-	-
09	1	1	93	88	-	-	79	77	80	57	56	81
09	1	2	-	-	55	54	75	-	-	-	-	-
09	2	1	79	82	-	-	86	77	80	57	57	81
09	2	2	-	-	59	58	NP	-	-	-	-	-
09	3	1	79	80	-	-	NP	78	88	63	64	86
09	3	2	-	-	58	58	89	-	-	-	-	-
10	1	1	86	NL	47	51	91	78	85	61	60	76
10	2	1	80	78	52	53	NL	76	74	53	53	78
10	3	1	71	72	44	44	83	78	81	54	52	74

NL: Non-linear; NP: Non-parallel; CD: Coded duplicate sample ratio >1.2

Table 3: Individual assay relative potency estimates, relative to current IS 12/104 - Type 3

Lab	Assay	Plate	In-house method					NIBSC method				
			24001	24002	24003	24004	BRP3	24001	24002	24003	24004	BRP3
01	1	1	243	255	207	210	281	274	286	205	206	291
01	2	1	321	275	NP	223	282	260	271	203	201	274
01	3	1	NL	260	169	165	256	243	NP	NP	176	NP
02	1	1	283	281	195	202	283	-	-	-	-	-
02	2	1	275	262	NL	NP	276	-	-	-	-	-
02	3	1	295	275	200	201	293	-	-	-	-	-
03	1	1	NP	299	263	NL/NP	NP	NP	299	263	NL/NP	NP
03	2	1	CD	CD	CD	CD	CD	CD	CD	CD	CD	CD
03	3	1	NL	284	NL	228	NP	NL	284	NL	228	NP
04	1	1	251	253	192	179	257	256	289	218	192	269
04	2	1	235	248	189	179	253	NP	312	193	208	224
04	3	1	237	274	197	201	273	225	267	195	220	250
05	1	1	116	121	NL	-	115	303	276	246	-	313
05	1	2	-	-	-	95	107	-	-	-	184	250
05	2	1	250	NL	187	-	223	322	331	282	-	404
05	2	2	-	-	-	181	203	-	-	-	237	309
05	3	1	267	272	NL	-	186	516	461	712	-	563
05	3	2	-	-	-	212	251	-	-	-	456	538
06	1	1	402	405	-	-	459	CD	CD	CD	CD	CD
06	1	2	-	-	293	296	439	-	-	-	-	-
06	2	1	260	257	-	-	288	286	265	196	217	258
06	2	2	-	-	188	196	295	-	-	-	-	-

06	3	1	271	288	-	-	283	267	281	218	242	254
06	3	2	-	-	208	221	279	-	-	-	-	-
07	1	1	249	264	-	-	234	-	-	-	-	-
07	1	2	-	-	CD	CD	CD	-	-	-	-	-
07	2	1	243	262	-	-	229	-	-	-	-	-
07	2	2	-	-	190	192	227	-	-	-	-	-
07	3	1	-	-	186	203	218	-	-	-	-	-
07	3	2	248	250	-	-	241	-	-	-	-	-
08	1	1	265	268	-	-	265	CD	CD	CD	CD	CD
08	1	2	-	-	211	208	255	-	-	-	-	-
08	2	1	267	269	-	-	NP	224	254	184	204	NL/NP
08	2	2	-	-	229	214	263	-	-	-	-	-
08	3	1	273	266	-	-	279	CD	CD	CD	CD	CD
08	3	2	-	-	213	213	267	-	-	-	-	-
09	1	1	289	309	-	-	263	263	253	206	205	240
09	1	2	-	-	192	200	NP	-	-	-	-	-
09	2	1	179	184	-	-	233	259	273	249	NL	208
09	2	2	-	-	201	200	270	-	-	-	-	-
09	3	1	179	184	-	-	233	297	NL	NL	223	243
09	3	2	-	-	200	200	270	-	-	-	-	-
10	1	1	271	NP	NP	161	251	304	276	199	215	252
10	2	1	237	244	NP	NP	197	297	332	235	215	252
10	3	1	CD	CD	CD	CD	CD	293	326	208	193	214

NL: Non-linear; NP: Non-parallel; CD: Coded duplicate sample ratio >1.2

Table 4: Laboratory geometric mean potency estimates relative to BRP3, using in-house methods (DU/ml) – Type 1

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	344	n/a	1	334	n/a	1	339	n/a	2	215	n/a	1	227	n/a	1	221	n/a	2
02	371	n/a	2	363	n/a	2	367	6.73	4	252	n/a	2	253	n/a	2	252	0.86	4
03	411	n/a	2	360	n/a	2	385	7.97	4	244	n/a	2	241	n/a	2	243	13.54	4
04	337	n/a	2	397	n/a	1	356	10.23	3	223	n/a	2	216	n/a	2	219	4.32	4
05	442	n/a	2	441	n/a	2	441	1.54	4	256	n/a	2	275	n/a	1	262	4.54	3
06	319	0.98	3	327	0.12	3	323	1.46	6	205	n/a	2	212	n/a	2	208	3.07	4
07	392	3.79	3	410	0.19	3	401	3.43	6	243	6.02	3	243	6.05	3	243	5.38	6
08	340	3.19	3	339	7.83	3	340	5.30	6	243	2.39	3	244	3.01	3	244	2.45	6
09	326	13.26	3	334	13.67	3	330	12.05	6	228	8.84	3	231	11.56	3	230	9.18	6
10	321	n/a	1	333	n/a	2	329	7.35	3	225	n/a	2	224	n/a	2	225	4.92	4
Overall	358	11.96	10	362	10.93	10	359	10.65	10	233	7.42	10	236	8.11	10	234	7.37	10

N = number of estimates used in calculations; n/a = not calculated as N < 3

Table 5: Laboratory geometric mean potency estimates relative to BRP3, using common NIBSC method (DU/ml) – Type 1

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	329	5.82	3	322	2.02	3	325	4.07	6	219	n/a	2	218	7.62	3	218	6.39	5
02		n/t			n/t			n/t			n/t			n/t			n/t	
03	411	n/a	2	360	n/a	2	385	7.97	4	244	n/a	2	241	n/a	2	243	13.54	4
04	337	9.03	3	349	n/a	2	342	7.92	5	229	9.00	3	248	11.75	3	238	10.42	6
05	286	n/a	1	303	n/a	1	294	n/a	2	190	n/a	1	218	4.75	3	210	8.16	4
06	342	6.05	3	367	5.39	3	354	6.45	6	250	n/a	2	257	n/a	2	254	6.15	4
07		n/t			n/t			n/t			n/t			n/t			n/t	
08	37*	n/a	1	38*	n/a	1	37*	n/a	2	23*	n/a	1	23*	n/a	1	23*	n/a	2
09	335	n/a	2	337	11.07	3	336	8.09	5	231	n/a	2	241	6.14	3	237	4.89	5
10	308	n/a	2	312	n/a	1	309	4.64	3	192	2.35	3	195	7.52	3	193	5.01	6
Overall	253	119.86	8	255	116.85	8	254	118.16	8	166	124.53	8	173	125.88	8	170	125.44	8
Overall**	334	11.88	7	335	7.60	7	334	9.28	7	221	11.65	7	230	10.20	7	227	10.02	7

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; *=outlier by Grubbs test; ** = results excluding outliers

Table 6: Laboratory geometric mean potency estimates relative to BRP3, using in-house methods (DU/ml) – Type 2

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N	GM	GCV	N
01	75	n/a	1	78	n/a	1	77	n/a	2	49	n/a	2	52	n/a	2	51	14.89	4
02	94	n/a	2	91	n/a	2	92	8.52	4	62	n/a	2	60	n/a	2	61	6.37	4
03	89	n/a	1	80	n/a	1	84	n/a	2	59	n/a	1	65	n/a	1	62	n/a	2
04	81	1.87	3	80	0.46	3	80	1.45	6	54	4.42	3	55	3.18	3	55	3.71	6
05	86	n/a	2	86	n/a	2	86	2.29	4	59	n/a	2	60	n/a	2	60	2.77	4
06	72	n/a	2	74	n/a	2	73	2.24	4	49	n/a	2	50	n/a	2	50	2.67	4
07	82	4.21	3	85	0.49	3	83	3.03	6	62	n/a	2	61	n/a	2	61	4.18	4
08	81	4.13	3	78	5.36	3	80	4.77	6	58	4.18	3	55	4.15	3	57	4.80	6
09	81	17.88	2	80	n/a	2	81	12.07	4	54	n/a	2	54	n/a	2	54	5.70	4
10	70	n/a	2	67	n/a	1	69	5.54	3	41	n/a	2	42	n/a	2	41	3.01	4
Overall	81	9.65	10	80	8.60	10	80	8.74	10	54	14.18	10	55	13.02	10	55	13.39	10

N = number of estimates used in calculations; n/a = not calculated as N < 3

Table 7: Laboratory geometric mean potency estimates relative to BRP3, using common NIBSC method (DU/ml) – Type 2

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	76	1.94	3	80	n/a	1	77	2.84	4	55	1.49	3	57	n/a	1	56	2.30	4
02		n/t			n/t			n/t			n/t			n/t			n/t	
03	89*	n/a	1	80	n/a	1	84	n/a	2	59	n/a	1	65*	n/a	1	62	n/a	2
04	73	n/a	2	75	n/a	1	74	5.36	3	57	n/a	2	57	n/a	2	57	11.35	4
05	76	n/a	2	72	n/a	2	74	6.17	4	75*	n/a	2	53*	2.87	3	61	33.39	5
06	74	n/a	1	74	n/a	1	74	n/a	2	56	n/a	1	57	n/a	1	56	n/a	2
07		n/t			n/t			n/t			n/t			n/t			n/t	
08		NP			NP			NP			NP			NP			NP	
09	73	2.67	3	78	2.56	3	76	4.20	6	56	1.83	3	56	3.23	3	56	2.35	6
10	79	5.14	3	82	9.59	3	81	7.09	6	57	8.97	3	56	8.87	3	57	8.06	6
Overall	77	7.20	7	77	5.13	7	77	5.43	7	59	11.52	7	57	6.35	7	58	4.41	7
Overall**	75	3.32	6	77	5.13	7	77	5.43	7	57	2.44	6	56	1.20	5	58	4.41	7

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; NP = non-parallel to reference sample; *=outlier by Grubbs test; ** = results excluding outliers

Table 8: Laboratory geometric mean potency estimates relative to BRP3, using in-house methods (DU/ml) – Type 3

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	286	n/a	2	278	5.72	3	281	11.09	5	201	n/a	2	209	11.37	3	206	9.26	5
02	289	0.62	3	277	2.88	3	283	2.99	6	197	n/a	2	201	n/a	2	199	2.12	4
03		NP			NP			NP			NP			NP			NP	
04	266	5.97	3	285	1.34	3	275	5.44	6	213	1.95	3	206	3.01	3	209	2.97	6
05	366	n/a	2	421	n/a	1	383	15.90	3	242	n/a	1	250	n/a	2	247	3.41	3
06	263	4.65	3	268	8.15	3	265	5.99	6	196	8.61	3	204	10.40	3	200	8.78	6
07	303	2.00	3	318	5.24	3	311	4.38	6	243	n/a	2	256	n/a	2	250	5.19	4
08	285	n/a	2	283	n/a	2	284	2.68	4	239	4.68	3	233	1.27	3	236	3.43	6
09	249	22.84	3	260	25.51	3	254	21.55	6	214	n/a	2	214	n/a	2	214	0.13	4
10	329	n/a	2	357	n/a	1	338	7.55	3		NP		185	n/a	1	185	n/a	1
Overall	291	12.58	9	301	17.14	9	295	13.79	9	217	9.78	8	216	11.25	9	215	10.95	9

N = number of estimates used in calculations; n/a = not calculated as N < 3; NP = non-parallel to reference sample

Table 9: Laboratory geometric mean potency estimates relative to BRP3, using common NIBSC method (DU/ml) – Type 3

Lab	24001			24002			24001/24002			24003			24004			24003/24004		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	272	n/a	2	284	n/a	2	278	2.42	4	208	n/a	2	208	n/a	2	208	2.56	4
02		n/t			n/t			n/t			n/t			n/t			n/t	
03		NP			NP			NP			NP			NP			NP	
04	267	n/a	2	336	16.14	3	307	18.15	5	235	5.16	3	240	14.73	3	238	9.79	6
05	257	10.58	3	242	4.32	3	249	7.94	6	255	37.00	3	225	7.42	3	240	23.99	6
06	312	n/a	2	307	n/a	2	309	3.91	4	233	n/a	2	258	n/a	2	245	9.73	4
07		n/t			n/t			n/t			n/t			n/t			n/t	
08		NP			NP			NP			NP			NP			NP	
09	341	7.25	3	338	n/a	2	340	9.69	5	291	n/a	2	254	n/a	2	272	17.39	4
10	359	8.40	3	374	17.94	3	367	12.56	6	257	11.65	3	250	3.35	3	253	7.73	6
Overall	299	15.00	6	311	16.70	6	306	14.79	6	245	12.08	6	239	8.67	6	242	9.29	6

N = number of estimates used in calculations; n/a = not calculated as N < 3; n/t=method not tested by lab; NP = non-parallel to reference sample

Appendix 3

NIBSC D-Antigen ELISA method for use with Mouse MAbs

- Add 50µl per well of sheep anti-polio capture polyclonal antibody diluted 1:500 in carbonate coating buffer. Overnight incubation at 2-8ºC
    - PV1: Sheep anti-polio 1 (13/218) 1:500 in Dilution Buffer
    - PV2: Sheep anti-polio 2 (13/220) 1:500 in Dilution Buffer
    - PV3: Sheep anti-polio 3 (13/222) 1:500 in Dilution Buffer
- Day of assay, wash plates x4 (wash buffer: PBS + 2% Milk + 0.5% Tween 20).
- Leave the 4th wash on the plate for 30 mins at room temperature to block
- Prepare sample dilutions in dilution buffer (PBS + 2% Milk) with starting dilutions as indicated in the protocol
- Add 50µl of each dilution to duplicate wells. Incubate at 37ºC for 2 hours.
- Prepare the mouse MAb detection antibody as below:
    - PV1: MAb 234 diluted 1:100 in Dilution Buffer
    - PV2: MAb 1050 diluted 1:100 in Dilution Buffer
    - PV3: MAb 520 diluted 1:100 in Dilution Buffer
- Wash plates x3 (wash buffer), add 50µl of mouse anti-polio detection monoclonal antibody diluted 1:100 in Dilution Buffer. Incubate at 37ºC for 1 hour
- Wash plates x3 (wash buffer), add 50µl anti-mouse IgG-HRP diluted 1:400. Incubate at 37ºC for 1 hour
- Wash plates x3 (PBS), add 50 µl OPD substrate buffer. Incubate at room temperature for 30 minutes in the dark.
- Stop reaction by adding 50 µl of 1 M H_2SO_4. Read OD at 492nm
- Data analysis

Reagents

- Carbonate coating buffer: $NaCO_3$ – Merck, $NaHCO_3$ –Fisher (1.590g $NaCO_3$ +2.930g $NaHCO_3$ in 1/L ultra-pure water, pH 9.6) store at +4ºC.
- Wash buffer: PBS + 2.0% dried milk power + 0.5% Tween 20
- Dilution buffer: PBS + 2.0% dried milk powder
- Milk: Dried milk powder. (Marvel bought at supermarket)
- Tween 20: Sigma - P7943
- Anti-mouse IgG: Sigma – A6782
- OPD: Sigma – P8412 - 100 TABS
- Substrate Buffer - Mix 12.15ml 0.1M Citric acid, 12.85ml 0.2M Na_2HPO_4 and 25ml distilled water. Prepare immediately before use.
- 1 M H_2SO_4

Recipe for substrate reagents

0.1M Citric acid - 19.2g made up to 1 litre with distilled H_20 in a measuring cylinder or
0.1M Citric acid.H_2O - 21.0g made up to 1 litre with distilled H_20 in a measuring cylinder.

0.2M Na_2HPO_4 - 28.4g made up to 1 litre with distilled H_20 in a measuring cylinder or
0.2M $Na_2HPO_4.12H_2O$ - 71.63g made up to 1 litre with distilled H_20 in a measuring cylinder.
(Storage is at room temperature)

OPD substrate - Prepare in a 50ml centrifuge tube: 1 x 30mg OPD tablet (Sigma) + 50ml substrate buffer + 50ul hydrogen peroxide (30%). Use within 1 hour of addition of tablet to buffer and add H_2O_2 immediately before use (Store in the dark).

Appendix 4

Proposed instruction for use

4th International Standard for Inactivated Polio Vaccine
08-143

"This material is not for *in vitro* diagnostic use"

1. INTRODUCTION

Preparation was established by the WHO Expert Committee on Biological Standardisation in 2026 as the 4th International Standard for inactivated poliovirus vaccine. It was shown to be suitable for the determination of antigenic in *in vitro* assays.
The preparation is a liquid trivalent blend of formaldehyde-inactivated monovalent pools of poliovirus type 1, 2 and 3.

2. UNITAGE

359 D-Ag/ml for type 1 antigen
81 D-Ag/ml for type 2 antigen
265 D-Ag/ml for type 3 antigen

3. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

The preparation does not contain material of human origin.

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 4th International Standard for IPV should be used to calibrate laboratory reference reagents to be used in *in vitro* assays for the determination of the antigenic content of inactivated poliovirus vaccines.

Unopened ampoules should be stored at ≤ -70°C. To remove the reagent from the ampoule, it is necessary to use some form of transfer pipette rather than volumetric pipette. The contents of the ampoules should not be assumed 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.

Please note, the 4th IS is provided as a reagent for calibrating your own in-house reference material(s). It is not intended that this product be used as a working reference.

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 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) ("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

4th International Standard for Inactivated Polio Vaccine NIBSC code 08-143 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)			
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.			