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Report on the WHO collaborative study to establish the 1st International Standard for Enterovirus A71 inactivated vaccine

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **27 September 2019** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

Enterovirus A71 (EV71) is the major causative agent of severe and fatal hand, foot and mouth disease (HFMD). Neurological complications induced by EV71 have become a serious public health problem in the Asia-Pacific Region. For this reason, vaccines for the effective prevention of HFMD caused by EV71 have been licensed and several others are under development across the region. The 1st WHO International Standard (IS) for anti-human enterovirus EV71 serum (Human) was established in 2015 to ensure that methods used to measure the serum neutralizing activity or antibody levels against EV71 are accurate, sensitive and reproducible.

A new collaborative study, reported here, was conducted with an aim to establish the 1st WHO IS for EV71 inactivated vaccine. Four candidate EV71 samples were provided by three different manufacturers and analysed by fourteen laboratories using their in-house ELISA method and a common method (designed by NIBSC and NIFDC) alongside a low titre vaccine sample. The Chinese National Standard (NS) for EV71 inactivated vaccine was also included in the study since it was the only reference available with an assigned potency value.

Within laboratory repeatability and between laboratory reproducibility were generally very good. There was largely good agreement between laboratories in EV71 antigen potency estimates whichever candidate was chosen as a standard and as a result all four candidate samples were found to be suitable as an IS. Candidate 18/116 is proposed as the 1st IS for EV71 inactivated vaccine because of the larger number of ampoules available, absence of detectable bacterial contamination and for showing as good or better overall results than the other candidates in terms of assay validity, within and between laboratory variability and thermal stability profiles. Candidates 18/120 and 18/156 are proposed as WHO Reference Reagents. These candidate standards have only been validated for use in *in vitro* assays to measure whole virus antigen content. A second phase of the study should include the evaluation of these samples for use in *in vivo* potency assays. Assays and reagents to specifically measure the quantity of empty and full virus particles present in EV71 vaccines and analysis of their relative contribution to the antigenic and immunogenic properties of different EV71 vaccine products will be fundamental to ensure appropriate standardization of EV71 vaccines used globally.

Introduction

Human Enterovirus 71 is one of the main causes of HFMD [1, 2]. Whilst the majority of infections are mild, EV71 can also cause severe neurological disease and even death and has been associated with sporadic cases or outbreaks of a wide spectrum [3]. EV71 was originally isolated in California in 1969 from a nine-month old child with encephalitis [4]. In recent years,

multiple epidemic outbreaks in China and South-East Asia have been reported with an increase in morbidity and mortality rates which is of extreme concern and a threat to public health [5-8]. The reasons for this increased association of EV71 infections with severe disease in the Asia-Pacific region are unknown but EV71 appears to circulate widely, including newly described endemic genogroups recently reported in Africa, India and Pakistan [9, 10]. Thus, the future implication of EV71 infection in causing outbreaks of severe neurological disease in regions other than the Asia-Pacific cannot be ruled out. Sporadic EV71 outbreaks causing severe disease have also occurred in other regions such as some recent examples in Europe [11].

Eight different EV71 genogroups (A to H) have been described in total. Antigenic differences between EV71 strains of the same or different genogroups do not appear to perfectly correlate with genetic classification but there is generally high cross reactivity with strains of different genogroups of antibodies raised against particular EV71 strains [12].

Three EV71 vaccines produced by the Institute of Medical Biology (CAMS), Sinovac Biotech and Wuhan Institute of Biological Products (vaccine developed by Vigoo Biologicals), have been approved by the China Food and Drug Administration (CFDA) since December 2015. More vaccines are currently under development, notably an EV71 vaccine developed by the National Health Research Institutes (NHRI), which is being produced by two manufactures, Medigen and Adimmune. These most advanced products are formalin inactivated whole virus vaccines which have been developed using either C4 or B4 virus strains, from the most prevalent genotypes in China [13-16]. Several studies have shown that serum from children or adults immunized with all these five EV71 vaccine products, showed a broad spectrum in cross-neutralization against EV71 strains from different genogroups [17, 18], suggesting that mono-genotype EV71 vaccines could be used to control HFMD epidemics in different countries. However, it must be noted that NHRI and CAMS vaccines had weak cross-neutralization against C1 or C2 sub-genogroup strains. Therefore, continuous monitoring of EV71 evolution in different countries and assessment of vaccine protective efficacy against emerging strains will be required.

Production of EV71 inactivated vaccines follow similar procedures to those successfully used to manufacture formalin-inactivated vaccines against poliovirus with a notable exception in that full and empty capsids are known to be present in EV71 inactivated vaccines, whereas IPV products only contain full virus particles. The possible implications of this phenomenon for antigen assays or vaccine immunogenicity, with a direct impact on vaccine standardization, are unknown.

Chinese National Standards for both EV71 antigen and neutralizing antibody, developed by NIFDC, already exist [19]. These standards are being used for the quality control, antigen quantification, and immunogenicity evaluation of sub-genogroup C4 EV71 vaccines in China. However, the need for EV71 international reference standards, applicable to all EV71 genogroups, to be used globally was identified. Proposals for establishing the 1st IS for EV71 anti-serum and the 1st IS for EV71 inactivated vaccines were endorsed by ECBS in 2012 (WHO TRS 980). The use of these WHO ISs will contribute to the standardized assessment of the quality and efficacy of vaccines used in immunization programs globally.

The 1st WHO IS for anti-human enterovirus EV71 serum (Human) was established in 2015 [20, 21]. The purpose of which was to standardise the measurement of anti-EV71 neutralizing antibodies in human sera, which is an important marker and diagnostic tool in assessing the

immune status of individuals. Results from this collaborative study confirmed the high cross-reactivity of human sera against EV71 strains of different genogroups although some degree of specificity towards genogroup C strains was observed. The need for international references for EV71 vaccines was also recognized with a view to harmonize vaccine potency assays.

This report describes the results of a collaborative study conducted with an aim to establish the 1st IS for EV71 inactivated vaccine. Candidate EV71 vaccine samples belonging to C4 or B4 genogroups were available for this study. The interchangeability of ELISA antigen assays used by different manufacturers and control laboratories, not demonstrated before, was assessed.

Aim of study

The aim of this study was to establish the 1st IS for EV71 inactivated vaccine. The study included samples from three manufacturers which were filled and freeze/dried at NIBSC. The primary aim of the study was to characterise the antigen content of four potential candidate EV71 inactivated vaccine standards and decide on a recommendation for the establishment of the 1st IS for EV71.

Materials and methods

Bulk materials and processing

Product summary

Materials for the preparation of the candidate IS were kindly donated to NIBSC and NIFDC by manufacturers. B4 vaccine materials were kindly donated through TFDA. Materials were shipped to NIBSC on ice and subsequently stored at 4°C thereafter. A pilot freeze drying trial was undertaken to validate the suitability of the material before a large-scale fill was completed. It was important to establish a formulation and cycle conditions that would give a reliable and stable product and therefore limit the loss of potency. This was completed on the 8th May 2018. The four candidates gave a white, robust cake that loosened on agitation but remained intact. Moisture and oxygen levels were within accepted range. The potency of these materials was tested using an ELISA to directly compare liquid and freeze-dried products. There was no loss in antigen potency following freeze-drying (data not shown) therefore indicating freeze-drying was a suitable preservation method.

Formulation, Filling and Freeze drying

Candidate materials were made to a formula of 5% sucrose and 1% BSA in PBS before filling commenced. Filling was completed from a homogenous stirred bulk maintained at 4°C throughout the filling using a Bausch and Strobel AFV5090 machine. 2 ml ampoules were filled with 0.25ml of material and in-line samples (4-5% of total filled ampoules) were taken for measurements of the fill volume and sterility. Freeze-drying was carried out directly after filling using a 4-day cycle. After completion of the freeze-drying the candidate materials were put at long term storage of -20°C. Product summary details for each of the four filled candidate samples are shown in Table 1.

Post-fill characterization of candidate material

Samples were taken before, during and at the end of the filling process for all candidate EV71 samples and were then tested for Ag content by ELISA at NIBSC. No significant differences were found between their Ag content (data not shown).

Sterility testing

Although fills at NIBSC are carried out in conditions to minimise microbial contamination, absolute sterility cannot be guaranteed. Therefore, sterility testing is performed on all automatic fills. For the routine sterility test, twenty-five individual ampoules are opened aseptically and transferred to sterile Petri dishes overlaid with Tryptone Soya Agar. The dishes are incubated at 37°C for seven days and observed for the presence of colony forming units (cfu). Low level microbial contamination was detected in two of the filled materials. Although the levels found were minimal and will not have an impact for *in vitro* ELISA assays, they might be an issue for the sample to be used in *in vivo* assays.

Study samples

A total of nine samples were provided to participants. The samples were shipped in dry ice and storage at ≤ -20°C was recommended. Participants were provided with a minimum of six ampoules per sample. A Sample Receipt Form was included with the shipment to give feedback on the date and condition the samples were received. Instructions for Use documents were also provided which included Material Safety Data sheets. The study samples are listed below, and participants were kindly asked to reconstitute candidates in 250µl of sterile distilled water:

WHO candidate IS for EV71, samples 18001 and 18006
Duplicate samples of candidate 18/116, belonging to genotype C4, were prepared from a concentrated bulk produced and kindly donated by a manufacturer.

WHO candidate IS for EV71, samples 18002 and 18007
Duplicate samples of candidate 18/120, also belonging to genotype C4 but a different strain from candidate 18/116 above, were prepared from a concentrated bulk produced and kindly donated by a second manufacturer.

WHO candidate IS for EV71, samples 18003 and 18008
Duplicate samples of candidate 18/122, belonging to genotype B4, were prepared from a concentrated bulk produced and kindly donated by a third manufacturer.

WHO candidate IS for EV71, samples 18004 and 18009
Duplicate samples of candidate 18/156, belonging to genotype B4 and representing the same material as candidate 18/122, were prepared from a concentrated bulk produced and kindly donated by the same third manufacturer. The reason for this was that candidate 18/122 had shown low levels of bacterial contamination a second attempt to prepare clean B4 material was carried out.

Low titre vaccine, sample 18005
An extra sample of candidate 18/122 was provided and participants were kindly asked to reconstitute in 2 ml of sterile distilled water to obtain a low potency sample.

Chinese National Standard (NS) for EV71 inactivated vaccine, sample EV71 NS
Ampoules of the Chinese EV71 NS were also included in the study to be used as reference as it was the only reference available with an assigned potency value [19]. The assigned potency is 1,600 EV71 Antigen Units/ml.

Participants

Fifteen laboratories were invited to participate in this study. All of them accepted, eight from Manufacturers and seven from National Control Laboratories (see Appendix 1). Fourteen participants received the study samples and returned data within the requested timeframe.

Design of the study

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

Participants were requested to:

- Determine the Ag content of the panel of 9 coded EV71 samples (18001 to 18009) using their routine in-house ELISA method and a common method validated by NIBSC using reagents provided by NIBSC and NIFDC. Details of the in-house methods used by the different participants and the common method are shown in Appendices 2 and 3, respectively.
- Perform three independent assays to determine the Ag content of the study samples using the Chinese EV71 NS as a 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.

Statistical analysis

All assay data were analysed at NIBSC using the approach described below. Study samples used as reference standards for the analysis were EV71 NS, candidate standard 18/116 (coded S18006), candidate 18/120 (coded S18007), candidate standard 18/122 (coded S18008) or candidate standard 18/156 (coded S18009).

Potency estimates relative to the sample selected as reference standard were calculated by parallel line analysis using a minimum of three dilutions in a linear section of the assay response range. Assay responses were log transformed where required to obtain a linear dose-response relationship and a consistent response transformation for all assays was used for each combination of laboratory and protocol. All calculations were performed using the R software program [https://www.R-project.org/]. Linearity was assessed by visual assessment and calculating an r^2 value. Samples with a value below 0.90 were excluded from further analysis. Test samples with response ranges that did not overlap with the range for the sample selected as reference standard were also excluded from the calculation of relative potency. Non-parallelism was assessed using the ratio of the slope of the test sample relative to the slope of the reference standard and ranges used for concluding acceptable parallelism are noted in the Results section of this report.

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). Due to possible outliers and anomalous results, Huber's robust mean was also calculated using the R package 'WRS2' [22].

Stability studies

The filled candidate standards were stored at -20°C intended for permanent storage. As stability studies are usually based on data from few time points and only three repeat assays per point, a single anomalous result can have a big impact. For this reason, a thorough analysis of real-time stability at the temperature intended for storage (-20°C) was conducted for all candidate standards using the Chinese EV71 NS as reference with several vials tested at various time points from the fill date up to 12 months. Additional samples were placed at +20°C, +37°C and +45°C for shorter time periods up to 6 weeks for accelerated-degradation analyses. In addition, candidate standards in their lyophilised state were subjected to multiple freeze/thaw cycles (from -70°C to room temperature). Furthermore, to test whether the standard is likely to be stable under conditions of normal storage and laboratory manipulation, samples were reconstituted and placed at +4°C for up to 4 weeks. The potencies of the samples were expressed as a percentage of those of the -20°C baseline samples. All assays were performed in triplicate and carried out at NIBSC.

Results of collaborative study

Data Received

No laboratory reported any problems with the condition of the study samples on receipt. Data were received from fourteen laboratories. All fourteen laboratories reported results using the common method. Laboratories 6, 13 and 15 did not return results from an in-house assay.

Validity criteria for parallelism

Using data from all assays by laboratories, slope ratios observed for the coded duplicate samples relative to each other (S18001 relative to S18006, S18002 relative to S18007, S18003 relative to S18008 and S18004 relative to S18009) were used to define acceptance criteria for parallelism. Using values of $m = \max(s, 1/s)$ where s denotes the slope ratio of the coded duplicates within an assay, a non-parametric upper tolerance bounds (95% coverage, 95% confidence) was calculated for m. The bound together with its reciprocal value was used to define an acceptable slope ratio range for concluding parallelism, giving 0.728 – 1.374. It should be noted that this range was intended for use in the analysis of data from this study only, in order to apply consistent criteria to all laboratories and assess the relative performance of the different standards. It should not be interpreted as suitable for routine use in the assessment of assay validity within the collaborating laboratories.

Assay validity

A small number of exclusions were made due to samples demonstrating non-linearity (Table 2). A summary of exclusions due to non-parallelism for the study samples is shown in Tables 3-7. Exclusions mostly affected potency calculations using the Chinese EV71 NS as a reference or values obtained for the Chinese EV71 NS and low titre sample 18005 using candidate samples as reference standards. There were generally more exclusions when using the common method than the in-house method. The most likely reason for exclusions was using a suboptimal dilution range. Summaries of potency estimates with details of determinations that were excluded and the reason for this are given in Appendix 6. Furthermore, 5 assays were excluded due to higher levels of intra-assay variability, as estimated by calculating relative potencies of coded duplicate samples. In the same manner as for the slope ratios, a non-parametric tolerance bound (95% coverage, 95% confidence) was calculated on the relative

potency estimates, giving a range of 0.815 – 1.227. Any assay where the relative potency estimates of one or more of the coded duplicate samples was outside of this range was excluded from further calculations.

Estimates of relative potency, intra-assay, intra-laboratory and inter-laboratory variability

A summary of potencies calculated across relative to EV71 NS, 18/116 (coded S18006), 18/120 (coded S18007), 18/122 (coded S18008), or 18/156 (coded S18009) together with GCV% values as estimates of intra-laboratory (between-assay) variability is provided in Tables 8-12 and Figures 1-5. The majority of laboratories achieved low intra-laboratory variability (87% of GCV values were below 15%). There were few individual high values which might be due to single anomalous results.

Coded duplicate GM relative potencies (as a measure of intra-assay variability) for all coded duplicate samples (Tables 9-12) showed good agreement with their expected value of 1 for all samples (98% of coded duplicate relative potency values were within 0.80 to 1.25).

Overall study GM potencies across samples and between-laboratory %GCV values are shown in Table 13. A summary of between-laboratory variability using median GCV values is shown in Table 14. There was generally good agreement between laboratories in potency estimates for all study samples when using either the in-house or common method with some exceptions in few laboratories. Between-laboratory variability was generally higher when using in-house methods than when using the common assay and particularly higher when measuring the potency of sample EV71 NS. However, this observed higher variability in potency estimates using in-house methods appeared to be largely due to some extreme results obtained in few laboratories, particularly laboratory 10. When %GCV values were re-calculated excluding laboratory 10's results, most of them markedly dropped (Tables 15 and 16). The ELISA potency assay used by laboratory 10 appeared to be biased towards the B4 strain used in candidate samples 18/122 and 18/156 as potencies for these samples when using an heterologous reference were higher (values >25% higher than GM values) than those obtained with in-house methods in other laboratories or with the common assay in all laboratories. Conversely, the potencies of heterologous vaccines measured with candidates 18/122 and 18/156 as reference were lower. Similarly, a degree of strain specificity was observed in ELISA methods used in laboratory 4 for the same B4 strain and in laboratories 3 and 5 for the C4/H05 strain used in candidate 18/120. Laboratories 3 and 5 used the same antibody reagents in their potency assays, polyclonal serum raised against strain C4/H05 for both capture and detection ELISA steps. Overall, the best agreement using either the in-house or common methods was clearly seen when measuring the potency of candidate samples using the same strain as reference (maximum between-laboratory GCV of 9%). This is somehow expected and has been observed with IPV before [23].

Potency assignment to candidate standards

A summary of overall potency estimates for 18/116, 18/120, 18/122 and 18/156 calculated relative to each of EV71 NS, 18/116, 18/120, 18/122 or 18/156 is given in Table 17. To mitigate the effects of any outliers or anomalous results, overall estimates are also shown as robust GM and median. Overall potency estimates expressed in EV71 Antigen Units calculated using the Chinese EV71 NS as reference are shown in Table 18.

Stability studies

Results of the analysis of real-time stability at the temperature intended for storage (-20°C) for all candidate standards are shown in Figures 6-9. The results showed no loss in potency at -20°C for any of the candidate standards. Accelerated-degradation of the candidates was investigated by storage of samples at +20°C, +37°C and +45°C for 1, 3 and 6 weeks. The results, shown in Table 19, showed no drop in potency at these temperatures up to week 6, suggesting a good maintenance of stability even at higher temperatures. However, an increase in potency was observed for different temperatures at different time points. The reasons for this observed increase in antigen potency are unknown but a possible explanation could be that high temperatures induce changes in EV71 vaccine antigenic structure resulting in increased binding to antibody reagents used in ELISA. These assays were only conducted at NIBSC so a thorough investigation using a variety of antibody reagents would be required to better understand this phenomenon. The effects of these temperature treatments on vaccine immunogenicity should also be investigated.

The effect of multiple rounds of freeze/thawing on potency for each candidate is shown in Table 20. The results revealed a good maintenance of stability for candidates 18/116, 18/120 and 18/156 up to three rounds of freeze/thawing. Candidate 18/122, however, exhibited a slight drop in potency from the first freeze/thaw cycle.

Reconstitution of the candidate standards, shown in Table 21, demonstrated a good maintenance of stability at +4°C for candidate 18/156 throughout the 4 weeks of incubation. Candidates 18/116 and 1/122 however, showed a slight drop in potency at week 4 whilst 18/120 revealed a greater loss in potency from week 2.

Overall, stability studies demonstrated that all four candidate materials are stable at temperatures used for long-term storage (-20°C) and short-term laboratory manipulation (+4°C to +20°C). A program to measure real-time stability is ongoing by regularly measuring the potency of samples stored at -20°C.

Discussion

The primary aim of this study was to characterise the antigen content of four potential candidate EV71 standards and decide on a recommendation for the establishment of the 1st International Standard for EV71 inactivated vaccines. There is currently no WHO IS for EV71 vaccines, so manufacturers and national control laboratories must rely on in-house reference materials that have been established independently and therefore comparison of EV71 products from different manufacturers is not possible. The four candidate EV71 samples were shown through preliminary studies to be fit for purpose. They were tested in fourteen laboratories from three continents, Europe, Asia and America.

Within laboratory repeatability in potency estimates for all candidate standards was generally very good, although some individual sample potency estimate values led to poor repeatability in very few cases. The overall geometric mean potency estimates for the duplicate samples of all four candidate standards showed excellent agreement between laboratories and good consistency between duplicates. There was generally very good agreement between laboratories whichever candidate was chosen as a standard to determine the antigen content of the candidate samples and when using either in-house or common methods. These results suggest that any of the four candidate standards would be suitable as IS.

Comparing the observed between-laboratory variability using in-house or common methods across laboratories could help identify the source of variability in more detail. Variability between results using the common method would likely be due to technical errors using the assay whereas using in-house methods might have additional sources of variability such as differences in binding specificity of antibody reagents to different EV71 strains. Between-laboratory variability was generally higher using in-house methods than common methods. However, this seemed to be largely due to some results in few laboratories. Methods used in few laboratories showed a degree of specificity towards one particular B4 or C4 strain leading to potency estimates for specific candidate samples obtained in those laboratories being somehow different to those obtained in other laboratories or in all laboratories using the common method. For this reason, the future need for a process to carefully validate potency assays to ensure cross-reactivity across different EV71 strains of different genogroups or to establish strain specific in-house references cannot be discarded.

A critical question not investigated in this study but important to resolve in the near future is to determine the relative contribution of empty and full virus particles, present in EV71 vaccines, to the antigenic and immunogenic properties of different EV71 vaccine products. Contrary to IPV, that is only composed of full virus particles, EV71 inactivated vaccines contain empty and full virus particles which are known to have structural differences [24, 25]. Antibody reagents used by different laboratories in this study included those binding to conformational or linear epitopes. However, the relative affinity of these antibody reagents to empty and full virus particles is largely unknown. Being able to accurately quantify the amount of empty and full virus particles in EV71 vaccines will be essential for the standardization of these vaccines. It will allow determining if the proportion of empty and full virus particles is similar between different production runs, which can be used as a measurement of production consistency. Ultimately, a fundamental task to resolve is to establish a correlation between *in vitro* and *in vivo* EV71 vaccine potency assays determining the relative contribution of empty and full virus particles on vaccine immunogenicity. Previous research reported that EV71 vaccine full virus particles are more immunogenic than empty particles [24].

Stability studies demonstrated that all four candidate materials are stable at temperatures used for long-term storage (-20°C) and short-term laboratory manipulation (+4°C to +20°C). However, sample 18/122 appeared to lose potency after freeze thawing. Stability after reconstitution remained high for all samples although 18/120 showed some loss of potency after two weeks at +4°C. A program to measure real-time stability is ongoing by regularly measuring the potency of samples stored at -20°C.

Given the results above, we concluded that all four EV71 vaccine candidates were suitable to serve as an IS. Assigned potency values expressed in EV71 Ag Units/ml using the Chinese NS were 14,500, 1,200, 1,000 and 1,000 EV71 Ag Units/ml for 18/116, 18/120, 18/122 and 18/156, respectively. In all cases, potency values were based on the robust GM and median values of the EV71 Ag/ml estimates relative to the Chinese EV71 NS from all assays in all laboratories. Candidate 18/116 was chosen because of the larger number of ampoules available, absence of detectable bacterial contamination and for showing good overall results in terms of assay validity, within and between laboratory variability and thermal stability profiles.

Recommendation

It is proposed that the candidate 18/116 should be established as the 1st WHO IS for EV71 inactivated vaccine. The assigned potency for this IS should be 14,500 EV71 International

Units (IU) of EV71 Antigen per ml. It is also proposed that candidates 18/120 and 18/156 are established as WHO Reference Reagents for EV71 inactivated vaccine with assigned potencies of 1,200 and 1,000 EV71 IU of EV71 Antigen per ml, respectively. For all three reference preparations the assigned potencies are those obtained when reconstituting the ampoules in 250µl of sterile distilled water as instructed. There are currently 3,472, 1,879 and 2,465 ampoules of 18/116, 18/120 and 18/156, respectively, stored at NIBSC.

These candidate standards have only been validated for use in *in vitro* assays to measure the whole-virus antigen content of EV71 inactivated vaccines. A second phase of the study should include the evaluation of these samples for use in *in vivo* assays.

The real-time stability of all three reference standards should be monitored periodically by testing samples stored at -20°C.

A summary of the comments received from participants is provided in Appendix 4. An example of the Instructions for use and the safety data sheet for the standards can be found in Appendix 5.

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NIBSC Code (study code)	18/116 (18001, 18006)	18/120 (18002, 18007)	18/122 (18003, 18008)	18/156 (18004, 18009)
Presentation	2.5 ml glass ampoule	2.5 ml glass ampoule	2.5 ml glass ampoule	2.5 ml glass ampoule
No. of containers	3816	2082	2944	2741
Mean fill mass	0.26g	0.26g	0.26g	0.26g
CV fill mass	0.87%	1%	0.87%	1.2%
Mean dry weight	0.01g	0.02g	0.02g	0.02g
CV of dry weight	1.53%	1.9%	2.48%	1.71%
Mean residual moisture	0.76%	1.14%	2%	2.68%
CV residual moisture	62.59%	22.16%	34.13%	14.01%
Mean oxygen headspace	0.52%	0.8%	0.49%	0.76%
CV of oxygen headspace	22.84%	12.88%	21.75%	16.37%
Date of fill	May 2018	May 2018	May 2018	September 2018
Storage temperature	-20ºC	-20ºC	-20ºC	-20ºC
Microbial contamination	None detected	Detected: >10 CFU	Detected: >10 CFU	None detected

Table 1. Product summaries of Candidate Standards

Table 2. Exclusions due to non-linearity for study samples

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH	6%	10%	6%	6%	6%	6%	13%	6%	8%	8%
Common	2%	2%	2%	2%	2%	2%	4%	8%	2%	4%
All	4%	6%	4%	4%	4%	4%	8%	8%	5%	6%

Table shows % of cases with non-linearity

Table 3. Exclusions due to non-parallelism for study samples, using EV71 NS as a reference

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH		7%	17%	23%	23%	10%	15%	20%	17%	14%
Common		17%	45%	33%	36%	7%	23%	37%	34%	28%
All		13%	33%	29%	30%	8%	19%	29%	27%	22%

Table shows % of cases with non-parallelism when EV71 NS is used as reference standard

Table 4. Exclusions due to non-parallelism for study samples, using 18006 as a reference

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH	15%	4%	4%	4%	0%	15%		0%	0%	0%
Common	23%	0%	8%	8%	3%	45%		0%	5%	3%
All	19%	1%	6%	6%	1%	33%		0%	3%	2%

Table shows % of cases with non-parallelism when 18006 is used as reference standard

Table 5. Exclusions due to non-parallelism for study samples, using 18007 as a reference

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH	20%	4%	3%	3%	3%	23%	0%		0%	0%
Common	37%	3%	3%	3%	0%	45%	0%		3%	0%
All	29%	3%	3%	3%	1%	35%	0%		1%	0%

Table shows % of cases with non-parallelism when 18007 is used as reference standard

Table 6. Exclusions due to non-parallelism for study samples, using 18008 as a reference

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH	17%	4%	7%	3%	7%	10%	0%	0%		0%
Common	34%	2%	7%	2%	2%	46%	5%	3%		0%
All	27%	3%	7%	3%	4%	31%	3%	1%		0%

Table shows % of cases with non-parallelism when 18008 is used as reference standard

Table 7. Exclusions due to non-parallelism for study samples, using 18009 as a reference

Method	Sample									
	EV71 NS	18001	18002	18003	18004	18005	18006	18007	18008	18009
IH	14%	7%	3%	3%	7%	7%	0%	0%	0%	
Common	28%	3%	10%	3%	3%	43%	3%	0%	0%	
All	22%	4%	7%	3%	4%	28%	2%	0%	0%	

Table shows % of cases with non-parallelism when 18009 is used as reference standard

Table 8. Potencies calculated relative to EV71 NS (Relative Potencies)

		18001		18002		18003		18004		18005		18006		18007		18008		18009	
Method	**Lab**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**
In-house	01	6.64	8%	0.57	3%	0.52	4%	0.56	7%	0.07	5%	6.17	4%	0.55	4%	0.49	4%	0.51	1%
In-house	02	8.35	5%	0.71	10%	0.64	8%	0.64	12%	0.09	6%	7.96	1%	0.73	6%	0.65	6%	0.68	16%
In-house	03	9.27	2%	1.18	3%	0.66	3%	0.69	3%	0.08	4%	9.00	6%	1.17	7%	0.64	3%	0.64	2%
In-house	04	7.15		0.63		0.72		0.76		0.11		7.30		0.65		0.73		0.74	
In-house	05	9.56	2%	1.17	3%	0.66	5%	0.65	3%	0.08	5%	9.41	5%	1.15	2%	0.67	4%	0.66	4%
In-house	07	6.65	30%	0.72	1%	0.54	6%	0.53	3%	0.08	19%	6.49	30%	0.73	16%	0.53	13%	0.51	8%
In-house	08	8.20	12%	0.68	1%	0.57	14%	0.57	20%	0.08	22%	8.54	10%	0.70	8%	0.58	9%	0.61	2%
In-house	09			0.55		0.50				0.08	7%			0.50		0.55		0.49	
In-house	10	14.10		1.21		1.91		1.61		0.25		10.16		1.02		1.90		1.54	
In-house	11	8.98	12%	0.69		0.53		0.49				9.48		0.80		0.61		0.56	
In-house	12	9.44								0.11									
Common	01	8.62	2%	0.76	9%	0.69	7%	0.68	7%	0.09	10%	9.27	2%	0.73	2%	0.63	5%	0.64	5%
Common	02	8.97	5%	0.72	2%	0.60	4%	0.60	4%	0.09	8%	9.32	12%	0.77	9%	0.57	6%	0.65	8%
Common	03	10.57	3%	0.78	1%	0.66	2%	0.62	7%	0.09	2%	10.46	4%	0.77	3%	0.66	2%	0.59	3%
Common	04	8.54	9%	0.75		0.61		0.60	3%	0.09	22%	8.89	12%	0.78	7%	0.60	9%	0.62	6%
Common	05	10.61	9%	0.94	5%	0.71	3%	0.69	9%	0.08	4%	9.15	11%	0.85	2%	0.70	6%	0.64	9%
Common	06									0.09									
Common	07	7.72	6%	0.56	10%	0.38	4%	0.40		0.07	5%	7.79	11%	0.55	7%	0.39		0.36	
Common	08	8.74		0.83		0.67		0.66		0.10		9.35		0.71		0.63		0.69	
Common	9	9.35	7%	0.76		0.62	2%	0.60	5%	0.09	8%	10.16	5%			0.64		0.61	2%
Common	10	11.33								0.09		10.80	2%			0.66		0.63	
Common	11	9.85								0.11	7%	9.89				0.65		0.60	
Common	12	8.15				0.49		0.47				8.79		0.54		0.42		0.45	
Common	13	9.27	5%	0.78	12%	0.60	14%	0.58	9%	0.09	17%	9.08		0.83		0.59		0.62	
Common	15	9.74	13%			0.74		0.74		0.09	6%	10.26		0.74		0.67		0.86	

Table 9. Potencies calculated relative to 18006 (Relative Potencies)

		18001		18002		18003		18004		18005		18007		18008		18009		EV71 NS	
Method	**Lab**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**
In-house	01	1.07	6%	0.09	7%	0.08	7%	0.09	11%	0.01	7%	0.09	8%	0.08	6%	0.08	4%	0.16	4%
In-house	02	1.06	5%	0.09	11%	0.08	8%	0.08	13%	0.01	6%	0.09	6%	0.08	5%	0.09	16%	0.13	1%
In-house	03	1.03	4%	0.13	6%	0.07	6%	0.08	6%	0.01	6%	0.13	6%	0.07	3%	0.07	4%	0.11	6%
In-house	04	0.97		0.09		0.10		0.11		0.02		0.09		0.11		0.11		0.14	
In-house	05	1.02	3%	0.12	3%	0.07	3%	0.07	4%	0.01	2%	0.12	3%	0.07	4%	0.07	5%	0.11	5%
In-house	07	1.03	1%	0.11	31%	0.09	38%	0.08	35%	0.01	54%	0.11	52%	0.08	51%	0.08	42%	0.15	30%
In-house	08	0.97	11%	0.08	10%	0.07	6%	0.07	31%	0.01	25%	0.08	11%	0.07	3%	0.07	14%	0.12	10%
In-house	09																		
In-house	10	1.20		0.11		0.15		0.14		0.02		0.09		0.16		0.13		0.10	
In-house	11	1.03		0.08		0.06		0.06	6%	0.01		0.08	11%	0.05	24%	0.05	18%	0.11	
In-house	12	0.96		0.09		0.08		0.09				0.09		0.08		0.08			
Common	01	0.93	4%	0.08	10%	0.07	8%	0.07	8%	0.01	9%	0.08	1%	0.07	4%	0.07	3%	0.11	2%
Common	02	0.96	15%	0.08	11%	0.06	10%	0.06	9%	0.01	16%	0.08	13%	0.06	7%	0.07	11%	0.11	12%
Common	03	1.01	1%	0.08	2%	0.06	1%	0.06	7%	0.01	2%	0.07	1%	0.06	4%	0.06	1%	0.10	4%
Common	04	0.95	11%	0.09	12%	0.07	14%	0.07	11%	0.01	22%	0.09	7%	0.07	13%	0.07	17%	0.11	12%
Common	05	1.16	5%	0.10	10%	0.08	11%	0.07	21%	0.01	9%	0.09	10%	0.08	12%	0.07	19%	0.11	11%
Common	06	1.17		0.08		0.06		0.06		0.01		0.11		0.06		0.06			
Common	07	1.06		0.07		0.05		0.05		0.01		0.07		0.06		0.06		0.14	
Common	08	0.96		0.08		0.07		0.06		0.01		0.08		0.07		0.06		0.11	
Common	09	0.91	10%	0.08	8%	0.06	4%	0.06	2%	0.01		0.08	6%	0.06	4%	0.06	6%	0.10	5%
Common	10	1.09	5%	0.10		0.07	11%	0.07	14%			0.08	2%	0.07	13%	0.06	14%	0.09	2%
Common	11	1.04	8%	0.08	17%	0.06	20%	0.05	22%			0.08	10%	0.06	20%	0.05	16%	0.10	
Common	12	0.92		0.07		0.05		0.05				0.06		0.05		0.05		0.11	
Common	13	1.05		0.09		0.07		0.07		0.01		0.09		0.07		0.07		0.11	
Common	15	0.99	3%	0.08	14%	0.07	4%	0.07	5%			0.08	10%	0.06	5%	0.07	11%	0.10	

Table 10. Potencies calculated relative to 18007 (Relative Potencies)

		18001		18002		18003		18004		18005		18006		18008		18009		EV71 NS	
Method	**Lab**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**
In-house	01	11.92	7%	1.03	2%	0.93	0%	1.01	3%	0.13	2%	11.02	8%	0.88	9%	0.92	4%	1.80	4%
In-house	02	11.58	6%	0.99	5%	0.89	3%	0.89	7%	0.12	6%	10.85	6%	0.90	8%	0.93	9%	1.37	6%
In-house	03	7.96	7%	1.01	5%	0.57	5%	0.59	5%	0.07	3%	7.72	6%	0.55	5%	0.55	5%	0.85	7%
In-house	04	10.76		0.99		1.16		1.23		0.18		10.75		1.17		1.17		1.54	
In-house	05	8.34	1%	1.03	0%	0.59	3%	0.57	1%	0.07	4%	8.21	3%	0.59	3%	0.58	5%	0.87	2%
In-house	07	8.95	52%	0.98	17%	0.75	16%	0.73	15%	0.11	2%	8.75	52%	0.73	6%	0.70	12%	1.37	16%
In-house	08	11.62	19%	0.99	10%	0.80	18%	0.93		0.10		11.82	11%	0.80	9%	0.86	11%	1.42	8%
In-house	09			1.03		0.97		0.99		0.14				1.06		0.91		1.99	
In-house	10	13.15		1.16		1.69		1.48		0.23		10.71		1.71		1.42		0.98	
In-house	11	12.94		0.97		0.68		0.73	8%	0.13		13.20	11%	0.68	13%	0.65	7%	1.25	
In-house	12	10.94		1.05		0.97		0.97				11.45		0.92		0.87			
Common	01	11.74	3%	1.04	9%	0.94	6%	0.93	6%	0.13	6%	12.69	1%	0.86	4%	0.87	4%	1.36	2%
Common	02	11.52	6%	0.92	10%	0.77	7%	0.77	7%	0.12	6%	12.10	13%	0.73	12%	0.85	18%	1.30	9%
Common	03	13.64	1%	1.02	1%	0.87	0%	0.82	7%	0.12	2%	13.44	1%	0.86	3%	0.78	1%	1.30	3%
Common	04	10.64	4%	0.97		0.78		0.75	11%	0.11	23%	11.25	7%	0.78	11%	0.77	15%	1.29	7%
Common	05	12.44	7%	1.11	3%	0.83	2%	0.80	9%	0.10	3%	10.71	10%	0.82	5%	0.75	8%	1.17	2%
Common	06	11.09		1.06		0.60		0.58				9.29				0.54			
Common	07	13.93	11%	1.01	7%	0.68	7%	0.70	7%	0.13		14.20	2%	0.66	20%	0.67	20%	1.82	7%
Common	08	11.54		0.98		0.79		0.76		0.09		12.13		0.81		0.80		1.40	
Common	09	11.60	10%	0.98	3%	0.77	3%	0.76	4%			12.71	6%	0.78	3%	0.76	1%		
Common	10	13.27	4%	1.15	5%	0.89	13%	0.85	17%			12.22	2%	0.85	14%	0.78	15%		
Common	11	12.46		1.00	8%	0.72	9%	0.68	10%			12.99	10%	0.72	9%	0.69	6%		
Common	12	14.63		1.21		0.88		0.85				15.97		0.76		0.77		1.84	
Common	13	11.40		1.05		0.78		0.73		0.12		10.82		0.71		0.74		1.21	
Common	15	13.09	10%	1.02	10%	0.89	9%	0.93	6%			13.10	10%	0.81	5%	0.95	21%	1.36	

Table 11. Potencies calculated relative to 18008 (Relative Potencies)

		18001		18002		18003		18004		18005		18006		18007		18009		EV71 NS	
Method	**Lab**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**
In-house	01	13.44	11%	1.17	7%	1.05	8%	1.14	12%	0.14	8%	12.45	6%	1.14	9%	1.05	5%	2.04	4%
In-house	02	13.04	3%	1.11	14%	1.00	12%	1.00	17%	0.13	3%	12.12	5%	1.12	8%	1.03	17%	1.54	6%
In-house	03	14.47	2%	1.83	3%	1.03	3%	1.07	3%	0.12	3%	14.05	3%	1.81	5%	1.00	1%	1.56	3%
In-house	04	9.53		0.85		1.00		1.05		0.15		9.44		0.85		0.99		1.36	
In-house	05	14.08	3%	1.73	4%	0.99	6%	0.96	5%	0.12	2%	13.87	4%	1.69	3%	0.98	1%	1.49	4%
In-house	07	12.20	51%	1.35	15%	1.02	10%	0.99	11%	0.15	9%	12.00	51%	1.38	6%	0.96	7%	1.91	13%
In-house	08	14.32	13%	1.22	9%	0.99	9%	1.17		0.11	22%	14.73	3%	1.25	9%	1.07	13%	1.73	9%
In-house	09					0.92		0.98		0.12				0.99		0.88		1.84	
In-house	10	7.97		0.66		1.02		0.87		0.13		6.35		0.58		0.84		0.53	
In-house	11	17.52		1.35		0.94		0.94		0.17		19.22	24%	1.48	13%	0.96	5%	1.64	
In-house	12	11.96		1.14		1.06		1.06				12.50		1.09		0.95			
Common	01	13.71	7%	1.21	6%	1.10	4%	1.09	5%	0.16	7%	14.82	4%	1.17	4%	1.02	6%	1.59	5%
Common	02	15.62	11%	1.24	5%	1.04	5%	1.03	4%	0.15	11%	16.60	7%	1.37	12%	1.17	7%	1.77	6%
Common	03	16.18	3%	1.19	2%	1.02	2%	0.96	6%	0.14	2%	15.89	4%	1.17	3%	0.90	3%	1.53	2%
Common	04	13.93	16%	1.20		0.97		0.97		0.14	13%	14.61	13%	1.29	11%	1.01	4%	1.66	9%
Common	05	15.31	7%	1.36	3%	1.02	4%	0.98	13%	0.12	8%	13.16	12%	1.22	5%	0.92	7%	1.43	6%
Common	06	21.08		1.56		1.08		1.01		0.10		17.92		1.66		1.02			
Common	07	18.76		1.51	26%	1.04	12%	1.07	13%	0.21		19.94		1.51	20%	1.02	7%	2.57	
Common	08	14.24		1.20		0.96		0.93		0.12		15.26		1.23		0.98		1.58	
Common	09	14.73	11%	1.25	5%	0.98	1%	0.96	4%			16.18	4%	1.28	3%	0.97	2%	1.55	
Common	10	15.57	19%	1.36	13%	1.06	7%	1.00	2%			14.23	13%	1.18	14%	0.91	11%	1.51	
Common	11	18.32	23%	1.37	8%	1.01	8%	0.95	4%			17.90	20%	1.40	9%	0.96	4%	1.55	
Common	12	19.18		1.43		1.14		1.16				20.60		1.33		1.03		2.40	
Common	13	16.04		1.44		1.10		1.03		0.16		15.27		1.41		1.05		1.69	
Common	15	15.90	5%	1.26	8%	1.11	7%	1.16	7%			16.03	5%	1.23	5%	1.17	17%	1.50	

Table 12. Potencies calculated relative to 18009 (Relative Potencies)

		18001		18002		18003		18004		18005		18006		18007		18008		EV71 NS	
Method	**Lab**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**	**GM**	**GCV**
In-house	01	12.81	7%	1.12	3%	1.00	4%	1.09	7%	0.13	5%	11.85	4%	1.08	4%	0.95	5%	1.95	1%
In-house	02	12.55	13%	1.07	5%	0.96	9%	0.96	9%	0.13	13%	11.72	16%	1.08	9%	0.97	17%	1.48	16%
In-house	03	14.42	1%	1.82	2%	1.03	3%	1.07	2%	0.12	3%	14.00	4%	1.81	5%	1.00	1%	1.56	2%
In-house	04	9.47		0.86		1.01		1.06		0.16		9.36		0.86		1.01		1.36	
In-house	05	14.30	4%	1.76	5%	1.01	8%	0.98	6%	0.13	3%	14.09	5%	1.72	5%	1.02	1%	1.51	4%
In-house	07	12.62	42%	1.40	9%	1.06	2%	1.03	4%	0.16	15%	12.39	42%	1.42	12%	1.04	7%	1.97	8%
In-house	08	13.41	14%	1.14	3%	0.93	17%	1.03		0.11	25%	13.76	14%	1.17	11%	0.93	13%	1.63	2%
In-house	09			1.08		1.06		1.12		0.13				1.14		1.14		2.03	
In-house	10	9.54		0.80		1.20		1.03		0.16		7.70		0.70		1.19		0.65	
In-house	11	17.97		1.45		1.01		1.00		0.17	16%	19.97	18%	1.54	7%	1.04	5%	1.78	
In-house	12	12.57		1.20		1.11		1.12				13.15		1.15		1.05			
Common	01	13.53	7%	1.19	12%	1.08	10%	1.07	9%	0.15	13%	14.66	3%	1.15	4%	0.98	6%	1.57	5%
Common	02	13.57	14%	1.11		0.92		0.91		0.14		14.46	11%	1.18	18%	0.85	7%	1.54	8%
Common	03	18.02	2%	1.32	2%	1.13	1%	1.07	7%	0.16	2%	17.66	1%	1.29	1%	1.11	3%	1.69	3%
Common	04	13.81	16%	1.31	22%	0.99	4%	0.97	5%	0.13	29%	14.58	17%	1.30	15%	0.99	4%	1.62	6%
Common	05	16.64	14%	1.48	9%	1.10	7%	1.07	8%	0.13	12%	14.29	19%	1.33	8%	1.08	7%	1.56	9%
Common	06	20.77		1.48		1.06		0.99		0.09		17.59		1.70		0.98			
Common	07	21.44		1.52		1.01	12%	1.05	12%	0.21		19.12		1.49	20%	0.98	7%	2.76	
Common	08	14.29		1.22		0.97		0.95		0.13		15.45		1.24		1.02		1.44	
Common	09	15.20	10%	1.28	3%	1.01	2%	0.99	5%	0.15		16.67	6%	1.31	1%	1.03	2%	1.63	2%
Common	10	16.94		1.23		1.08		1.05				15.24		1.27		1.03		1.60	
Common	11	19.01	20%	1.43	9%	1.05	10%	0.98	8%			18.58	16%	1.45	6%	1.04	4%	1.68	
Common	12	18.62		1.48		1.11		1.12				20.48		1.29		0.97		2.23	
Common	13	15.34		1.37		1.05		0.98		0.16		14.60		1.34		0.96		1.61	
Common	15	13.65	12%	1.07	26%	0.95	13%	0.99	14%			13.72	11%	1.05	21%	0.85	17%	1.17	

Table 13. Potency summary

		Reference														
		EV71 NS			18006			18007			18008			18009		
Method	Sample	GM	GCV	n	GM	GCV	n	GM	GCV	n	GM	GCV	n	GM	GCV	n
In-house	EV71 NS				0.12	19%	9	1.30	33%	10	1.48	47%	10	1.53	39%	10
In-house	18001	8.63	25%	10	1.03	7%	10	10.67	19%	10	12.58	25%	10	12.76	21%	10
In-house	18002	0.78	36%	10	0.10	19%	10	1.02	5%	11	1.19	36%	10	1.21	30%	11
In-house	18003	0.66	48%	10	0.08	31%	10	0.87	37%	11	1.00	4%	11	1.03	7%	11
In-house	18004	0.67	43%	9	0.08	29%	10	0.88	34%	11	1.02	9%	11	1.04	5%	11
In-house	18005	0.10	44%	10	0.01	37%	9	0.12	45%	10	0.14	15%	10	0.14	15%	10
In-house	18006	8.17	19%	9				10.32	19%	10	12.23	34%	10	12.43	29%	10
In-house	18007	0.77	33%	10	0.10	19%	10				1.16	38%	11	1.20	33%	11
In-house	18008	0.67	47%	10	0.08	34%	10	0.86	38%	11				1.03	8%	11
In-house	18009	0.65	39%	10	0.08	29%	10	0.84	33%	11	0.97	8%	11			
Common	EV71 NS				0.11	10%	13	1.39	17%	10	1.69	20%	13	1.67	23%	13
Common	18001	9.29	12%	13	1.01	9%	14	12.30	10%	14	16.20	14%	14	16.29	18%	14
Common	18002	0.76	15%	9	0.08	12%	14	1.03	7%	14	1.32	10%	14	1.31	12%	14
Common	18003	0.61	21%	11	0.06	13%	14	0.80	13%	14	1.04	6%	14	1.03	6%	14
Common	18004	0.60	20%	11	0.06	13%	14	0.77	14%	14	1.02	7%	14	1.01	6%	14
Common	18005	0.09	9%	13	0.01	20%	10	0.11	16%	8	0.14	25%	9	0.14	22%	10
Common	18006	9.44	9%	13				12.30	14%	14	16.19	13%	14	16.10	14%	14
Common	18007	0.72	17%	10	0.08	14%	14				1.31	11%	14	1.31	12%	14
Common	18008	0.59	20%	13	0.06	12%	14	0.78	8%	13				0.99	8%	14
Common	18009	0.60	23%	13	0.06	13%	14	0.76	14%	14	1.01	8%	14			

Table 14. Between-laboratory variability summary for study samples

Measure of Variability	Method	Reference				
		EV71 NS	**18006**	**18007**	**18008**	**18009**
Median between-laboratory GCV	In-house	39%	29%	33%	25%	21%
	Common	17%	13%	14%	11%	12%

Table 15. Potency summary (excluding laboratory 10 in-house results)

		Reference													
		EV71 NS			18006			18007			18008			18009		
Method	Sample	GM	GCV	n	GM	GCV	n	GM	GCV	n	GM	GCV	n	GM	GCV	n
In-house	EV71 NS				0.13	18%	8	1.34	33%	9	1.67	14%	9	1.68	15%	9
In-house	18001	8.17	16%	9	1.01	4%	9	10.42	19%	9	13.24	18%	9	13.18	18%	9
In-house	18002	0.74	32%	9	0.10	20%	9	1.01	3%	10	1.27	26%	9	1.26	27%	10
In-house	18003	0.59	14%	9	0.08	19%	9	0.81	26%	10	1.00	4%	10	1.02	5%	10
In-house	18004	0.60	16%	8	0.08	21%	9	0.84	28%	10	1.03	8%	10	1.04	5%	10
In-house	18005	0.09	17%	9	0.01	25%	8	0.11	36%	9	0.14	16%	9	0.14	16%	9
In-house	18006	7.95	18%	8				10.27	20%	9	13.15	21%	9	13.11	22%	9
In-house	18007	0.75	33%	9	0.10	20%	9				1.25	27%	10	1.26	27%	10
In-house	18008	0.60	14%	9	0.08	21%	9	0.81	28%	10				1.01	6%	10
In-house	18009	0.60	15%	9	0.08	22%	9	0.79	27%	10	0.99	6%	10			
Common	EV71 NS				0.11	10%	13	1.39	17%	10	1.69	20%	13	1.67	23%	13
Common	18001	9.29	12%	13	1.01	9%	14	12.30	10%	14	16.20	14%	14	16.29	18%	14
Common	18002	0.76	15%	9	0.08	12%	14	1.03	7%	14	1.32	10%	14	1.31	12%	14
Common	18003	0.61	21%	11	0.06	13%	14	0.80	13%	14	1.04	6%	14	1.03	6%	14
Common	18004	0.60	20%	11	0.06	13%	14	0.77	14%	14	1.02	7%	14	1.01	6%	14
Common	18005	0.09	9%	13	0.01	20%	10	0.11	16%	8	0.14	25%	9	0.14	22%	10
Common	18006	9.44	9%	13				12.30	14%	14	16.19	13%	14	16.10	14%	14
Common	18007	0.72	17%	10	0.08	14%	14				1.31	11%	14	1.31	12%	14
Common	18008	0.59	20%	13	0.06	12%	14	0.78	8%	13				0.99	8%	14
Common	18009	0.60	23%	13	0.06	13%	14	0.76	14%	14	1.01	8%	14			

Table 16. Between-laboratory variability summary for study samples (excluding laboratory 10 in-house results

Measure of Variability	Method	Reference				
		EV71 NS	**18006**	**18007**	**18008**	**18009**
Median between-laboratory GCV	In-house	16%	20%	27%	16%	16%
	NIBSC	17%	13%	14%	11%	12%

Table 17. Overall relative potency summary for 18/116, 18/120, 18/122 and 18/156

Reference	Sample	Method	GM	GCV	n	Robust GM	Median
EV71 NS	18/116 (18001 & 18006)	In-house	8.34	19%	10	8.45	8.63
		Common	9.31	10%	13	9.35	9.14
		All	8.87	15%	23	9.03	9.13
	18/120 (18002 & 18007)	In-house	0.77	35%	10	0.74	0.71
		Common	0.73	17%	11	0.76	0.76
		All	0.75	26%	21	0.74	0.75
	18/122 (18003 & 18008)	In-house	0.66	48%	10	0.61	0.61
		Common	0.60	20%	13	0.63	0.64
		All	0.63	33%	23	0.62	0.64
	18/156 (18004 & 18009)	In-house	0.65	41%	10	0.61	0.62
		Common	0.60	20%	13	0.62	0.61
		All	0.62	30%	23	0.61	0.61
18006	18/116 (18001 & 18006)	In-house	1.03	7%	10	1.02	1.03
		Common	1.01	9%	14	1.00	1.00
		All	1.02	8%	24	1.01	1.02
	18/120 (18002 & 18007)	In-house	0.10	19%	10	0.10	0.09
		Common	0.08	11%	14	0.08	0.08
		All	0.09	18%	24	0.09	0.09
	18/122 (18003 & 18008)	In-house	0.08	34%	10	0.08	0.08
		Common	0.06	11%	14	0.06	0.06
		All	0.07	27%	24	0.07	0.07
	18/156 (18004 & 18009)	In-house	0.08	29%	10	0.08	0.08
		Common	0.06	12%	14	0.06	0.06
		All	0.07	25%	24	0.07	0.07
18007	18/116 (18001 & 18006)	In-house	10.51	19%	10	10.99	11.20
		Common	12.32	10%	14	12.33	12.18
		All	11.53	17%	24	11.79	11.76
	18/120 (18002 & 18007)	In-house	1.02	5%	11	1.01	1.01
		Common	1.03	7%	14	1.02	1.02
		All	1.03	6%	25	1.02	1.02

	18/122 (18003 & 18008)	In-house	0.86	38%	11	0.85	0.89
		Common	0.78	12%	14	0.79	0.79
		All	0.81	26%	25	0.81	0.80
	18/156 (18004 & 18009)	In-house	0.86	33%	11	0.87	0.91
		Common	0.77	14%	14	0.78	0.78
		All	0.81	24%	25	0.80	0.80
18008	18/116 (18001 & 18006)	In-house	12.47	31%	10	12.86	12.75
		Common	16.18	13%	14	16.10	15.81
		All	14.52	26%	24	14.84	14.63
	18/120 (18002 & 18007)	In-house	1.17	36%	11	1.21	1.15
		Common	1.32	9%	14	1.30	1.28
		All	1.25	24%	25	1.28	1.27
	18/122 (18003 & 18008)	In-house	1.00	4%	11	1.01	1.00
		Common	1.04	6%	14	1.04	1.04
		All	1.02	5%	25	1.02	1.02
	18/156 (18004 & 18009)	In-house	0.99	7%	11	1.00	1.00
		Common	1.01	7%	14	1.01	1.01
		All	1.00	7%	25	1.01	1.00
18009	18/116 (18001 & 18006)	In-house	12.67	26%	10	12.78	12.68
		Common	16.25	15%	14	16.26	15.67
		All	14.65	25%	24	14.85	14.20
	18/120 (18002 & 18007)	In-house	1.20	32%	11	1.24	1.16
		Common	1.32	12%	14	1.32	1.31
		All	1.27	22%	25	1.29	1.30
	18/122 (18003 & 18008)	In-house	1.03	7%	11	1.03	1.01
		Common	1.01	7%	14	1.02	1.02
		All	1.02	7%	25	1.02	1.02
	18/156 (18004 & 18009)	In-house	1.04	5%	11	1.04	1.03
		Common	1.01	6%	14	1.01	0.99
		All	1.02	6%	25	1.03	1.03

Table 18. Overall potency summary (EV71 Ag/ml) for 18/116, 18/120, 18/122 and 18/156 using EV71 NS (1,600 EV71 Ag/ml) as a reference

Sample	Method	GM	GCV	n	Robust GM	Median
18/116 (18001 & 18006)	In-house	13345	19%	10	13514	13812
	Common	14893	10%	13	14953	14626
	All	14199	15%	23	14450	14603
18/120 (18002 & 18007)	In-house	1227	35%	10	1179	1131
	Common	1172	17%	11	1215	1220
	All	1198	26%	21	1182	1195
18/122 (18003 & 18008)	In-house	1060	48%	10	976	972
	Common	963	20%	13	1005	1032
	All	1004	33%	23	986	1029
18/156 (18004 & 18009)	In-house	1036	41%	10	982	996
	Common	956	20%	13	991	976
	All	990	30%	23	977	976

Table 19. D-Ag content of stability samples (% of -20ºC) after storage at +20ºC, +37ºC or +45ºC for 1, 3 or 6 weeks.

Temperature	18/116	18/120	18/122	18/156
1 Week				
+20°C	115.2	107.5	111.5	110.1
+37ºC	117.2	123.7	111.2	124.2
+45ºC	120.5	135.5	116.9	134.3
3 Weeks				
+20°C	133.2	145.2	135	129.8
+37ºC	106.1	99.3	104	94.2
+45ºC	118.5	102.6	106.7	96.9
6 Weeks				
+20°C	114.3	101.1	112.5	121.5
+37ºC	135.6	115.6	124.2	130.6
+45ºC	114.8	129.5	121.2	150.4

Table 20. EV71 Antigen content of stability samples (% of -20ºC) after 1, 2 or 3 freeze / thaw cycles.

Freeze/thaw cycle	18/116	18/120	18/122	18/156
1	113	102.7	76.9	92.4
2	123.8	100.7	82.1	105.1
3	123.4	114.2	88.2	103.2

Table 21. D-Ag content of stability samples (% of -20ºC) after reconstitution and storage at +4ºC.

Storage time (+4 ºC)	18/116	18/120	18/122	18/156
1 Week	109.2	91.4	113	109.6
2 Week	107.5	89	118.7	111.1
3 Week	113.9	75.9	114.9	109.8
4 Week	90.8	86.3	96.4	116.1

Figure 1. Laboratory Geometric Mean Potency Estimates

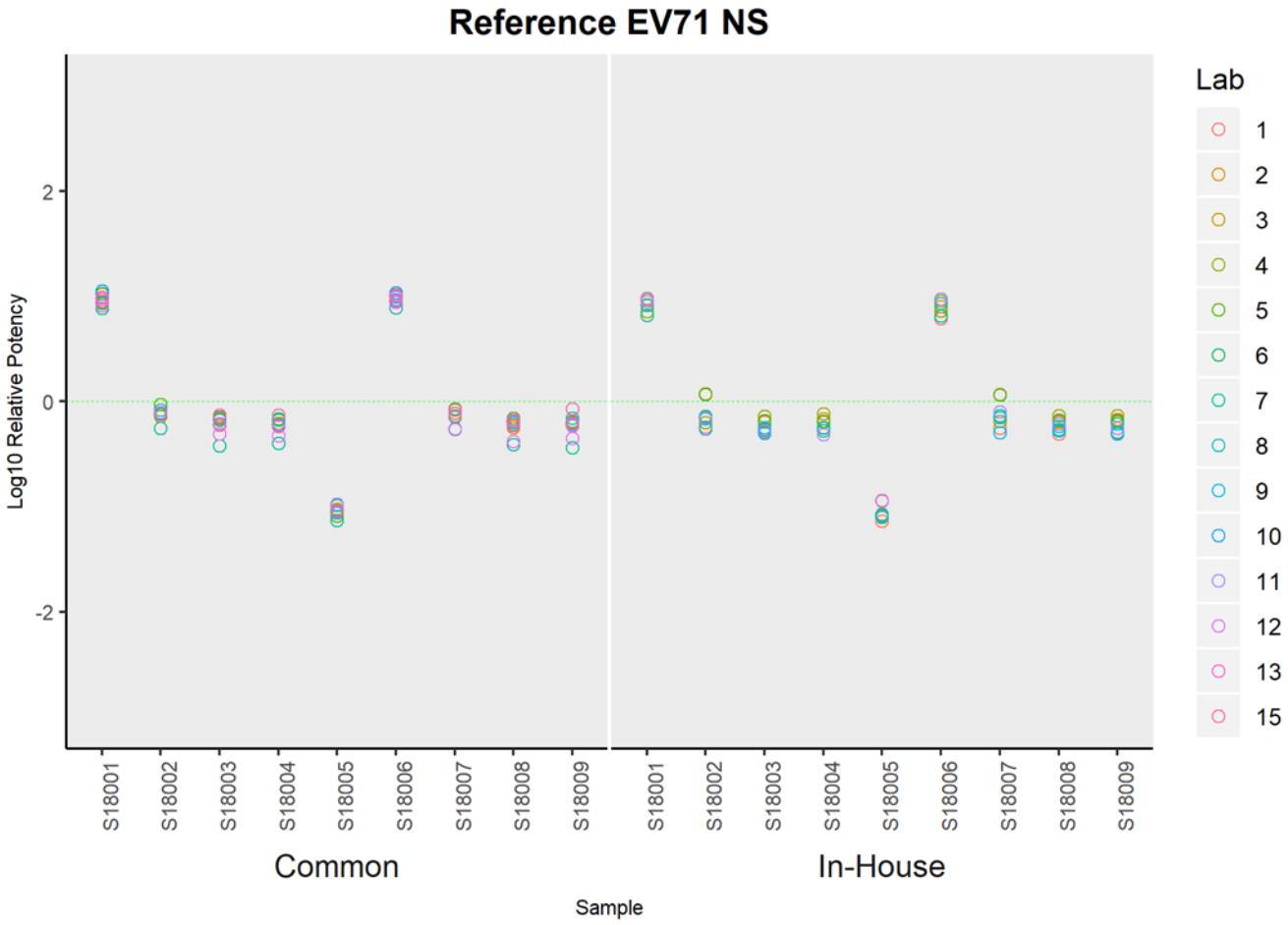


Figure 2. Laboratory Geometric Mean Potency Estimates

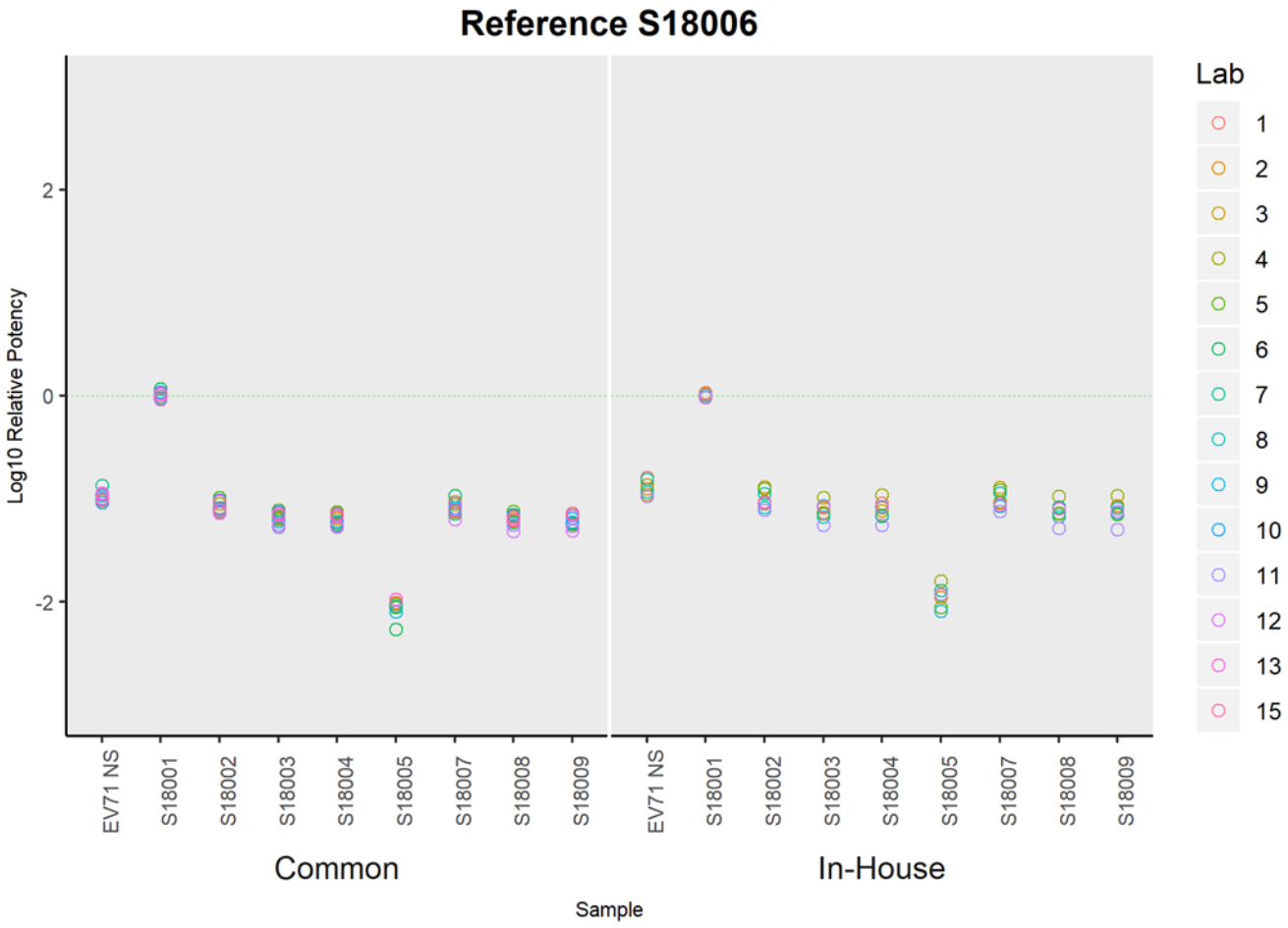


Figure 3. Laboratory Geometric Mean Potency Estimates

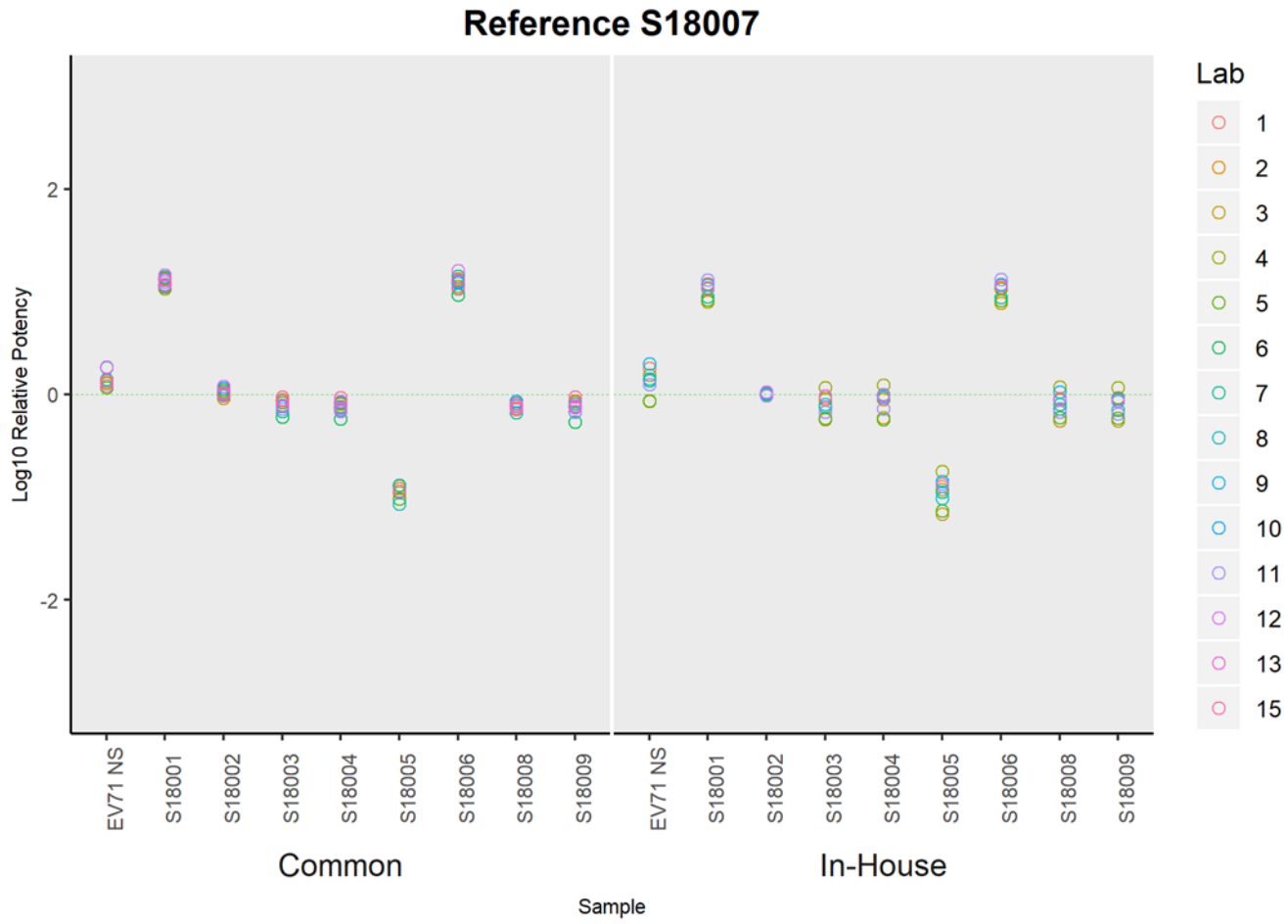


Figure 4. Laboratory Geometric Mean Potency Estimates

Figure 5. Laboratory Geometric Mean Potency Estimates

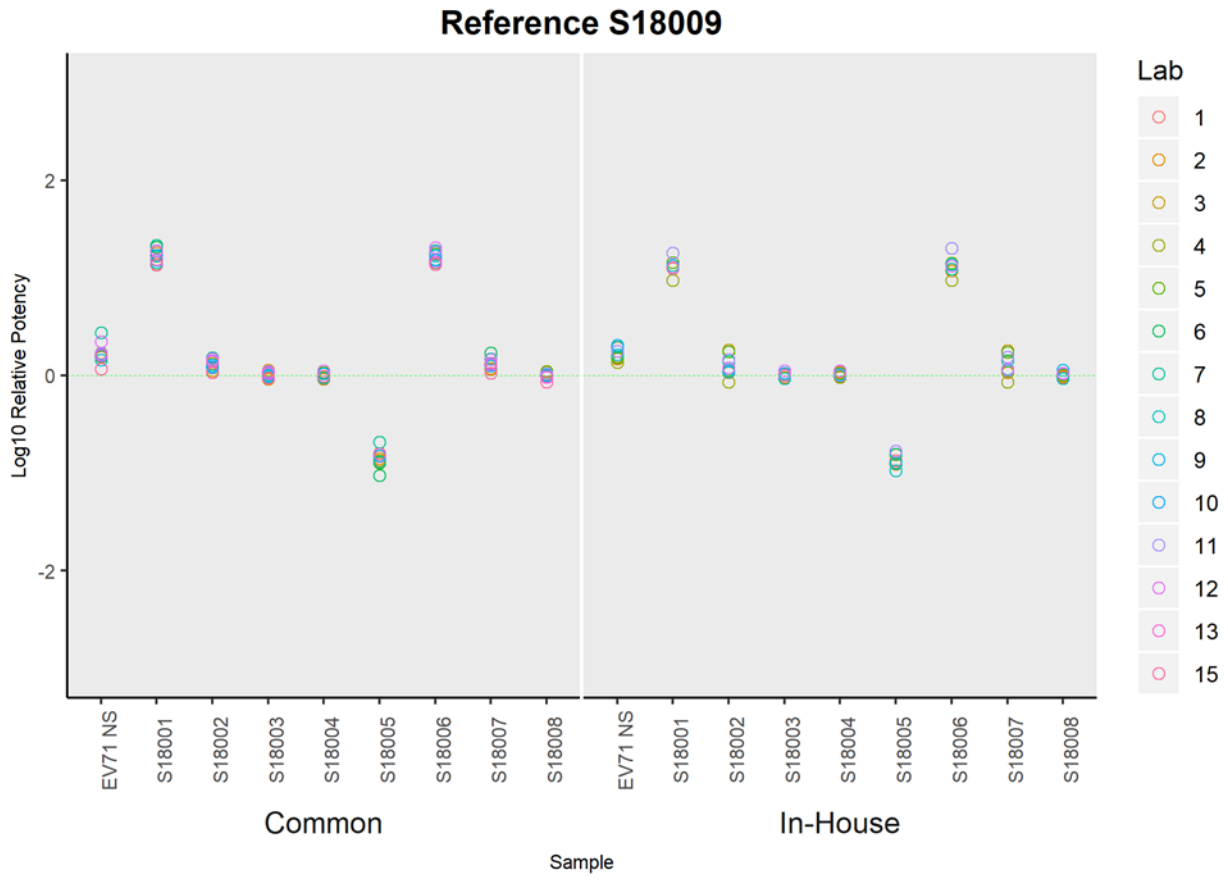


Figure 6. Real-time stability of candidate sample 18/116 at -20°C. Potency expressed in EV71 Antigen Units using EV71 NS as reference.

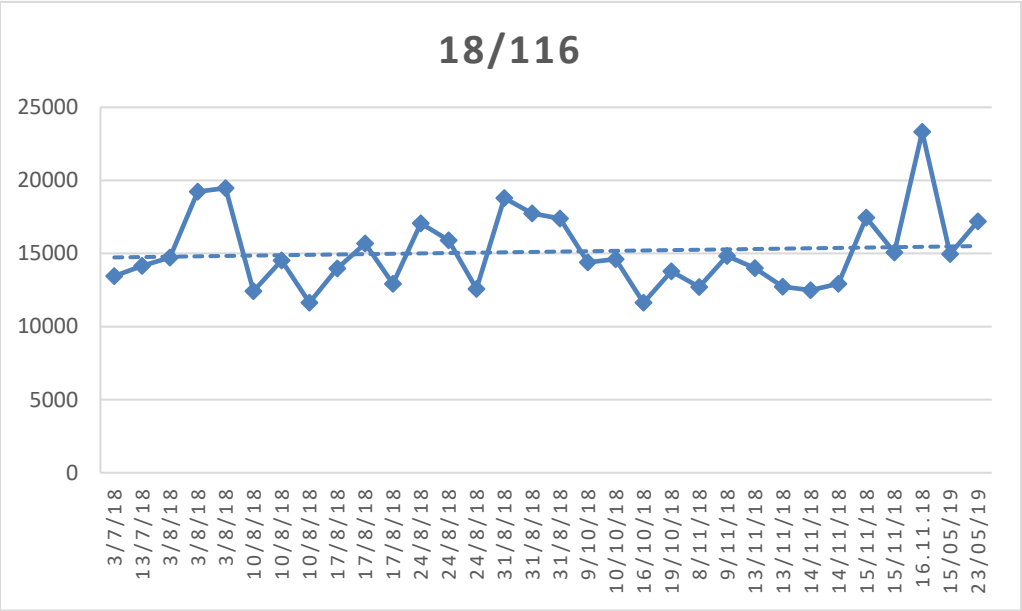


Figure 7. Real-time stability of candidate sample 18/120 at -20°C. Potency expressed in EV71 Antigen Units using EV71 NS as reference.

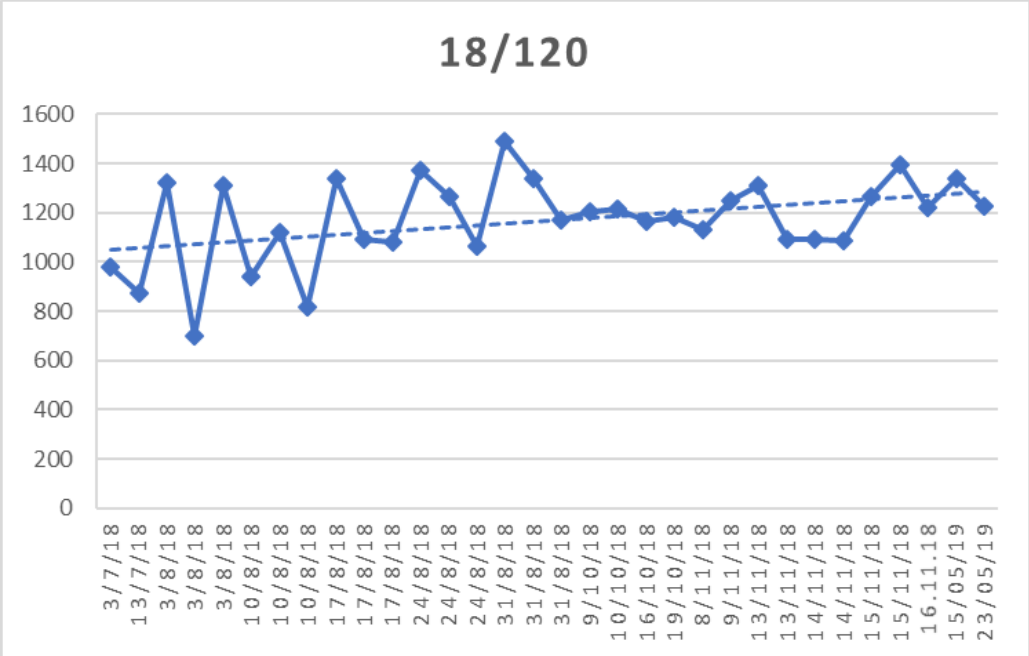


Figure 8. Real-time stability of candidate sample 18/122 at -20°C. Potency expressed in EV71 Antigen Units using EV71 NS as reference.

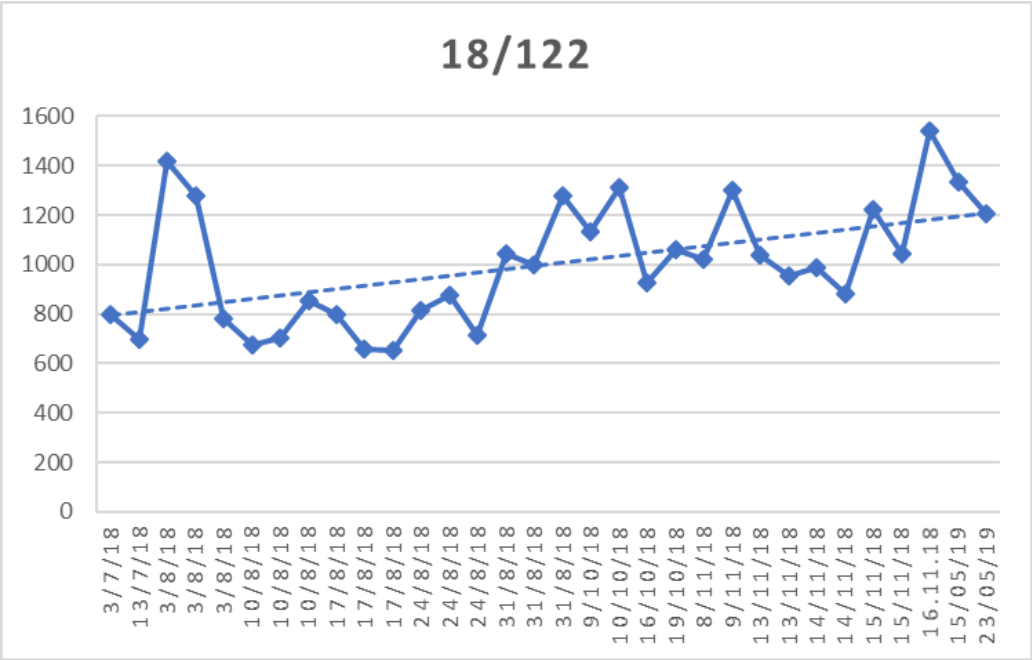
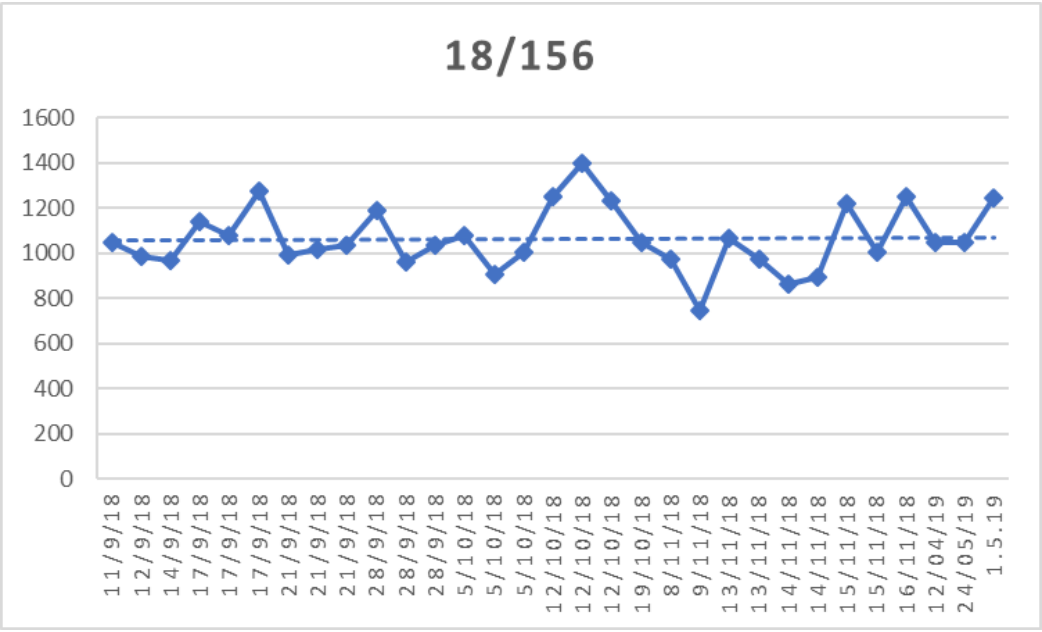


Figure 9. Real-time stability of candidate sample 18/156 at -20°C. Potency expressed in EV71 Antigen Units using EV71 NS as reference.



Appendix 1

Collaborative study participants

Name	Laboratory
Tong Wu Jason MacLaurin	Health Canada https://www.canada.ca/en/health-canada.html
Qiang Zou	Beijing Zhifei Lvzhu Biopharmaceutical Co., Ltd http://www.vaccine.com.cn/index/en/
Wen-qi An	Hualan Biological Vaccine Co., Ltd http://english.hualanbio.com/
Tong Cheng	National Institute of Diagnostics and Vaccine Development in Infectious Diseases (Xiamen University) http://nidvd.xmu.edu.cn/en/
Xiuling Li	National Vaccine and Serum Institute http://www.cnvsi.com/
Gao Fan	National Institutes for Food and Drug Control https://www.nifdc.org.cn/nifdc/index.html
Chaoming Zhou	Shanghai Bovax Biotechnology Co., Ltd
Dan Yu	Sinovac Biotech Co., Ltd. http://www.sinovac.com/
Shuo Shen	Wuhan Institute of Biological Products Co., Ltd http://www.wibp.com.cn/Chs/Default.aspx
Charlie Chen	Adimmune Corporation http://www.adimmune.com.tw/en/index.php
Scott Hsiang-Chi Lee	Medigen Vaccine Biologics Corp. http://www.medigen.com.tw/en/home/
Min-Shi Lee	National Health Research Institutes http://english.nhri.org.tw/
Po-Chih Wu	TFDA https://www.fda.gov.tw/EN/
Alison Tedcastle Elaine Pegg	National Institute for Biological Standards and Control https://www.nibsc.org/

Appendix 2

Details of in-house assay methods used by participants

	Capture antibody			Detection antibody				
Lab	**Antibody**	**Animal**	**Genotype/ strain**	**Antibody**	**Animal**	**Genotype**	**No. of replicas/ dilutions**	**Substrate**
1	Polyclonal	Rabbit	C4/H07	Monoclonal	Mouse	C4/H07	2/4	TMB
2	Polyclonal	Rabbit	C4/H07	Monoclonal	Mouse	C4/H07	2/4	TMB
3	Polyclonal	Rabbit	C4/E150	Polyclonal	Rabbit	C4/E150	2/4	TMB
4	Polyclonal	Rabbit		Monoclonal	Mouse	C4/CT11F9		
5	Polyclonal	Rabbit	C4/E150	Polyclonal	Rabbit	C4/E150	2/4	TMB
7	Monoclonal	Mouse	C4	Monoclonal	Mouse	C4	2/4	TMB
8	Polyclonal	Rabbit	C4	Monoclonal	Mouse	C4/CT11F9		
9	Polyclonal	Chicken	A/BrCr	Monoclonal	Mouse	A/BrCr	2/4	TMB
10	Polyclonal	Rabbit	B4	Monoclonal	Mouse	A/BrCr	2/3	TMB
11	Polyclonal	Rabbit	B4/E59	Monoclonal	Mouse	A/BrCr		
12	Monoclonal	Mouse	B5	Polyclonal	Rabbit	C2	2/4	OPD
15	Polyclonal	Rabbit	C4	Monoclonal	Mouse	C4/CT11F9	2/4	OPD

Appendix 3

NIBSC-NIFDC EV71 Antigen ELISA method

Test all samples in the same ELISA plate and include sample EV71 NS in each assay as well as the in-house reference (if available)

Protocol

- Add 50µl per well of rabbit anti-EV71 capture polyclonal antibody diluted 1:2000 in carbonate coating buffer. Overnight incubation at 2-8ºC
- Day of assay wash plates x4 (wash buffer: PBS +2% Milk + 0.5%Tween 20).
- Leave the 4th wash for 30 mins at RT
- Prepare sample dilutions in dilution buffer (PBS +2% Milk) with starting dilutions as indicated. The same dilutions used for the in-house method can be used for this method provided both assays are done at the same time.
- Add 50µl of each dilution to duplicate wells using the plate layout suggested in the method protocol sent to participants. At the same time, add 50µl of dilution buffer to the blank wells to act as a control. Incubate at 37ºC / 2 hours.
- Prepare the mouse MAb detection antibody CT11F9 to 2ug/ml (starting concentration is 1.8mg/ml) in dilution buffer.
- Wash plates x4 (wash buffer), add 50µl of mouse anti-EV71 detection monoclonal antibody. Incubate at 37ºC / 1 hour
- Wash plates x4 (wash buffer), add 50µl anti-mouse IgG-HRP diluted 1:400. Incubate at 37oC / 1 hour
- Wash plates x2 (wash buffer) and x2 (PBS), add 50 µl OPD substrate buffer. Incubate at room temperature for 30 minutes in the dark.
- Stop reaction by adding 50µl of 1 M H_2SO_4. Read OD at 492nm
- Data analysis

Reagents

- Carbonate coating buffer: NaCO3 – Merck, NaHCO3 – 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 - P7949
- 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 H20 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 + 50µl 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.

Summary of Participants Comments on the Report

Some participants suggested minor editorial changes on the report and proposed Instructions for Use and some requested additional information, in particular, clarification on statistical criteria used for data exclusions. Some laboratories also emphasized the importance of measuring the antigen content of both empty and full virus particles present in EV71 vaccines. Comments were also made on the different reactivity of some antibody reagents with strains from different genogroups and the possible need of establishing genogroup-specific International Standards in the future. The importance of understanding which epitopes are immunogenically relevant for different strains from different genogroups was also commented. One laboratory inquired if the stability data would support the possibility of reconstituting the IS in larger volume and store the aliquots at -20C for future use. The answer is that this practice would not be recommended but if used, this process should be fully validated in-house. These suggestions have been addressed in the revised report which includes some changes. None of the participants expressed disagreement with the proposals made in the report.

Appendix 5.

Proposed instruction for use*

1st International Standard for EV71 inactivated vaccine
18/116

"This material is not for in vitro diagnostic use"

1. INTRODUCTION

This preparation was established by the WHO Expert Committee on Biological Standardization in 2019 as the 1st International Standard (IS) for EV71 inactivated vaccine It was shown to be suitable for determination of the antigenic content of EV71 inactivated vaccine products by *in vitro* assays.

The original material was prepared by a manufacturer using EV71 inactivated vaccine produced with an EV71 genogroup C4 strain. The preparation is a freeze-dried blend and has been tested for the absence of adventitious agents.

2. UNITAGE

The 1st WHO IS 18/116 for EV71 inactivated vaccine for use in standardization of *in vitro* vaccine potency assays has an assigned potency of 14,500 IU of EV71 antigen per ml when reconstituted in 0.25ml sterile distilled water as instructed.

3. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS.

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

4. DIRECTIONS FOR OPENING THE DIN AMPOULE

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of

the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

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

5. USE OF AMPOULED MATERIAL

The 1st WHO International Standard for EV71 inactivated vaccine should be used to calibrate laboratory reference reagents to be used in in vitro assays for the determination of the antigenic content of EV71 vaccine products.

Unopened ampoules should be stored at ≤ -20°C. Ampoules should be reconstituted by adding exactly 0.25 ml of sterile distilled water ideally on the day of the assay, but reconstituted ampoules can be used for up to 4 weeks if stored at 2-8°C. To remove the reagent from the ampoule it is necessary to use some form of transfer pipette rather than a volumetric pipette. The contents of the ampoules should not be assumed to be sterile.

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

Please note that the 1st IS is provided as a reagent for calibrating your own in-house reference material(s). Recipients should remember that the supply of this reagent will be limited to 3 vials per organization per year.

6. STABILITY

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

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

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

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

7. CITATION

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

8. PRODUCT LIABILITY AND LOSS

8.1 Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx) ("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 8.3:

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

1st International Standard for EV71 Inactivated Vaccine NIBSC Code 18/116 Physical properties (at room temperature)			
Physical appearance	Freeze dried and has a small white/yellowish cake		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	Contains inactivated enterovirus A71		
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 an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.			

* Similar documents will be created for WHO Reference Reagents 18/120, 18/122 and 18/156

Appendix 6

Relative potency estimates by assay, with EV71 NS as a reference (shaded cells are in-house assays)

Lab	S18001			S18002			S18003			S18004			S18005			S18006			S18007			S18008			S18009		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	6.18	7.21	6.57	0.56	0.57	0.59	0.49	0.52	0.54	0.52	0.55	0.60	0.07	0.07	0.08	6.14	6.45	5.92	0.53	0.56	0.58	0.51	0.48	0.48	0.51	0.52	0.51
2	8.10	8.13	8.83	0.76	0.64	0.75	0.68	0.59	0.64	0.72	0.57	0.64	0.08	0.08	0.09	7.93	7.94	8.01	0.76	0.69	0.75	0.62	0.65	0.69	0.73	0.57	0.74
3	9.21	9.18	9.44	1.14	1.20	1.20	0.64	0.68	0.66	0.67	0.70	0.69	0.08	0.08	0.08	8.78	8.63	9.62	1.08	1.20	1.24	0.63	0.63	0.66	0.63	0.64	0.66
4	7.15			0.63			0.72			0.76			0.11			7.30			0.65			0.73			0.74		
5	9.36	9.59	9.74	1.14	1.16	1.20	0.63	0.66	0.69	0.63	0.64	0.67	0.08	0.09	0.08	8.90	9.73	9.61	1.12	1.16	1.17	0.65	0.70	0.66	0.65	0.70	0.64
7	8.95	5.58	5.88	0.73	0.72	0.71	0.52	0.58	0.52	0.52	0.55	0.51	0.07	0.09	0.10	8.76	5.48	5.70	0.62	0.78	0.81	0.46	0.58	0.54	0.48	0.55	0.50
8	8.29	9.13	7.30	0.68	0.69	0.68	0.62	0.60	0.49	0.46	0.65	0.61	0.09	0.06	0.08	9.43	8.55	7.74	0.75	0.65	0.71	0.64	0.56	0.54	0.61	0.61	0.63
9	*5.93*	*6.74*	*9.98*	*0.53*	0.55	*0.77*	*0.52*	0.50	*0.65*	*0.54*	*0.50*	*0.66*	0.08	0.08	0.09	*5.73*	*7.93*	*13.11*	*0.55*	0.50	*0.93*	0.54	*0.55*	*0.89*	0.49	*0.49*	*0.77*
10	12.82	14.10	*11.55*	1.24	1.28	1.14	*2.43*	2.13	1.71	2.12	1.82	1.42	0.28	0.26	0.24	9.72	10.96	9.41	1.04	1.03	1.01	2.29	1.99	1.81	1.86	1.63	1.45
11	8.07	8.92	10.06	*0.57*	0.55	0.88	*0.43*	0.44	0.64	*0.47*	0.40	0.60	*0.10*	*0.10*	*0.12*	*8.13*	*7.56*	9.48	*0.61*	*0.51*	0.80	*0.47*	*0.34*	0.61	*0.45*	*0.35*	0.56
12	9.44	*10.09*	8.66	*1.00*	*0.88*	*0.80*	*0.87*	*0.84*	*0.75*	*0.84*	*0.95*	*0.74*	0.11	0.12	0.10	*10.74*	*9.74*	*11.79*	*0.93*	*0.88*	*0.68*	*0.83*	*0.81*	*0.60*	*0.78*	*0.79*	0.59
1	8.49	8.79	8.58	0.83	0.76	0.70	0.75	0.69	0.65	0.73	0.68	0.64	0.10	0.09	0.08	9.24	9.09	9.48	0.73	0.72	0.74	0.66	0.60	0.62	0.64	0.60	0.66
2	8.67	8.76	9.49	0.73	0.73	0.71	0.57	0.62	0.61	0.58	0.62	0.60	0.09	0.09	0.10	8.63	10.65	8.80	0.70	0.79	0.83	0.55	0.61	0.54	0.67	0.69	0.59
3	10.90	10.27	10.54	0.79	0.77	0.79	0.67	0.65	0.67	0.59	0.60	0.67	0.09	0.09	0.10	10.84	10.07	10.48	0.78	0.75	0.78	0.64	0.65	0.67	0.60	0.57	0.60
4	9.26	8.56	7.85	*0.94*	0.81	0.70	*0.63*	0.60	0.62	0.59	0.62	0.60	0.07	0.10	0.09	8.53	10.06	8.19	0.77	0.83	0.73	0.54	0.63	0.64	0.58	0.61	0.66
5	11.44	9.69	10.77	0.99	0.91	0.92	0.73	0.71	0.69	0.64	0.75	0.67	0.08	0.08	0.09	9.68	8.08	9.79	0.86	0.85	0.84	0.74	0.70	0.66	0.65	0.69	0.59
6	*11.47*	*13.07*	*14.00*	*0.96*	*0.93*	*1.35*	*0.80*	*0.68*	*0.83*	*0.76*	*0.65*	*0.74*	0.09	0.09	0.09	*13.52*	*11.63*	*11.72*	*0.96*	*1.02*	*1.24*	*0.84*	*0.67*	*0.68*	*0.76*	*0.70*	*0.68*
7	7.54	8.24	7.40	0.50	0.60	0.58	0.38	0.36	0.40	0.38	*0.37*	0.42	0.07	0.07	0.08	7.00	7.80	*8.64*	0.51	0.55	0.59	0.41	*0.33*	0.37	0.41	0.32	*0.40*
8	8.74	*10.44*	9.30	0.70	0.98	*0.74*	0.61	0.75	0.56	0.59	0.75	*0.59*	0.09	0.11	0.09	9.35	*13.70*	*11.12*	0.71	*1.01*	*0.89*	0.63	*0.80*	*0.69*	0.60	0.80	*0.61*
9	9.78	8.67	9.64	*0.75*	0.76	*0.76*	0.62	0.63	0.60	0.63	0.59	0.58	0.09	0.08	0.09	10.70	10.11	9.71	*0.78*	*0.79*	*0.77*	0.64	0.65	*0.61*	0.60	0.63	0.61
10	11.33	*12.36*	*11.82*	*0.99*	*0.97*	*0.97*	*0.86*	*0.73*	*0.82*	*0.78*	*0.67*	*0.85*	*0.07*	0.09	0.09	10.64	10.73	11.04	*0.83*	*0.87*	*0.89*	*0.80*	0.66	*0.82*	*0.68*	0.63	*0.82*
11	9.10	*9.89*	10.65	*0.68*	*0.62*	*0.88*	*0.55*	*0.49*	*0.69*	*0.54*	*0.46*	*0.65*	0.10	0.10	0.11	*9.60*	*9.48*	9.89	*0.71*	*0.64*	*0.81*	*0.58*	*0.48*	0.64	*0.57*	*0.48*	0.60
12	8.27	8.82	8.04	*0.60*	*0.75*	*0.65*	*0.49*	*0.59*	0.49	*0.52*	*0.58*	0.47	*0.07*	0.08	*0.06*	8.79	8.24	*8.86*	*0.57*	*0.61*	0.54	*0.46*	0.49	0.42	0.46	*0.53*	0.44
13	8.81	9.35	9.68	0.69	0.84	0.83	0.52	0.65	0.65	0.53	0.59	0.63	0.08	0.09	0.10		8.88	9.27		0.83	0.83		0.59	0.59		0.62	0.62
15	9.13	8.99	11.24	*0.77*	*0.67*	*0.83*	*0.63*	*0.67*	0.74	*0.65*	*0.71*	0.74	0.09	0.09	0.10	*9.15*	9.40	11.19	*0.72*	0.70	0.77	*0.60*	*0.59*	0.67	*0.59*	*0.71*	0.86

Potencies marked in **purple** are from assays where at least one of the test or reference samples are non-linear
Potencies marked in **red** are from assays where the test and reference samples are non-parallel

Relative potency estimates by assay, with 18006 as a reference (shaded cells are in-house assays)

Lab	EV71 NS			S18001			S18002			S18003			S18004			S18005			S18007			S18008			S18009		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	0.16	0.16	0.17	1.00	1.12	1.10	0.09	0.09	0.10	0.08	0.08	0.09	0.09	0.09	0.10	0.01	0.01	0.01	0.09	0.09	0.10	0.08	0.08	0.08	0.08	0.08	0.09
2	0.13	0.13	0.12	1.04	1.03	1.12	0.10	0.08	0.10	0.09	0.08	0.08	0.09	0.07	0.08	0.01	0.01	0.01	0.10	0.09	0.09	0.08	0.08	0.09	0.09	0.07	0.09
3	0.11	0.12	0.10	1.06	1.06	0.98	0.13	0.14	0.12	0.07	0.08	0.07	0.08	0.08	0.07	0.01	0.01	0.01	0.12	0.14	0.13	0.07	0.07	0.07	0.07	0.07	0.07
4	0.14			0.97			0.09			0.10			0.11			0.02			0.09			0.11			0.11		
5	0.11	0.10	0.10	1.04	1.00	1.00	0.13	0.12	0.12	0.07	0.07	0.07	0.07	0.07	0.07	0.01	0.01	0.01	0.13	0.12	0.12	0.07	0.07	0.07	0.07	0.07	0.07
7	0.11	0.18	0.18	1.02	1.03	1.05	0.08	0.14	0.13	0.06	0.11	0.10	0.06	0.11	0.09	0.01	0.02	0.02	0.07	0.14	0.15	0.05	0.11	0.10	0.05	0.11	0.09
8	0.11	0.12	0.13	0.88	1.09	0.95	0.07	0.08	0.09	0.07	0.07	0.06	0.05	0.08	0.08	0.01	0.01	0.01	0.08	0.08	0.10	0.07	0.07	0.07	0.06	0.07	0.08
9	***0.17***	***0.13***	***0.08***	***1.15***	***0.74***	***0.93***	***0.08***	***0.07***	***0.05***	***0.08***	***0.06***	***0.07***	***0.09***	***0.04***	***0.08***	***0.01***	***0.01***	***0.01***	***0.09***	***0.05***	***0.03***	***0.09***	***0.05***	***0.05***	***0.08***	***0.04***	***0.06***
10	0.10	0.09	0.11	1.26	1.23	1.17	0.11	0.11	0.11	0.19	0.16	0.15	0.18	0.14	0.13	0.02	0.02	0.02	0.10	0.09	0.10	0.19	0.15	0.16	0.16	0.13	0.13
11	***0.12***	***0.13***	0.11	1.02	***1.34***	1.04	0.07	***0.08***	0.09	0.05	***0.06***	0.06	0.05	0.05	0.06	***0.01***	***0.01***	0.01	0.08	0.07	0.08	0.05	0.04	0.06	0.05	0.04	0.06
12	***0.09***	***0.10***	***0.08***	0.88	1.04	***0.80***	0.09	0.09	***0.07***	0.08	0.09	***0.07***	0.07	0.10	***0.07***	***0.01***	***0.01***	***0.01***	0.08	0.09	***0.06***	0.08	0.08	***0.05***	0.07	0.08	***0.05***
1	0.11	0.11	0.11	0.91	0.97	0.91	0.09	0.08	0.07	0.08	0.08	0.07	0.08	0.08	0.07	0.01	0.01	0.01	0.08	0.08	0.08	0.07	0.07	0.07	0.07	0.07	0.07
2	0.12	0.09	0.11	1.00	0.82	1.07	0.08	0.07	0.08	0.07	0.06	0.07	0.07	0.06	0.07	0.01	0.01	0.01	0.08	0.07	0.09	0.06	0.06	0.06	0.08	0.06	0.07
3	0.09	0.10	0.10	1.00	1.01	1.00	0.07	0.08	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.01	0.01	0.01	0.07	0.07	0.08	0.06	0.06	0.06	0.06	0.06	0.06
4	0.12	0.10	0.12	1.05	0.85	0.96	0.10	0.08	0.09	0.07	0.06	0.08	0.06	0.06	0.07	0.01	0.01	0.01	0.09	0.08	0.09	0.07	0.06	0.08	0.07	0.06	0.08
5	0.10	0.12	0.10	1.18	1.21	1.10	0.10	0.11	0.09	0.08	0.09	0.07	0.07	0.09	0.07	0.01	0.01	0.01	0.09	0.10	0.09	0.08	0.09	0.07	0.07	0.08	0.06
6	***0.07***	***0.09***	***0.09***	0.80	1.15	1.20	0.07	0.08	***0.12***	0.05	0.05	0.07	0.05	0.05	0.06	0.00	***0.01***	0.01	0.07	***0.08***	0.11	0.06	0.05	0.06	0.06	0.06	0.06
7	0.14	0.13	***0.12***	1.06	1.06	***0.84***	0.07	0.08	***0.07***	0.05	***0.05***	***0.05***	0.05	***0.05***	***0.05***	***0.01***	0.01	***0.01***	0.07	0.07	***0.07***	0.06	***0.04***	***0.04***	0.06	***0.04***	***0.05***
8	0.11	***0.07***	***0.09***	0.94	0.98	0.82	0.08	0.08	0.07	0.07	***0.06***	***0.05***	0.06	0.06	0.05	0.01	***0.01***	***0.01***	0.08	0.09	***0.09***	0.07	0.06	0.06	0.07	0.06	0.05
9	0.09	0.10	0.10	0.90	0.83	1.01	0.07	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06	***0.01***	0.01	***0.01***	0.07	0.08	0.08	0.06	0.06	0.06	0.06	0.06	0.06
10	0.09	0.09	0.09	1.06	1.16	1.07	0.10	***0.09***	***0.09***	0.08	0.07	0.07	0.07	0.06	0.08	***0.01***	***0.01***	***0.01***	0.08	0.08	0.08	0.08	0.06	0.08	***0.06***	0.06	0.07
11	***0.10***	***0.11***	0.10	0.95	1.09	1.07	0.07	0.07	0.09	0.05	0.05	0.07	0.05	0.04	0.06	***0.01***	***0.01***	***0.01***	0.08	0.07	0.08	0.06	0.05	0.06	0.06	0.05	0.06
12	0.11	0.12	***0.11***	0.93	1.09	0.91	0.07	0.10	0.08	0.05	0.07	0.05	0.06	0.07	0.05	***0.01***	***0.01***	***0.01***	***0.06***	0.08	0.06	0.05	0.06	***0.05***	0.05	0.06	0.05
13		0.11	0.11		1.05	1.05		0.10	0.09		0.07	0.07		0.07	0.07		0.01	0.01		0.09	0.09		0.07	0.06		0.07	0.07
15	***0.11***	0.11	0.09	1.01	0.96	1.01	0.09	0.07	0.08	0.07	0.07	0.07	0.07	0.07	0.07	***0.01***	***0.01***	***0.01***	0.08	0.08	0.07	0.07	0.06	0.06	0.06	0.08	0.08

Potencies marked in **purple** are from assays where at least one of the test or reference samples are non-linear
Potencies marked in **red** are from assays where the test and reference samples are non-parallel

Relative potency estimates by assay, with 18007 as a reference (shaded cells are in-house assays)

Lab	EV71 NS			S18001			S18002			S18003			S18004			S18005			S18006			S18008			S18009		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	1.89	1.80	1.73	11.56	12.93	11.32	1.06	1.02	1.02	0.93	0.93	0.93	0.98	1.00	1.04	0.13	0.13	0.13	11.47	11.53	10.14	0.96	0.87	0.82	0.96	0.93	0.88
2	1.32	1.46	1.33	10.84	11.96	11.98	1.02	0.94	1.01	0.92	0.88	0.88	0.97	0.85	0.87	0.11	0.12	0.12	10.42	11.57	10.60	0.82	0.96	0.91	0.96	0.84	0.99
3	0.92	0.83	0.81	8.55	7.69	7.65	1.06	1.00	0.96	0.60	0.57	0.54	0.62	0.59	0.56	0.07	0.07	0.07	8.12	7.27	7.81	0.59	0.53	0.54	0.59	0.54	0.53
4	1.54			10.76			0.99			1.16			1.23			0.18			10.75			1.17			1.17		
5	0.89	0.86	0.86	8.33	8.41	8.28	1.03	1.03	1.03	0.58	0.58	0.61	0.57	0.56	0.58	0.07	0.08	0.07	7.98	8.41	8.26	0.59	0.61	0.57	0.59	0.60	0.55
7	1.62	1.28	1.23	14.52	7.05	7.01	1.17	0.93	0.87	0.87	0.75	0.65	0.85	0.71	0.64	0.11	0.11	0.11	14.18	6.93	6.81	0.75	0.76	0.68	0.78	0.71	0.62
8	1.33	1.55	1.40	10.83	14.23	10.20	0.91	1.10	0.96	0.81	0.93	0.68	***0.62***	1.02	0.86	***0.10***	***0.07***	0.10	12.16	12.94	10.49	0.82	0.85	0.73	0.77	0.94	0.87
9	***1.81***	1.99	***1.08***	***12.30***	***15.61***	***27.28***	0.93	1.14	***1.24***	0.93	1.02	***1.45***	0.99	0.99	***1.59***	***0.10***	0.14	***0.07***	***11.57***	***20.28***	***38.38***	1.01	***1.10***	***1.40***	0.88	***0.95***	***1.43***
10	0.96	0.97	0.99	12.97	14.11	12.26	1.16	1.20	1.12	1.98	1.80	1.58	1.81	1.60	1.36	0.25	0.23	0.23	10.11	11.38	10.08	1.96	1.74	1.69	1.63	1.46	1.38
11	***1.64***	***1.96***	1.25	13.65	***19.96***	12.26	0.93	***1.21***	1.02	0.63	***0.91***	0.73	0.70	0.79	0.69	***0.11***	***0.11***	0.13	13.15	14.63	11.96	0.70	0.59	0.75	0.67	0.60	0.68
12	***1.07***	***1.13***	***1.48***	10.37	11.55	12.94	1.09	1.01	1.17	0.96	0.98	1.12	0.86	1.09	1.08	***0.11***	***0.13***	0.12	11.90	11.02	***15.92***	0.91	0.92	0.85	0.84	0.90	0.88
1	1.36	1.39	1.34	11.49	12.18	11.57	1.12	1.06	0.95	1.00	0.96	0.88	0.98	0.95	0.87	0.13	0.14	0.12	12.78	12.56	12.73	0.89	0.83	0.84	0.87	0.84	0.90
2	1.43	1.26	1.21	12.22	10.97	11.40	1.02	0.91	0.84	0.82	0.77	0.72	0.82	0.77	0.71	0.12	0.11	0.12	12.30	13.53	10.64	0.79	0.76	0.64	0.97	0.89	0.71
3	1.28	1.33	1.28	13.72	13.79	13.42	1.01	1.03	1.01	0.87	0.88	0.87	0.77	0.82	0.88	0.12	0.12	0.12	13.60	13.39	13.33	0.83	0.88	0.87	0.77	0.77	0.78
4	1.30	1.20	1.37	11.09	10.23	10.61	***1.07***	0.98	0.97	***0.71***	0.71	0.86	0.68	0.73	0.83	0.09	0.12	0.13	10.64	12.18	10.99	0.72	0.74	0.88	0.70	0.72	0.91
5	1.16	1.17	1.19	13.21	11.62	12.54	1.15	1.10	1.09	0.85	0.83	0.82	0.75	0.89	0.79	0.09	0.09	0.10	11.20	9.58	11.44	0.86	0.81	0.78	0.76	0.81	0.70
6	***1.04***	***0.98***	***0.81***	10.92	***14.08***	11.09	0.93	***0.98***	1.06	0.70	***0.62***	0.60	0.68	***0.59***	0.58	0.06	***0.06***	***0.04***	13.63	***12.03***	9.29	0.82	***0.60***	***0.56***	0.75	***0.64***	0.54
7	1.96	1.81	1.71	14.58	14.95	12.39	0.96	1.09	0.97	0.74	0.65	0.66	0.75	0.65	0.71	***0.14***	0.13	0.13	13.85	14.29	***14.45***	0.82	0.58	0.62	0.81	0.57	0.66
8	1.40	***0.99***	***1.13***	12.16	10.95	***9.38***	0.98	0.98	***0.79***	0.84	0.74	***0.56***	0.82	0.70	***0.59***	***0.12***	0.09	***0.08***	12.70	11.58	***10.92***	0.88	0.75	***0.68***	0.83	0.78	***0.61***
9	***1.29***	***1.27***	***1.30***	12.18	10.37	12.38	0.96	0.97	1.01	0.77	0.78	0.74	0.80	0.74	0.74	***0.12***	***0.10***	***0.12***	13.50	12.73	11.96	0.80	0.79	0.75	0.76	0.76	0.76
10	***1.21***	***1.15***	***1.13***	13.18	13.82	12.83	1.21	1.13	1.11	1.02	0.80	0.89	0.93	0.70	0.93	***0.08***	***0.11***	***0.12***	12.49	12.04	12.13	0.93	0.73	0.91	***0.76***	0.69	0.90
11	***1.41***	***1.56***	***1.23***	12.32	***16.02***	12.61	0.92	0.99	1.08	0.69	0.69	0.80	0.69	0.62	0.74	***0.12***	***0.11***	***0.15***	12.73	14.42	11.93	0.74	0.65	0.77	0.71	0.64	0.71
12	***1.76***	***1.63***	1.84	***14.58***	14.39	14.63	***1.10***	1.33	1.21	***0.83***	0.95	0.88	***0.88***	0.92	0.85	***0.14***	***0.15***	***0.14***	***15.76***	13.04	15.97	***0.74***	0.76	0.76	***0.77***	0.84	0.77
13		1.21	1.21		11.18	11.62		1.08	1.01		0.78	0.79		0.71	0.76		0.11	0.12		10.58	11.07		0.71	0.71		0.75	0.74
15	***1.39***	1.42	1.31	12.23	12.64	14.52	1.07	0.91	1.07	0.81	0.92	0.95	0.87	0.96	0.97	***0.12***	***0.13***	***0.14***	11.94	13.13	14.35	0.79	0.79	0.86	0.78	0.98	1.13

Potencies marked in **purple** are from assays where at least one of the test or reference samples are non-linear
Potencies marked in **red** are from assays where the test and reference samples are non-parallel

Relative potency estimates by assay, with 18008 as a reference (shaded cells are in-house assays)

Lab	EV71 NS			S18001			S18002			S18003			S18004			S18005			S18006			S18007			S18009		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	1.96	2.06	2.10	11.99	14.83	13.65	1.10	1.17	1.25	0.97	1.07	1.13	1.02	1.15	1.27	0.13	0.14	0.15	11.91	13.21	12.26	1.04	1.15	1.22	1.00	1.08	1.08
2	1.62	1.55	1.46	13.35	12.60	13.19	1.26	0.98	1.11	1.13	0.91	0.97	1.19	0.88	0.95	0.14	0.13	0.14	12.66	12.15	11.58	1.22	1.04	1.09	1.18	0.87	1.08
3	1.58	1.59	1.51	14.70	14.45	14.26	1.81	1.89	1.80	1.02	1.07	1.01	1.06	1.10	1.05	0.12	0.13	0.12	13.88	13.72	14.55	1.71	1.88	1.86	1.00	1.01	1.00
4	1.36			9.53			0.85			1.00			1.05			0.15			9.44			0.85			0.99		
5	1.53	1.43	1.52	14.09	13.64	14.52	1.73	1.66	1.80	0.97	0.94	1.06	0.96	0.91	1.01	0.12	0.12	0.13	13.45	13.72	14.45	1.69	1.63	1.75	0.99	0.99	0.97
7	2.19	1.72	1.84	19.54	9.00	10.33	1.58	1.21	1.28	1.14	0.99	0.94	1.12	0.93	0.94	0.15	0.15	0.17	19.15	8.92	10.11	1.34	1.32	1.47	1.05	0.94	0.91
8	1.57	1.78	1.87	12.96	16.51	13.71	1.10	1.27	1.30	0.98	1.09	0.91	***0.74***	1.18	1.17	0.12	0.09	0.13	14.70	15.15	14.34	1.22	1.17	1.37	0.94	1.10	1.19
9	1.84	***1.81***	***1.12***	***11.50***	***14.89***	***17.48***	***0.93***	***1.06***	***0.93***	0.92	***0.93***	***1.14***	0.98	***0.89***	***1.25***	0.12	***0.12***	***0.08***	***10.86***	***19.97***	***21.55***	0.99	***0.91***	***0.71***	0.88	***0.85***	***1.07***
10	0.44	0.50	0.55	6.82	8.25	7.71	0.61	0.67	0.64	1.07	1.07	0.96	0.95	0.93	0.81	0.12	0.13	0.13	5.16	6.52	6.18	0.51	0.58	0.59	0.85	0.85	0.83
11	***2.15***	***2.91***	1.64	18.70	***30.45***	16.42	1.32	***1.86***	1.38	0.90	***1.39***	1.00	0.94	***1.22***	0.94	***0.14***	0.17	0.18	18.39	24.36	15.85	1.43	1.69	1.34	0.96	1.01	0.91
12	***1.21***	***1.23***	***1.67***	11.46	12.49	14.98	1.19	1.09	1.38	1.05	1.06	1.31	0.95	1.18	1.27	***0.12***	***0.14***	0.13	13.11	11.92	***18.94***	1.10	1.08	1.17	0.93	0.97	1.02
1	1.50	1.66	1.61	12.74	14.63	13.82	1.24	1.27	1.14	1.11	1.15	1.05	1.08	1.14	1.04	0.15	0.17	0.15	14.22	15.06	15.20	1.12	1.20	1.19	0.97	1.01	1.08
2	1.81	1.65	1.85	15.32	14.28	17.40	1.27	1.17	1.28	1.03	0.99	1.09	1.03	1.00	1.08	0.15	0.14	0.17	15.54	17.83	16.51	1.26	1.32	1.55	1.23	1.20	1.09
3	1.55	1.53	1.49	16.79	16.02	15.75	1.22	1.18	1.17	1.05	1.01	1.01	0.92	0.94	1.03	0.14	0.14	0.14	16.59	15.48	15.63	1.20	1.14	1.16	0.92	0.88	0.91
4	1.84	1.58	1.57	16.32	13.56	12.20	***1.65***	1.31	1.10	***1.09***	0.95	0.98	***1.02***	0.99	0.95	0.13	0.16	0.14	15.26	16.13	12.67	1.39	1.35	1.14	1.03	0.97	1.03
5	1.35	1.44	1.52	15.50	14.19	16.31	1.34	1.34	1.40	0.98	1.02	1.05	0.86	1.09	1.01	0.11	0.11	0.13	13.08	11.74	14.83	1.16	1.23	1.28	0.89	0.99	0.89
6	***1.19***	***1.49***	***1.48***	13.23	22.04	20.15	1.16	1.56	***1.92***	0.87	1.02	1.14	0.84	0.97	1.05	0.08	***0.10***	0.10	16.53	18.98	16.92	1.22	***1.66***	***1.79***	0.91	1.06	0.98
7	2.44	***3.02***	2.70	17.99	***24.60***	19.57	1.19	1.91	1.52	0.91	1.12	1.08	0.92	1.14	1.15	***0.18***	***0.20***	0.21	17.00	***23.29***	***23.40***	1.23	1.73	1.62	1.00	0.98	1.10
8	1.58	***1.25***	***1.45***	13.81	14.69	13.21	1.12	1.30	1.13	0.96	0.95	0.79	0.93	0.93	0.83	0.14	0.11	***0.11***	14.53	16.03	16.10	1.14	1.33	***1.47***	0.95	1.02	0.87
9	1.56	1.55	***1.63***	15.21	13.11	16.04	1.21	1.22	1.32	0.97	0.98	0.98	0.99	0.92	0.96	***0.14***	***0.12***	***0.15***	16.85	15.90	15.83	1.25	1.27	1.33	0.95	0.97	1.00
10	***1.25***	1.51	***1.22***	14.08	18.95	14.13	1.33	1.55	1.22	1.11	1.10	0.98	1.00	0.99	1.03	***0.08***	***0.15***	***0.13***	13.27	16.33	13.31	1.08	1.36	1.10	***0.81***	0.94	0.99
11	***1.72***	***2.08***	1.55	16.06	23.16	16.53	1.25	1.45	1.42	0.93	1.04	1.06	0.91	0.94	0.99	***0.15***	***0.17***	***0.19***	16.97	21.88	15.44	1.35	1.54	1.31	0.97	0.99	0.92
12	***2.19***	2.03	2.40	19.01	18.53	19.35	1.43	1.72	***1.60***	1.10	1.25	1.18	1.19	1.22	1.13	***0.17***	***0.18***	***0.17***	20.60	16.99	***21.54***	***1.35***	1.31	1.31	1.02	1.10	1.04
13		1.68	1.69		15.72	16.38		1.46	1.42		1.10	1.10		0.99	1.06		0.15	0.17		14.90	15.65		1.40	1.41		1.05	1.04
15	***1.67***	***1.70***	1.50	15.28	15.63	16.83	1.37	1.17	1.25	1.05	1.19	1.11	1.10	1.25	1.13	***0.15***	***0.15***	***0.16***	15.08	16.38	16.67	1.26	1.27	1.16	0.98	1.27	1.31

Potencies marked in **purple** are from assays where at least one of the test or reference samples are non-linear
Potencies marked in **red** are from assays where the test and reference samples are non-parallel

Relative potency estimates by assay, with 18009 as a reference (shaded cells are in-house assays)

Lab	EV71 NS			S18001			S18002			S18003			S18004			S18005			S18006			S18007			S18008		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	1.96	1.92	1.95	12.02	13.80	12.68	1.10	1.09	1.16	0.97	0.99	1.05	1.02	1.07	1.18	0.13	0.13	0.14	11.93	12.27	11.37	1.04	1.07	1.13	1.00	0.93	0.93
2	1.38	1.75	1.35	11.29	14.39	12.16	1.06	1.12	1.03	0.96	1.06	0.89	1.01	1.02	0.88	0.11	0.15	0.13	10.82	13.85	10.73	1.04	1.20	1.01	0.85	1.15	0.93
3	1.58	1.56	1.53	14.64	14.29	14.34	1.80	1.87	1.81	1.02	1.06	1.01	1.06	1.09	1.06	0.12	0.13	0.12	13.83	13.56	14.62	1.70	1.86	1.87	1.00	0.99	1.00
4	1.36			9.47			0.86			1.01			1.06			0.16			9.36			0.86			1.01		
5	1.54	1.44	1.56	14.16	13.82	14.96	1.74	1.68	1.85	0.98	0.96	1.09	0.97	0.93	1.04	0.12	0.13	0.13	13.51	13.89	14.89	1.70	1.65	1.80	1.01	1.01	1.03
7	2.10	1.81	2.01	18.70	9.53	11.27	1.52	1.28	1.40	1.08	1.05	1.04	1.07	0.98	1.03	0.14	0.15	0.18	18.33	9.43	11.00	1.28	1.40	1.61	0.96	1.06	1.10
8	1.65	1.64	1.59	13.76	15.05	11.65	1.17	1.15	1.10	1.05	0.99	0.77	*0.78*	1.07	0.98	0.13	0.08	0.11	15.68	13.76	12.08	1.30	1.06	1.15	1.07	0.91	0.84
9	2.03	*2.03*	*1.29*	*13.83*	*18.74*	*14.74*	1.08	*1.21*	*0.88*	1.06	*1.07*	*1.07*	1.12	*1.04*	*1.17*	0.13	*0.13*	*0.09*	*13.21*	*26.16*	*17.17*	1.14	*1.05*	*0.70*	1.14	*1.18*	*0.94*
10	0.54	0.61	0.69	8.18	9.78	9.30	0.72	0.80	0.79	1.24	1.25	1.15	1.11	1.09	0.98	0.15	0.15	0.16	6.30	7.82	7.59	0.61	0.69	0.72	1.17	1.18	1.20
11	*2.21*	*2.89*	1.78	*19.48*	*29.99*	17.97	1.38	*1.84*	1.52	0.94	*1.37*	1.10	0.97	*1.20*	1.03	0.14	0.17	0.19	19.20	23.98	17.30	1.50	1.66	1.46	1.05	0.99	1.09
12	*1.29*	*1.26*	1.68	12.32	12.83	14.73	1.29	1.12	1.34	1.14	1.09	1.27	1.03	1.21	1.23	*0.13*	*0.14*	0.14	14.10	12.25	*18.42*	1.19	1.11	1.14	1.08	1.03	0.98
1	1.55	1.66	1.51	13.16	14.57	12.91	1.28	1.26	1.05	1.14	1.14	0.97	1.12	1.13	0.96	0.16	0.16	0.13	14.71	15.02	14.26	1.16	1.19	1.11	1.03	0.99	0.92
2	1.50	1.45	1.68	12.70	12.42	15.84	1.05	*1.01*	1.18	0.84	*0.84*	1.01	0.84	*0.84*	0.99	0.12	*0.12*	0.16	12.80	15.75	15.00	1.03	1.13	1.41	0.81	0.83	0.92
3	1.67	1.75	1.66	17.92	18.47	17.67	1.31	1.35	1.30	1.14	1.15	1.12	1.00	1.07	1.14	0.15	0.16	0.16	17.69	17.76	17.52	1.29	1.30	1.27	1.08	1.14	1.10
4	1.72	1.63	1.52	15.94	13.99	11.81	1.58	1.35	1.06	1.02	0.98	0.95	0.97	1.02	0.92	0.10	0.17	0.14	15.22	16.62	12.25	1.44	1.39	1.10	0.97	1.03	0.97
5	1.54	1.45	1.70	17.69	14.27	18.26	1.53	1.34	1.57	1.10	1.03	1.18	0.97	1.10	1.13	0.12	0.12	0.14	14.86	11.82	16.61	1.31	1.24	1.43	1.13	1.01	1.12
6	*1.31*	*1.42*	*1.46*	14.50	20.84	20.69	1.26	1.48	*2.00*	0.97	0.96	1.17	0.93	0.91	1.08	0.09	*0.09*	0.09	18.15	17.93	17.26	1.33	*1.56*	1.87	1.10	0.94	1.02
7	2.45	3.11	*2.50*	18.08	25.43	*17.70*	1.20	1.94	*1.36*	0.92	1.15	0.99	0.93	1.16	1.06	*0.18*	0.21	0.20	17.07	*24.09*	*21.41*	1.23	1.77	1.52	1.00	1.03	0.91
8	1.67	1.25	*1.63*	14.57	14.01	15.14	1.18	1.26	1.28	1.01	0.94	0.90	0.98	0.91	0.96	0.15	0.12	*0.14*	15.38	15.52	18.50	1.20	1.29	*1.64*	1.06	0.98	1.15
9	1.65	1.59	1.65	16.07	13.55	16.11	1.27	1.26	1.32	1.02	1.01	0.99	1.05	0.95	0.96	0.15	*0.12*	*0.15*	17.74	16.41	15.91	1.31	1.31	1.32	1.06	1.03	1.00
10	*1.47*	1.60	*1.22*	*17.21*	20.20	14.21	*1.66*	*1.66*	1.23	*1.37*	1.18	0.99	*1.22*	1.06	1.03	*0.09*	*0.15*	*0.13*	*16.09*	17.35	13.38	*1.32*	1.45	1.11	*1.24*	1.06	1.01
11	*1.74*	*2.09*	1.68	16.43	23.31	17.93	1.30	1.46	1.54	0.95	1.05	1.16	0.93	0.94	1.08	*0.15*	*0.17*	*0.20*	17.42	22.03	16.71	1.40	1.55	1.41	1.03	1.01	1.08
12	2.17	*1.87*	2.29	18.39	16.93	18.85	1.36	1.57	1.60	1.07	1.13	1.14	1.15	1.11	1.09	*0.17*	*0.17*	*0.16*	19.80	15.45	21.18	*1.29*	1.19	1.29	0.98	0.91	0.96
13		1.61	1.62		14.96	15.72		1.38	1.37		1.04	1.06		0.94	1.02		0.15	0.17		14.19	15.01		1.33	1.36		0.95	0.96
15	*1.70*	*1.41*	1.17	15.62	12.69	12.83	1.40	0.92	0.95	1.07	0.94	0.84	1.13	0.98	0.87	*0.15*	*0.13*	*0.12*	15.44	13.22	12.67	1.29	1.02	0.89	1.02	0.79	0.76

Potencies marked in **purple** are from assays where at least one of the test or reference samples are non-linear
Potencies marked in **red** are from assays where the test and reference samples are non-parallel