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Development and Characterisation of the Proposed 5th WHO International Standard for Pertussis Vaccine (Whole Cell)

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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 **23 March 2026** and should be addressed to:

Product Standards, Specifications and Nomenclature
Department of Medicines and Health Products Policies and Standards
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Comments may also 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 and Proposal

Here we report details of the production and characterisation of the proposed 5th WHO International Standard (IS) for Whole Cell Pertussis (wP) Vaccine, including the results of an international collaborative study to establish the candidate 5th IS (coded 24/116) as a replacement for the 4th WHO IS for wP Vaccine (coded 94/532). The aims of the collaborative study were to assess the suitability of the candidate to serve as the 5th IS for wP Vaccine, to assign a unitage to the candidate in International Units (IU), and to assess continuity of the IU. In addition, a thermal degradation study was conducted at MHRA in order to confirm suitable stability of the candidate standard.

The intracerebral mouse protection test (Kendrick test) was used to estimate the potency of the candidate standard relative to the 4th IS. This was performed using data from 12 laboratories in 8 countries and there was good agreement in the results obtained (intra-laboratory GCV ranging from 8% to 24% and inter-laboratory GCV of 24%). The results from an accelerated thermal degradation study, after storage of ampoules of 24/116 at elevated temperatures for 1 year, indicate that the candidate standard will be sufficiently stable for both shipping at ambient temperature and for long-term storage at the recommended storage temperature.

Based on the results of this study, and with the agreement of the participants, it is proposed that 24/116 be established as the 5th WHO IS for wP Vaccine, and that it be assigned an activity of 60 IU per ampoule on the basis of its estimated potency relative to the 4th WHO IS in the Kendrick test.

Introduction

Pertussis vaccine is available in two formulations - as wP vaccine and acellular pertussis (aP) vaccine. Despite aP vaccines being associated with fewer mild adverse events after immunisation compared to wP-containing combination vaccines [1, 2], wP vaccines continue to be the most widely used globally, particularly across Africa, South and Southeast Asia, Latin America, and the Western Pacific [3]. This widespread use of wP vaccines in the national immunisation programmes of low- and middle-income countries is mainly driven by economic reasons. In addition to the lower cost of producing wP vaccines [4], WHO recommends that countries that currently use wP vaccines continue to do so, because generally they provide longer lasting protection against pertussis (whooping cough) compared to aP vaccines [3, 5], which is also a consideration where periodic booster doses and maternal immunisation are not affordable, and is of benefit where vaccination has been interrupted or when coverage has markedly decreased [6].

The potency of wP vaccines is measured in IU, using the Kendrick test [7]. The Kendrick test remains the only official and internationally recognised potency test for wP vaccines, and therefore the availability of an IS calibrated in IU is critical for the routine potency control testing of wP vaccines.

The 4th WHO IS for wP Vaccine (94/532) was established in 2006 [8, 9] and is now in limited supply. A project was subsequently endorsed by WHO ECBS in 2021 [10, 11] to develop a replacement lyophilized wP preparation, and to assess its suitability to serve as a WHO IS for use in the Kendrick test.

Bulk material and processing

Six litres of inactivated *Bordetella pertussis* (whole cell) vaccine bulk suspension was kindly donated by Serum Institute of India Pvt. Ltd., Pune in 2023, with a stated optical density value of 180 (±20) International Opacity Units in terms of the International Opacity Standard, and an estimated potency of approximately 60 IU/mL. Potency testing of the liquid vaccine bulk in the Kendrick test to demonstrate fitness for purpose was performed at MHRA (data not shown).

Trial fills were performed at MHRA with the undiluted liquid bulk material freeze-dried (1 mL per 5 mL DIN ampoule) in three different formulations using dextran and trehalose as stabilisers. The 4th IS (94/532) was formulated with dextran and glucose, but trehalose was the preferred alternative to glucose to avoid the Maillard reaction [12]. Two of the formulations were stabilised with either 8% high molecular weight (77 kDa) or 8% low molecular weight (10 kDa) dextran alone, and the third formulation included both 8% low molecular weight (10 kDa) dextran and 5% trehalose as excipients. Product appearance was good across all formulations, with robust, homogeneous cakes formed, and moisture and oxygen content was satisfactory for all formulations. The formulation of 8% dextran (10 kDa) and 5% trehalose was chosen as the preferred option for the definitive fill due to its similarity to the formulation of 94/532, and subsequently one ampoule of this trial formulation was tested at MHRA in the Kendrick test, with the potency estimated to be 51 IU/mL (equivalent to 51 IU/ampoule).

Definitive fill

The bulk material was processed for filling and freeze-drying at MHRA on 9th May 2024. The material was formulated with 8% (w/v) dextran (Sigma, product code D9260, MW 9,000 – 11,000) and 5% (w/v) trehalose (Sigma, product code 90210) prior to filling (1 mL per 5 mL DIN ampoule) using a Bausch & Strobel filling machine (AFV5090). During filling, the material was stirred constantly, and the temperature was maintained between +4°C to +8°C. The ampoule contents were freeze-dried using a Serail CS100 freeze dryer (Le Coudray St Garmer, France) following a four-day cycle (4 hours freezing, 43 hours freeze-drying, 30 hours secondary drying, and 20 hours thermal tempering). Ampoules were sealed and stored in the dark at -20°C. A total of 6401 ampoules were filled, and the finished product was coded 24/116.

An unusually high proportion (21.7%) of the ampoules were rejected and discarded due to burnt ampoule tops, material present within and above the ampoule necks, and net outside tolerance for weight of the fill. The most likely reason for this is the configuration of the filling apparatus not being optimal for the viscosity of the material, as dripping from the filling needle was observed periodically during the production run which not only caused the fill weights to be outside the predetermined acceptable range, but caused the product to splash onto the necks of a number of ampoules, some of which was subsequently burnt black during flame sealing. Despite this issue, there was no impact on the quality of the contents of the candidate ampoules in the final batch.

Characterization of freeze-dried candidate standard

After filling and freeze-drying, the candidate material (24/116) was examined for appearance, residual moisture content, oxygen head space and microbial contamination. The precision of fill was determined by weighing representative ampoules from across the production run after filling. An accelerated degradation study was initiated at MHRA in order to assess the stability of the candidate standard. The potency of the freeze-dried product was initially estimated at MHRA in a single Kendrick test to assess fitness for purpose.

All Kendrick tests undertaken at MHRA were performed under a Project Licence granted by the UK Home Office (PP4174842) and reviewed and approved by the MHRA Animal Welfare and Ethical Review Body.

Collaborative study design and methods

Study design

An international collaborative study (coded CS728) was organised by MHRA to assess the suitability of the candidate standard (24/116) to serve as a WHO IS for wP vaccine and, subject to its suitability, to assign units to maintain continuity of the International Unit. In this study, the potency of 24/116 was estimated in IU relative to the 4th IS (94/532) using the Kendrick test.

A total of 14 laboratories from 9 countries agreed to participate in the study. Each laboratory was randomly assigned a unique code number by which they are referred to in this report. The participants are listed alphabetically by country in Appendix A, and the randomly assigned code numbers are not related to the order of listing. Data was not returned from laboratories 7 and 13 within the timeframe of the study.

Participants were provided with study guidelines to aid with the planning of the tests and preparation of the samples, including the provision of a nominal potency of 50 IU/ampoule (based in the result of a single fitness for purpose Kendrick test performed at MHRA) for 24/116 to aid with the selection of suitable dilutions to prepare for the Kendrick test.

Intracerebral Mouse Protection Test (Kendrick Test)

Each laboratory was asked to perform a minimum of two independent Kendrick tests using their own routine procedures, reagents and analytical methods, and to use locally sourced mice along with the strain of *B. pertussis* in routine use in the individual laboratory.

Sufficient ampoules of 94/532 and 24/116 were provided to each laboratory to allow up to a maximum of five independent tests to be performed using fresh ampoules for each test. Participants were encouraged to include their own in-house reference preparation in each test (if available), and manufacturers were asked to include an anonymised vaccine sample in each test (if possible) so that potency results could be compared when estimated against both 94/532 and 24/116 to assess continuity of the International Unit.

Reporting of data and statistical analysis

All test details and raw data were returned to MHRA using the provided summary form to permit independent analysis.

Data from all assays were analysed by probit parallel-line bioassay analysis [13], comparing transformed assay responses to log dose using CombiStats software [14]. For all assays, data for each test preparation was analysed separately against the reference preparation chosen for that analysis, and the resulting potency estimates are therefore based on direct pair-wise comparisons. Linearity and parallelism were assessed by analysis of variance, using the 5%

level as a threshold for significance, and Grubbs' Test [15] was applied to each set of log-transformed potency estimates to identify possible outliers.

All relative potency estimates were combined to generate an unweighted geometric mean (GM) potency per laboratory. The laboratory GM values were then used to calculate an overall unweighted GM potency, median and robust GM. The robust GM was calculated with the R package 'WRS2' using Huber's M-Estimator [16]. Variability between assays and laboratories 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 study

An accelerated thermal degradation study was initiated at MHRA to assess whether the candidate standard is suitably stable for both shipping at ambient temperature and for long-term storage. Representative ampoules of 24/116 were stored at elevated temperatures (+4°C, +20°C, +37°C and +45°C), in addition to the recommended storage temperature of -20°C.

The evaluation of stability in the study to establish the 4th IS demonstrated that ampoules stored at +37°C for one year, and ampoules stored at both +4°C and +20°C for up to 32 months, showed no loss of activity relative to that for ampoules stored continuously at -20°C [8, 9]. To confirm the previous findings, and to provide more information on storage at higher temperatures, in this study we tested ampoules of 24/116 stored for one year at +37°C and +45°C in a single Kendrick test. One ampoule from each of the two elevated temperatures was tested in the same assay relative to two ampoules that had been stored for one year at the recommended temperature of -20°C.

Stability after reconstitution will not be assessed in the Kendrick test as, after reconstitution, the material is not expected to be stored for a prolonged period. Furthermore, experience with material of a similar type to the candidate standard suggests that such material may not be stable after reconstitution, and it is recommended that freshly reconstituted material should be used for each Kendrick test [9].

Results and Data Analysis

Characterization of freeze-dried product, 24/116

The final product batch contained ampoules with both homogeneous and heterogeneous (material separated into visible layers) cake structures, although all cakes produced throughout the fill were of a robust composition.

The mean fill mass was 1.008 g with a coefficient of variation (CV) of 0.25% (n = 360). Ampoules were sealed under boiled off gas from high purity liquid nitrogen (99.99%) and measurement of the mean oxygen head space (0.18%, CV 66%, n = 12) after sealing was used as a measure of ampoule integrity. The mean dry weight was 0.142 g with a CV of 0.6% (n = 6). Residual moisture content (0.67%, CV 21%, n = 12) was measured using thermogravimetric analysis coupled with mass spectrometry, with total moisture expressed as a percentage of the mean dry weight of the ampoule contents. No microbial contamination was detected in representative samples (n = 4).

An ampoule of 24/116 was included in a Kendrick test performed at MHRA, and the potency was estimated to be 49 IU/ampoule (95% confidence interval (CI) 21-109), and this potency was included in the study guidelines (as a nominal potency of 50 IU/ampoule) provided to the collaborative study participants to help with the selection of suitable dilutions to prepare for the Kendrick test. The pre-filled formulated liquid bulk was also tested in the same assay, and the potency was estimated to be 58 IU/mL (95% CI 27-120).

A summary of the characterization testing of 24/116 is provided in Table 1.

Collaborative study data received

Data from 31 assays was returned by participants, across 12 laboratories (including MHRA). Nine laboratories reported data for an in-house reference sample, and 3 reported data for an anonymised vaccine sample. All laboratories performed between 2 to 4 assays.

A summary of the details of the assays performed by the participants is provided in Tables 2a to 2f.

Assay validity and data exclusions

Some assay results were excluded due to non-linearity of either the reference or test sample, or due to non-parallelism between reference and test samples. Across the study, one result (0.8%) was excluded due to non-linearity, and seven results (5.6%) were excluded due to non-parallelism, none of which were results that were used to calibrate 24/116 against 94/532. One estimate was excluded due to being identified as an outlier.

Estimated potency of the candidate International Standard

All assays that estimated the potency of the candidate IS relative to the current IS were valid, and only one value was excluded as an outlier. The inter-laboratory variation, where there were sufficient assays to measure it, was below 25% in all cases. The GM potency across laboratory mean values was 59.0 IU/ampoule (95% CI 51.5-67.7) with an overall GCV of 24%. The results are shown in Table 3 and Figures 1 and 2. Additionally, the robust GM and median value for this data set were calculated as 58.9 IU/ampoule and 55.6 IU/ampoule, respectively. The GM potency across laboratory mean values for the candidate IS relative to the in-house reference was a similar potency of 60.8 IU/ampoule, but with a higher GCV value of 37%. These results can be seen in Table 4 and Figures 3 and 4.

Continuity of the International Unit

The potencies of the laboratory in-house references and anonymised vaccine samples included in the study were estimated in terms of both the 4th IS and the candidate IS to establish if the measured IU was consistent when transitioning from the current IS to the new IS. This is to give end users confidence that they will see good continuity of results for IU potency estimates when testing whole cell pertussis vaccines.

The potency estimates of the in-house reference samples relative to both 94/532 and 24/116 are reported in Tables 5 and 6, and Figure 5. All assays performed by one laboratory were invalid due to non-parallelism when estimates were calculated relative to the candidate IS. For most laboratories the results are close to the reported potencies of the in-house references, and the percentage difference from the expected value is included in Tables 5 and 6. Furthermore, in all cases where each in-house reference has been tested within a laboratory relative to both 94/532 and 24/116, the 95% CI of the potency estimates overlap indicating that there is not likely to be any significant difference between the estimates.

The potency estimates of the anonymised vaccine samples relative to 94/532, 24/116, and the in-house references, are reported in Tables 7, 8 and 9, and Figure 6. Only one estimate was invalid due to non-linearity. Overall, estimates for the same vaccine sample were comparable, regardless of the reference used, with the maximum difference between any two estimates for the same vaccine sample being 35%. Furthermore, in all cases where each vaccine sample has been tested relative to either 94/532, 24/116 or the in-house reference, the 95% CI of the potency estimates overlap, indicating that there is not likely to be any significant difference between the estimates.

Furthermore, the potency of the candidate IS calculated relative to the 4th IS or the in-house reference in each laboratory (Tables 3 and 4 and Figures 1 to 4), also indicates good continuity of the IU, because, if the laboratories have calibrated their in-house references accurately against the 4th IS, we would expect to see a similar potency estimate for the candidate when it is calculated relative to either the 4th IS or the in-house reference, as is the case here.

Stability study

Ampoules of 24/116 which had been stored for one year at +37°C, +45°C, and the recommended storage temperature of -20°C, were tested together in a Kendrick test performed at MHRA to assess whether the candidate standard is suitably stable.

The relative potency estimates of the +37°C and the +45°C samples, calculated relative to the -20°C samples are shown in Table 10, and these were put into an analysis to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [17] and hence predict the degradation rate when stored at -20°C. However, the model could not be fitted to the data, and no prediction could be made due to the low precision of estimates obtained from the Kendrick test, where the width of the 95% CI is approximately 50-200% of the estimates obtained. For both high temperature samples, the upper CI was above 1, demonstrating that there is no significant difference between the calculated potency estimates for either the +37°C or the +45°C sample and the -20°C sample. This data supports that the candidate standard is suitably stable for shipment at ambient temperature and is likely to be very stable when stored at the recommended storage temperature.

Summary and Recommendation

Whole cell pertussis vaccine, coded 24/116, was confirmed to be fit-for-purpose in terms of potency, precision of fill, residual moisture content and ampoule integrity.

Although the appearance across the batch was a mix of homogeneous and heterogeneous cakes (the latter separated into visible layers), subsequent testing undertaken at MHRA of ampoules representative of both presentations did not indicate any difference in potency. All other ampoules of 24/116 were shipped to participants without recording their appearance. As phase separation was not observed in the trial fills, it can be speculated that the reason for the heterogeneous appearance could be due in some part to the process involved in the scale up to the definitive fill.

A Hamilton mLab filling machine was used for the trial fill in this study, which is not fully comparable to the Bausch & Strobel filling machine used for the definitive fill. Subsequently, the production team at MHRA has acquired an additional Telstar freeze-dryer, which is comparable to the Bausch & Strobel filling machine in terms of both machines having comparative vacuum gauge profiles in addition to thermocouples. Whereas thermocouples only report the temperature of the ampoules they are inserted into, using the gauges gives an indication of when the entire batch has dried sufficiently. Having this capability in both trial fill freeze-dryers will help to ensure the final production cycle is optimised when scaling up from a small-scale fill to a production batch.

The dripping and splashing issue, which led to a number of ampoules being rejected from the final stock, could have been due to the filling pistons used not being optimal for the viscosity of the material being filled. Whilst we have not observed similar issues during previous fills of wP vaccine, one future consideration would be to take a viscosity measurement of the material prior to filling using a rheosense viscometer to aid in the selection of the appropriate equipment configuration.

Extensive homogeneity testing was not undertaken as the low precision of the Kendrick test [18] would not give sufficient statistical power to distinguish heterogeneity between ampoules from the method repeatability. Furthermore, as there is no alternative to the Kendrick test for potency testing of wP vaccine, the large number of animals that would be required for this purpose would not be ethically justified.

Data from the accelerated degradation study confirms that the candidate standard is suitable for shipment at ambient temperature, and, along with similar material having previously been demonstrated to be stable when stored long-term at -20°C [8, 9], gives no indication that there will be any concerns about long-term stability.

The data generated from the testing of in-house references and anonymised vaccines samples included in the study provides confidence that good continuity of results for potency reported in IU will be achieved when transitioning to the new IS.

The inter-laboratory GCV was 24% (n = 12) for the Kendrick tests performed in this study. This compares favourably to the inter-laboratory variability of the previous collaborative study to establish the 4th IS (GCV 37%, n = 30), and the much smaller scale study to establish the 3rd IS (GCV 64%, n = 4).

The results from this study confirm that 24/116 is suitable as a **WHO International Standard for Whole Cell Pertussis Vaccine**. The proposed assigned units are 60 IU/ampoule. MHRA will act as custodian of the IS which will be stored under assured temperature-controlled conditions within the Agency's Centre for Biological Reference Materials.

A total of 6401 ampoules of 24/116 were filled at MHRA. After the collaborative study, in-house measurements, accelerated degradation studies, and containers rejected during the manufacturing process, **4742** ampoules remain available. Based on historical use, it can be predicted that this will be sufficient stock to last for at least 20 years.

Comments from participants

All participants who returned data were sent a draft of this report and asked to comment on the content and conclusions where necessary, and to confirm that their data had been reported accurately. All participants responded and all agreed with the findings of the report and the following specific comments were received:

1. Comment: Laboratory 1 highlighted that there is variability in the mouse strains used across laboratories and the number of mice per cage is inconsistent. They questioned whether the tests performed by one laboratory with an interval of 21 days between immunisation and challenge should be considered a modified Kendrick test as it does not comply with the WHO recommendations. They also noted that the average time between preparation of the challenge dose and administration of the challenge in the last mouse varies among laboratories, and that this may affect the consistency and comparability of results. Finally, they stated that it would be interesting to note the viable count of bacteria post-challenge and the total LD_{50} injected.
Response: There is no particular mouse strain specified by WHO, and in the study to establish the 4th IS there was also a similar range of mouse species used. It was noted that reporting the number of mice per cage is not informative and instead we have now included the number of mice per group in the report which will offer a more useful comparison between laboratories. The interval between immunisation and challenge as specified by WHO is only a recommendation. We note the differences in the times between preparation of the challenge dose and administration of the challenge in the last mouse, however we asked participants to follow their routine method, and we found the results of the study to be robust with a comparatively low GCV. We did not ask participants to provide a viable count before and after challenge, but we note that this could perhaps have been useful information to collect, however not all laboratories determine this routinely.

2. Comment: Laboratory 2 questioned why two laboratories used an obsolete Indian regional in-house reference (RWRS 01/11).
Response: The two relevant laboratories were contacted for comment. One laboratory replied to explain that they still have stock of RWRS 01/11 available and monitoring of the ED_{50} shows that it is still stable. They added that they are in the process of calibrating their own national reference which they will be switching to soon. The second laboratory replied to explain that they still have stock of RWRS 01/11 available and that it continues to serve as a valid reference.

3. Comment: Lab 4 asked for clarity on why some results obtained from analysis of their raw data at MHRA differed from the results they had calculated themselves.
Response: It was established that there was a misinterpretation of the final number of mice per group used in the analysis and that this had led to an error in the analysis performed at MHRA which was subsequently rectified.

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Abbreviations Used

aP: acellular pertussis, CFU: colony-forming units, CI: confidence interval, CV: coefficient of variation, GCV: geometric coefficient of variation, GM: geometric mean, IU: international units, IS: International Standard, WHO: World Health Organization, wP: whole cell pertussis.

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Table 1. Summary of the characterization testing of 24/116

MHRA product code	**24/116**
Bulk material manufacturer	Serum Institute of India Pvt. Ltd.
No. ampoules available	4742
Appearance	Off-white, freeze-dried cake
Mean fill mass[$]	1.008 g (CV 0.25%) (*n* = 360)
Mean dry weight	0.142 g (CV 0.6%) (*n* = 6)
Mean residual moisture[*]	0.67% (CV 21%) (*n* = 12)
Mean oxygen head space[#]	0.18% (CV 66 %) (*n*=12)

[$] Fill precision - <1% for a 1.0 mL fill considered acceptable for this type of material.
[*] Residual moisture - <1% historically provides adequate long-term stability.
[#] Oxygen content - ≤1.13% is used to indicate back-fill with nitrogen and container closure integrity.

Table 2a. Summary of mouse strain and immunisation details for Kendrick tests (as reported by participants)

Lab code	Mouse strain	Sex	No. mice per group	Age or weight range at immunisation	Interval between immunisation & challenge
1	NIH	M,F	24 (10 for controls)	14-16 g	14 days
2	Swiss webstar	M,F	20 (10 for controls)	13-16 g	14-15 days
3	Jcl:ICR	M	20 (10 for controls)	13-17 g	14 days
4	ICR	M,F	20 (12 for controls)	3-5 weeks, 10-18 g	14 days
5	Swiss	M,F	18 (12 for controls)	14-16 g	14-16 days
6	LACA	M,F	20 (10 for controls)	4-5 weeks, 13-16 g	14 days
8	Swiss	M	24 (10 for controls)	14-16 g	14 days
9	ICR	F	24 (10 for controls)	12-16 g	14 days
10	ddY	M	10 (10 for controls)	13-15 g	19 days
11	ddY	M,F	16 (10 for controls)	3-4 weeks, 12-16 g	14-17 days
12	NIH	M,F	20 (10 for controls)	10-12 g	21 days
14	NIH	M,F	20 (20 for controls)	3-4 weeks	14 days

Table 2b. Summary of challenge strain used in Kendrick tests (as reported by participants)

Lab code	Bacterial strain for challenge	Growth medium	Incubation temperature	No. subcultures	Age of challenge culture	Medium used to prepare challenge	Average CFU in challenge suspension
1	18323	N/A – ready to use culture	N/A	N/A	N/A	Tryptone solution	32
2	18323	N/A – ready to use culture	N/A	N/A	N/A	1% casamino acid	59
3	18323	Bordet Gengou agar	37°C	2	24 hrs	1% casamino acid	13.27%
4	18323	Bordet Gengou agar	35°C	6	24-30 hrs	1% casamino acid	148
5	18323	Bordetella selective medium (Thermo Fisher)	37°C	A few loops of bacteria	24 hrs	Casamino acid	Not done
6	18323	N/A – ready to use culture	N/A	N/A	N/A	1% casamino acid	93.9
8	18323	Bordet Gengou agar	35.5°C	2	22-24 hrs	1% casamino acid	41%
9	18323	N/A – frozen stock of bacteria	N/A	N/A	N/A	1% casamino acid	≤ 300
10	18323	Bordet Gengou agar	35°C	1	6 days	Casamino acid	80-400
11	18323	Bordet-Gengou agar	35°C	2	20-24 hrs	1% casamino acid	76.75
12	18323	Bordet Gengou agar	37°C	3	24 hrs	Protein peptone water	Not done
14	18323	Charcoal agar	37°C	2	20 hrs	1% casamino acid	1 x 10^4 CFU/dose

Table 2c. Summary of inoculation and challenge for Kendrick tests (as reported by participants)

Lab code	**Route**	**Challenge volume**	**Challenge dose (CFU/mL)**	**Intended LD_{50} of challenge dose**	**Titration performed**	**Average CFU per LD_{50}**	**Average time from preparation of challenge to challenge of last mouse**
1	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	375	1 hr 35 mins
2	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	219	1 hr 47 mins
3	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	333	3 hrs 23 mins
4	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	262	2 hrs
5	IC	30 µL	3.3×10^6	Approx. 100 x LD_{50}	Yes	143	2 hrs
6	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	625	1 hr 10 mins
8	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	324	1 hr 22 mins
9	IC	25 µL	4×10^6	100-1000 x LD_{50}	Yes	244	< 2.5 hrs
10	IC	30 µL	3.3×10^6	100-1000 x LD_{50}	Yes	440	2 hrs 8 mins
11	IC	25 µL	4×10^6	100-1000 x LD_{50}	Yes	152	1 hr 55 mins
12	IC	30 µL	2.7×10^6	100-1000 x LD_{50}	Yes	191	2 hrs 30 mins
14	IC	20 µL	9.1×10^4	100-1000 x LD_{50}	Yes	21.1	2 hrs 53 mins

Table 2d. Summary of in-house references included in the study (as reported by participants)

Lab code	Identifier	Supplier	Unitage	Presentation	Storage temperature
1	IPRS/20/PERT	Central Drugs Laboratory, India	75 IU/vial	Freeze-dried	-20°C
2	IPRS/20/PERT	Central Drugs Laboratory, India	75 IU/vial	Freeze-dried	-20°C
3	RWRS 01/11	Central Drugs Laboratory, India	63 IU/vial	Freeze-dried	-20°C
4	No. 5	In-house	40 IU/ampoule	Freeze-dried	-20°C
5	Per 15	National manufacturer	44.55 IU/ampoule	Freeze-dried	-20°C
6	IPRS/20/PERT	Central Drugs Laboratory, India	75 IU/vial	Freeze-dried	-20°C
8	N/A	N/A	N/A	N/A	N/A
9	N/A	N/A	N/A	N/A	N/A
10	2015-1	In-house	60 IU/vial	Freeze-dried	+4°C
11	RWRS 01/11	Central Drugs Laboratory, India	63 IU/vial	Freeze-dried	+4°C
12	260008-201607	NIFDC	15 IU/ampoule	Freeze-dried	-80°C
14	N/A	N/A	N/A	N/A	N/A

Table 2e. Summary of anonymised vaccine batches included in the study (as reported by participants)

Lab code	Vaccine type
1	One batch of DTPHBHib vaccine
2	N/A
3	N/A
4	One batch of Combined bacterial vaccine
5	N/A
6	N/A
8	N/A
9	N/A
10	Two batches of Pentavalent vaccine
	Two batches of whole cell pertussis final bulk
11	N/A
12	N/A
14	N/A

Table 2f. Summary of Kendrick test validity criteria (as reported by participants)

Lab code	Assay validity criteria
1	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel.
2	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel. For proceeding to challenge, no more than 6% of animals per dilution group may die.
3	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. Probit results must not be significantly non-linear or non-parallel.
4	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel.
5	The ED_{50} must lie within the dose range used. The challenge dose must be approximately 100 x LD_{50}. The statistical analysis must not be significantly non-linear or non-parallel.
6	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel.
8	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel. The percentage viable count should range from 12.5% to 62.5%.
9	The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU.
10	Not less than 94% of immunized mice must survive prior to challenge. The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. Regression must be significant at the 5% level (0.05). The statistical analysis must not be significantly non-linear or non-parallel.
11	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. The statistical analysis must not be significantly non-linear or non-parallel. The decrease in bacterial colonies that grow before and after challenge should not be more than 50%.
12	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The statistical analysis must not be significantly non-linear or non-parallel.
14	The ED_{50} must lie within the dose range used. The challenge dose must fall between 100-1000 x LD_{50}. The LD_{50} must contain no more than 300 CFU. Regression must be significant at the 5% level (0.05). The statistical analysis must not be significantly non-linear or non-parallel.

Table 3 Potency estimates (IU/ampoule) for 24/116 calculated relative to 94/532

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM	GCV	*n*
1	72.2	82.5			77.2	-	2
2	86.5	74.2	84.2		81.4	9%	3
3	48.7	43.2			45.8	-	2
4	43.5	51.3			47.2	-	2
5	47.5	55.6	51.8		51.5	8%	3
6	75.2	45.6			58.5	-	2
8	45.7	48.6			47.1	-	2
9	64.9	105.3			82.6	-	2
10	58.3	60.5	47.9	55.3	55.3	11%	4
11	49.2	57.4	53.5		53.3	8%	3
12	76.5	75.6	54.1		67.9	22%	3
14	49.0	182.9[$] & 74.3[*]	57.9 & 46.1[*]		55.9	24%	4

Summary of Assays		Summary of Lab GMs	
GM	59.0	GM	59.0
95% CI	54.2 – 64.2	95% CI	51.5 – 67.7
GCV	27%	GCV	24%
n	32	*n*	12

[$] Outlier according to Grubb's Test, excluded from summary calculations
[*] Ampoules with material separated into visible layers

Figure 1. Laboratory geometric mean potency estimates for 24/116 relative to 94/532. Laboratory code numbers are indicated within each box.

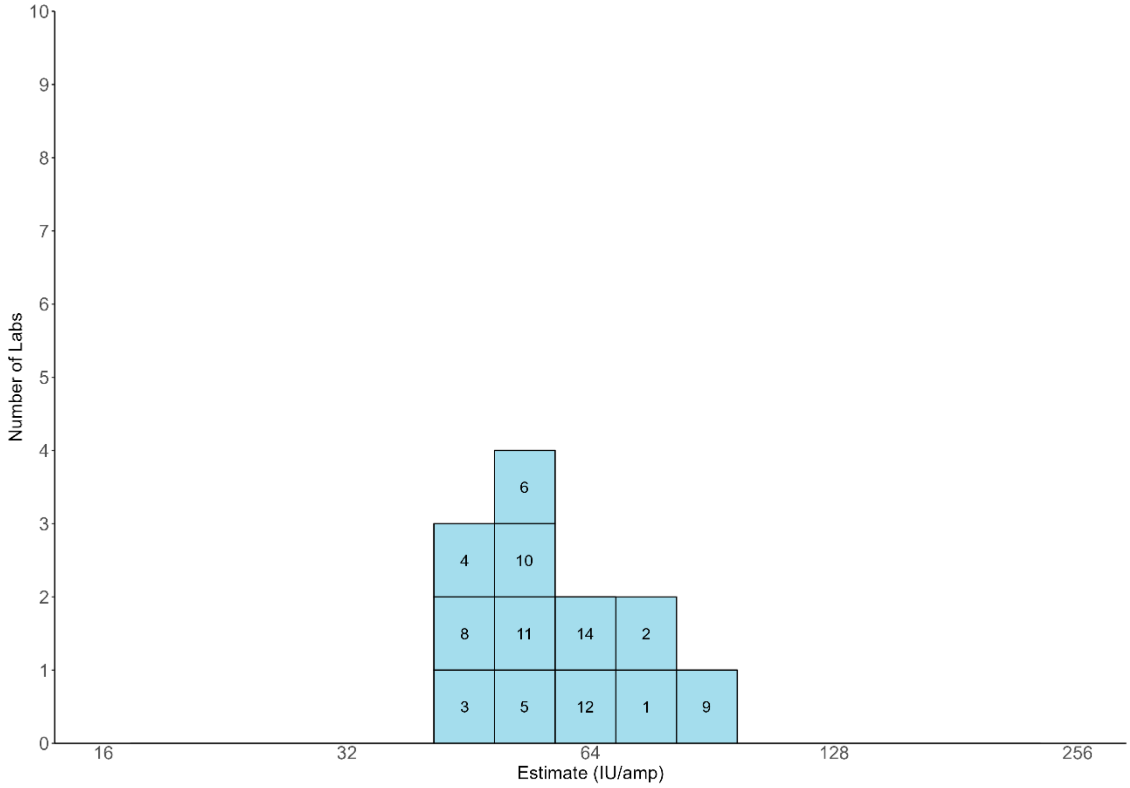

Figure 2. Potency estimates for 24/116 relative to 94/532. Laboratory code numbers are indicated within each box. * Indicates outlier according to Grubb's Test

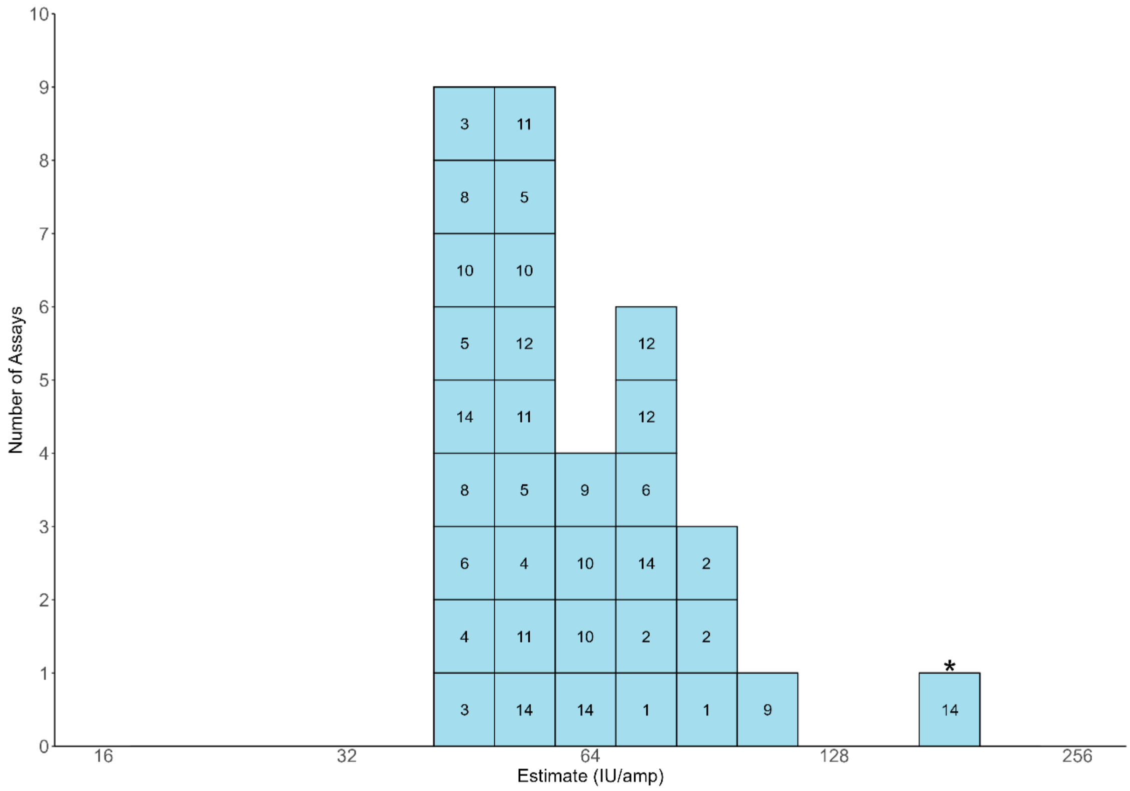

Table 4. Potency Estimates (IU/ampoule) for 24/116 calculated relative to in-house references

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM	GCV	*n*
1	63.0	65.6			64.2		2
2	132.1	114.5	97.5		113.8	16%	3
3	48.0	40.3			44.0		2
4	49.2	44.6			46.9		2
5	40.5	63.8	51.7		51.1	26%	3
6	NP	NP					
10	50.1	66.3	50.8	66.2	57.8	17%	4
11	49.1	55.0	54.4		52.8	7%	3
12	85.6	75.6	76.5		79.1	7%	3

Summary of Assays		**Summary of Lab GMs**	
GM	62.1	GM	60.8
95% CI	53.9 – 71.6	95% CI	46.7 – 79.0
GCV	38%	GCV	37%
n	22	*n*	8

NP = non-parallel

Figure 3. Laboratory geometric mean potency estimates for 24/116 relative to in-house references. Laboratory code numbers are indicated within each box.

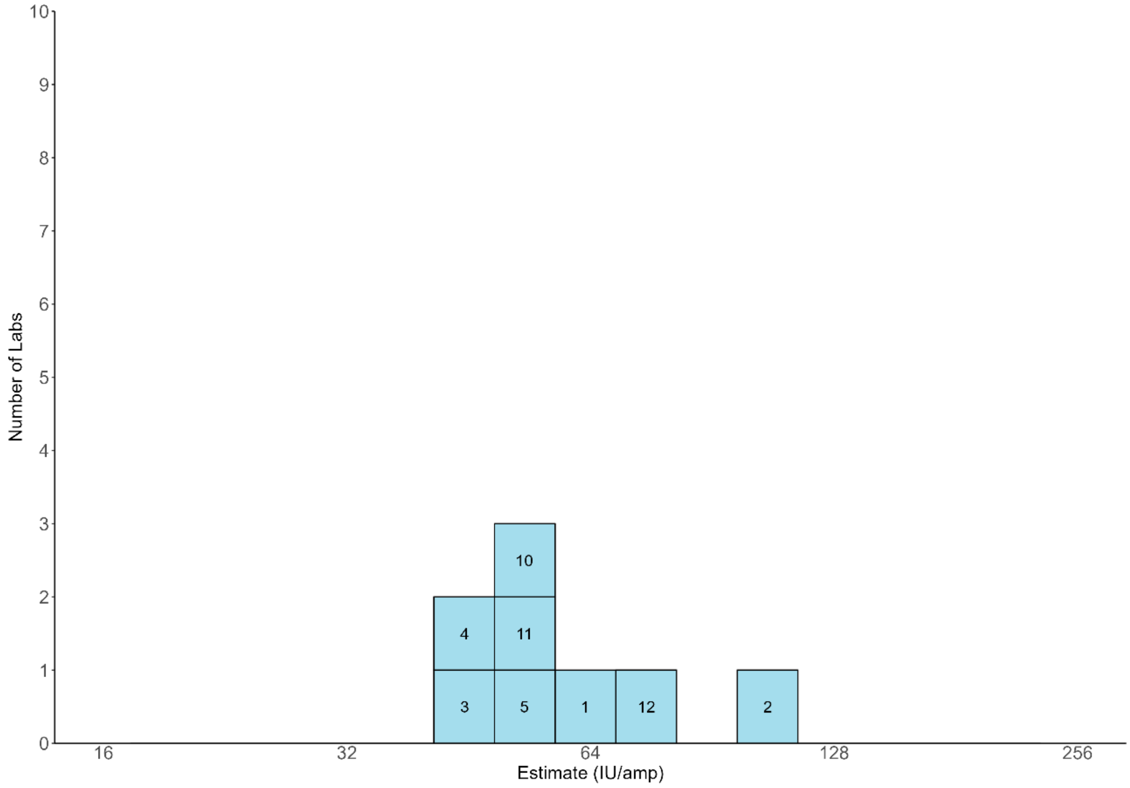

Figure 4. Potency estimates for 24/116 relative to in-house references. Laboratory code numbers are indicated within each box.

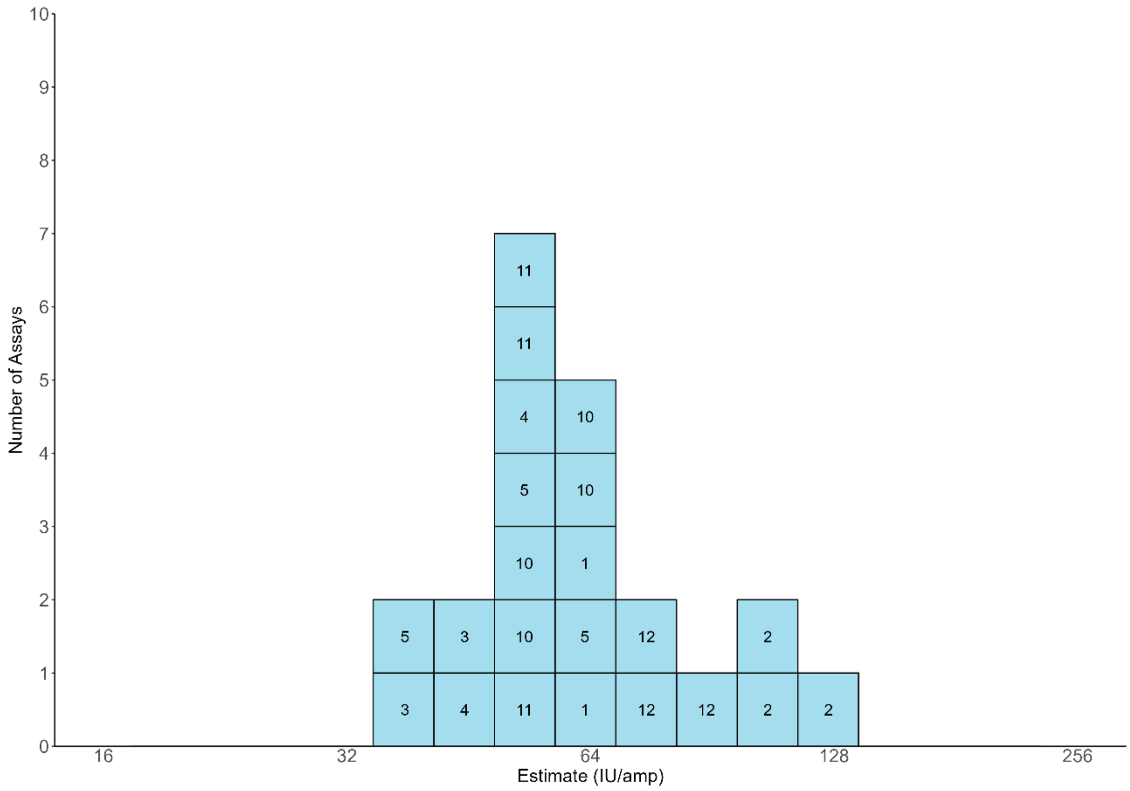

Table 5. Potency estimates (IU/ampoule or vial) for in-house references calculated relative to 94/532

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM (% difference from expected)	GCV	*n*
1	85.1	93.3			89.1 (15.8)		2
2	48.7	47.9	64.7		53.3 (29.0)	18%	3
3	64.7	NP			64.7 (2.6)		1
4	35.5	46.0			40.4 (1.0)		2
5	52.2	40.6	44.4		45.5 (2.1)	14%	3
6	43.2	NP			43.2 (42.4)		1
10	70.8	57.3	56.2	53.3	59.1 (1.5)	13%	4
11	NP	63.8	60.9		62.3 (1.1)		2
12	12.8	14.4	10.3		12.4 (17.3)	18%	3

NP = non-parallel

Table 6. Potency estimates (IU/ampoule or vial) for in-house references calculated relative to 24/116

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM (% difference from expected)	GCV	*n*
1	71.5	68.6			70.0 (6.7)		2
2	34.1	39.3	46.1		39.5 (47.3)	16%	3
3	78.8	93.8			86.0 (26.7)		2
4	48.7	53.8			51.2 (21.9)		2
5	66.1	41.9	51.7		52.3 (14.8)	26%	3
6	NP	NP			n/a		
10	71.8	54.3	70.9	54.4	62.3 (3.7)	17%	4
11	77.0	68.7	69.4		71.6 (12.0)	7%	3
12	10.5	11.9	11.8		11.4 (24.0)	7%	3

NP = non-parallel

Figure 5. Assay potency estimates (IU/ampoule or vial), with 95% CI, for in-house references calculated relative to 94/532 and 24/116

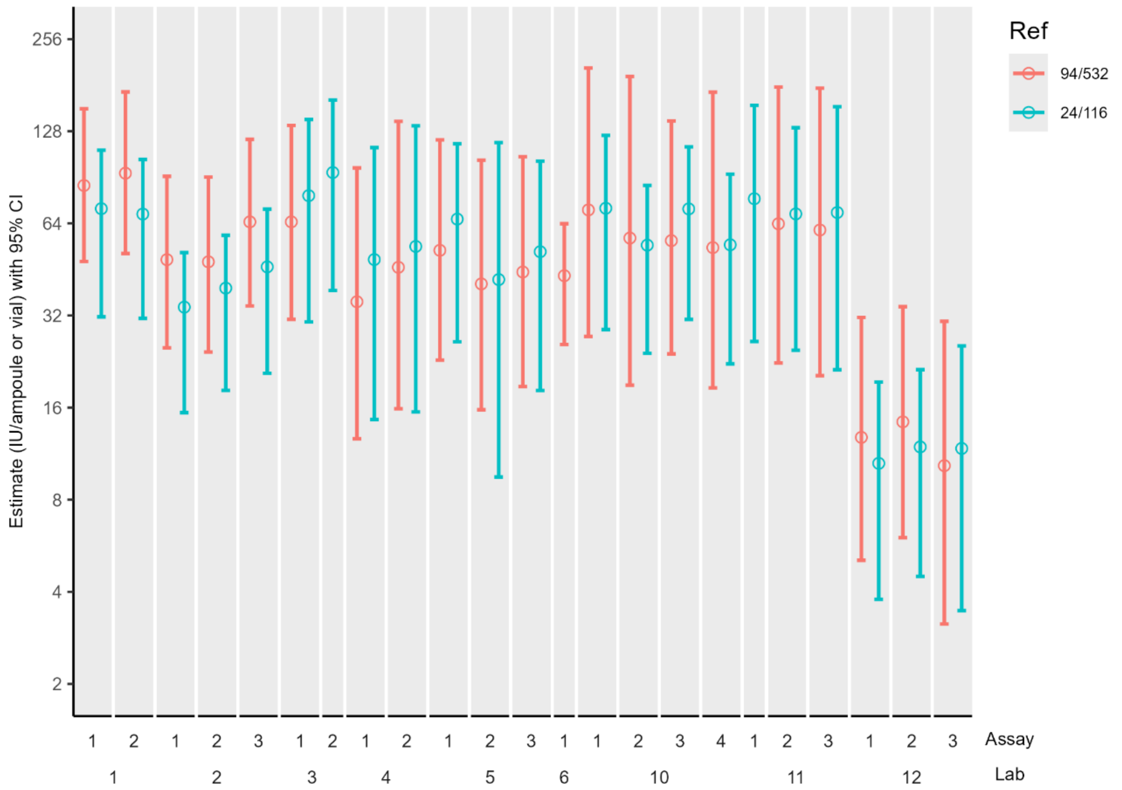

Table 7. Potency estimates (IU/dose) for anonymised vaccine samples calculated relative to 94/532. One dose = 0.5 mL

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM	GCV	*n*
1	6.0	5.7			5.9		2
4	5.8	5.9			5.9		2
10	4.4$	4.5$	3.9*	4.3*	4.3	7%	4

$ Pentavalent vaccine
* wP final bulk

Table 8. Potency estimates (IU/dose) for anonymised vaccine samples calculated relative to 24/116. One dose = 0.5 mL

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM	GCV	*n*
1	5.1	4.2			4.6		2
4	8.0	6.9			7.4		2
10	4.5$	NL$	4.9*	4.5*	4.6	5%	3

NL = non-linear
$ Pentavalent vaccine
* wP final bulk

Table 9. Potency estimates (IU/dose) for anonymised vaccine samples calculated relative to in-house references. One dose = 0.5 mL

Lab	Assay 1	Assay 2	Assay 3	Assay 4	Lab GM	GCV	*n*
1	5.3	4.6			4.9		2
4	6.4	5.2			5.8		2
10	3.7$	4.8$	4.1*	4.9*	4.4	13%	4

$ Pentavalent vaccine
* wP final bulk

Figure 6. Assay potency estimates (IU/dose) with 95% CI for anonymised vaccine samples relative to 94/532, 24/116 and in-house references (IHR). One dose = 0.5 mL. Laboratory 10 results are comprised of 2 different products - pentavalent vaccine (assays 1 and 2) and wP final bulk (assays 3 and 4).

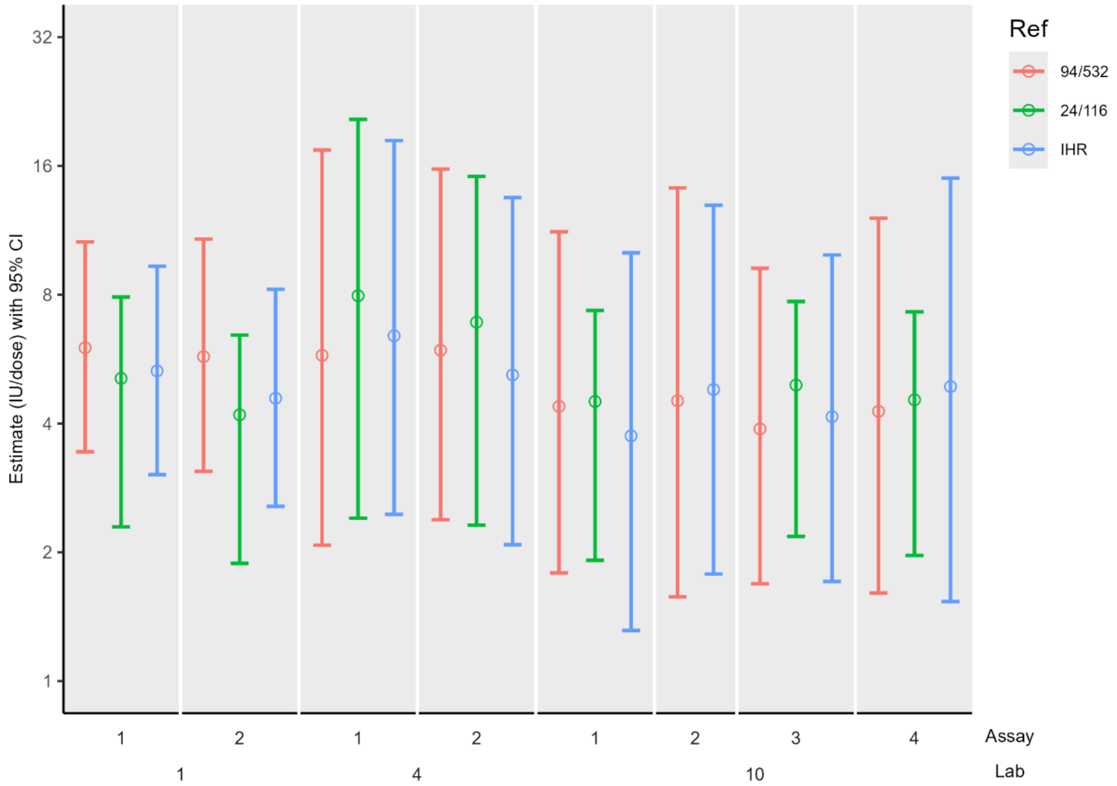

Table 10. Relative potency of 24/116 accelerated thermal degradation samples, calculated relative to 24/116 stored at -20°C

Temperature	Storage time	Relative Potency	95% CI
37°C	1 year	1.02	0.48 – 2.16
45°C	1 year	0.57	0.30 – 1.06

Appendix A - List of participants (alphabetical order by country)

BULGARIA	Radostin Dimitrov BB-NCIPD EAD 26 Yanko Sakazov boulevard 1504 Sofia
CHINA	Wang Lichan National Institutes for Food and Drug Control No. 31 Huatuo St. Daxing District Beijing
INDIA	Dr. Pradip Kumar Das Biological E Limited Plot No. 1 Biotech Park Phase II Kolthur Village – 500 078 Shameerpet Medchal-Malkaigiri District Telangana
INDIA	Mr. Sushil Kumar Sahu Central Drugs Laboratory Kasauli CDSCO (Ministry of Health & Family Welfare India) Main Campus Central Research Institute Kasauli District Solan Tehsil Kasauli (H.P)-173204
INDIA	Dr. Maya Ramdas Panacea Biotec Limited Malpur Baddi District Solan Himachal Pradesh - 173205
INDIA	Dr. Sunil Gairola Serum Institute of India Pvt. Ltd. 212/2 Hadapsar Maharashtra Pune 411 028
INDONESIA	Dwi Damayanti Badan Pengawas Obat dan Makanan (The Indonesian Food and Drug Authority) Biological Products Laboratory – Center for National Quality Control Laboratory of Drugs and Food JI. Percetakan Negara No. 23

	Jakarta Pusat - 10560
INDONESIA	Vinca Medica Lestari Bio Farma Pasteur 28 Bandung 40161
KOREA	Kyung Sik Yoon Ministry of Food and Drug Safety / National Institute of Food and Drug Safety Evaluation Osong Health Technology Administration Complex 187 Osongsaemyeong 2-ro Osong-eup Heungdeok-gu Cheongju-si Chungcheongbuk-do 28159
POLAND	Marta Prygiel, Ewa Mosiej, Karol Wdowiak and Aleksandra Anna Zasada National Institute of Public Health National Institute of Hygiene – National Research Institute Chocimska street 24 00-791 Warsaw
THAILAND	Dr. Koraphong Pinyosukee Institute of Biological Products Department of Medical Sciences Ministry of Public Health 88/7 Soi 14 Tiwanon Road Tald-Kwan subdistrict Mueang Nonthaburi district Nonthaburi Province 11000
UNITED KINGDOM	Cathy Asokanathan, Alex Douglas-Bardsley, Dr. Kevin Markey Medicines and Healthcare Regulatory products Agency Blanche Lane South Mimms EN6 3QG

Appendix B Instructions for Use (IFU)

Medicines & Healthcare products Regulatory Agency

Standard Type

WHO International Standard
5th International Standard for Whole Cell Pertussis Vaccine
NIBSC code: 24/116
Instructions for use
(Version 1.0, Dated 28/01/2026)

1. INTENDED USE

This material is a freeze-dried preparation of inactivated whole cell pertussis vaccine. It is intended for use as a reference in the intracerebral mouse protection test (Kendrick test) for potency testing of whole cell pertussis vaccines.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

The material is not of human or bovine origin. 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 should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

Each ampoule contains 60 International Units (IU). This value was assigned in a multi-laboratory collaborative study using the results obtained in the Kendrick test [1].

4. CONTENTS

Country of origin of biological material: India.

Each ampoule contains the freeze-dried residue from 1 mL of inactivated whole cell pertussis vaccine, formulated in 8% dextran and 5% trehalose (equivalent to 180 International Opacity Units in terms of the International Opacity Standard). The source material was obtained from Serum Institute of India.

5. STORAGE

Unopened ampoules should be stored in the dark at -20°C.

Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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9. REFERENCES

[1] Tierney, R; Hockley, J; Stickings, P; Markey, K; Hassall, L. Collaborative study: Calibration of a Replacement International Standard for Whole Cell Pertussis Vaccine. WHO Report BS/2026.2502.

10. ACKNOWLEDGEMENTS

Not applicable.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering.aspx
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

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

World Health Organization

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Medicines & Healthcare products Regulatory Agency

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties			
Physical appearance: Freeze-dried powder		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contains material of biological origin.		
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 an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.			

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.142 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. **Attached**: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established

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WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards https://www.who.int/publications/m/item/annex2-trs932(revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

World Health Organization

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