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**Development, production and characterisation of the proposed 1st WHO
International Standard for Antibodies to *Shigella* serotypes *S. flexneri* 2a,
3a, 6 and *S. sonnei***

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**Listed in Appendix 1*

NOTE:

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

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

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Summary

We report here the development of a candidate standard intended for the calibration of immunoassays that measure antibody levels against four epidemiologically relevant *Shigella* serotypes: *S. flexneri* 2a, 3a, 6 and *S. sonnei*. The candidate standard was produced from a pool of 28 human serum samples collected from individuals who participated in a clinical trial involving a tetravalent bioconjugate vaccine targeting the above serotypes. The serum pool was filled into ampoules and lyophilised, resulting in a homogeneous batch with a good stability profile. Results obtained from an international collaborative study involving expert *Shigella* laboratories in countries across 5 WHO regions suggest that the candidate standard, coded 25/160, is fit for purpose and that results expressed relative to the candidate standard improve the agreement between laboratories (compared to results expressed as end-point titres). Product coded 25/160 is therefore recommended for establishment as the 1st WHO International Standard for Antibodies to *Shigella* serotypes *S. flexneri* 2a, 3a, 6 and *S. sonnei*, with assigned values of 50 IU per ampoule for each of the four serotypes. A separate product, coded 25/170, was prepared from a pool of 2 human serum samples from the same clinical trial and was confirmed to have low but detectable responses against all 4 serotypes. Product coded 25/170 is recommended for establishment as a WHO International Reference Reagent for Antibodies to *Shigella* serotypes *S. flexneri* 2a, 3a, 6 and *S. sonnei* (low positive).

1. Introduction

Shigellosis is an acute diarrhoeal disease caused by Gram negative bacilli of the *Shigella* genus. The global burden of disease is high, especially in children under 5 in low- and middle-income countries [1]. Treatment is focused on hydration and restoration of electrolyte balance. Antibiotics are recommended for children presenting with dysentery, but increasing antimicrobial resistance of *Shigella* spp. resulted in a move from the “medium” to “high” priority category in the 2024 update of the WHO Bacterial Priority Pathogens List [2]. The Global Enteric Multicentre Study investigated the burden and aetiology of moderate-to-severe diarrhoeal illness over 3 years at multiple sites in Africa and Asia and found that nearly 90% of case isolates were attributable to *S. flexneri* or *S. sonnei*, and suggested that a multivalent vaccine targeting serotypes from these species might provide broad coverage against global disease [3].

There is currently no licensed vaccine for *Shigella*, although several vaccine candidates are in clinical development [4] and WHO has published its preferred product characteristics for vaccines against *Shigella* [5]. Measurement of antigen-specific serum IgG is a cornerstone of sample analysis in vaccine trials, and data from epidemiological studies and vaccine efficacy trials have provided strong evidence that anti-lipopolysaccharide (LPS) serum IgG is associated with protection from disease [6, 7, 8]. However, different approaches to measurement of antigen-specific antibody responses make it difficult to compare between laboratories and highlight the need for standardisation in this area [9]. The WHO Expert Committee on Biological Standardisation endorsed a proposal to develop a serum standard for *Shigella* immunoassays at its 66th meeting held in Geneva in 2015 [10]. Since then, the Gates Foundation has convened a number of workshops where *Shigella* experts involved in vaccine development and immunogenicity assays have further highlighted the need for standardisation in this area [unpublished meeting notes].

To develop the serum standard, we obtained large volume bleeds from 37 healthy adults who were participants in a Phase 2b, double-blind, placebo-controlled efficacy challenge study conducted in the USA using a tetravalent bioconjugate *Shigella* vaccine targeting 4 *Shigella* serotypes: *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6 and *S. sonnei*. The blood samples used for production of the candidate preparation were taken 28 days after injection in step 1 of the clinical trial (prior to any challenge step). This report describes the use of this material to produce the candidate standard together with the results of an international multi-laboratory collaborative study that was conducted to evaluate its performance in immunoassays used by expert *Shigella* laboratories worldwide.

2. Bulk Material

The source material used to produce the candidate standard was obtained from healthy adults and was tested at MHRA and shown to be negative for antibodies to HIV, HBsAg and HCV RNA. The source material was provided as serum, and MHRA was blinded to the treatment received (placebo, low dose vaccine or high dose vaccine). All 37 samples were tested at MHRA to estimate anti-LPS IgG titres for the 4 serotypes present in the vaccine to help inform the pooling strategy for production of the candidate standard. This was done using an ELISA protocol obtained from the Walter Reed Army Institute of Research (WRAIR) [9]. LPS from each serotype was used as coating antigen and was provided by WRAIR. The LPS used by MHRA was obtained from the following strains: *S. flexneri* 2a (2457T), *S. flexneri* 3a (J17B), *S. flexneri* 6 (SSU2415) and *S. sonnei* (Moseley). Results were expressed as end point titres (EPT, defined as the highest serum dilution giving an O.D 405 nm $\geq$ 0.2)

3. Processing of the candidate WHO International Biological Reference Standard(s)

Of the 37 samples that were screened at MHRA, 9 samples had very low end point titres for at least 2 of the 4 serotypes (EPT $\leq$ 300). These 9 samples were not considered for inclusion in a large volume pool for preparation of the candidate standard. However, 2 of the samples which were very low across all 4 serotypes were used to prepare a low positive control. Twenty-eight samples were selected for pooling to produce the candidate standard. Aliquots were taken from some samples for use in the collaborative study sample panel (and potentially in future studies that will focus on functional assays). To prepare the large volume pool, the volumes for each sample were not equal (min. 63 mL and max. 109 mL) and a volume-adjusted mini-pool was prepared to match the relative amounts of each donation that were to be used for the large volume pool. Prior to preparation of the large volume pool, this mini-pool was tested in all 4 LPS ELISAs to confirm that a suitable dose response (significant regression and a curve plateauing at the upper and lower asymptotes of the response range) was obtained for each serotype. A total volume of ~2.4L was obtained after pooling the 28 selected serum samples.

Serum samples were removed from storage at -80°C two days before filling and held at +2 to +8°C. On the day before filling, the samples were pooled in a sterile vessel and filtered using a positive pressure filtration system through sequential filters of decreasing pore size (1 μ M and 0.22 μ M). The pooled serum was filled into 2.5 mL DIN type I glass ampoules with a target fill weight of 0.5 g per ampoule. Filling was performed at the MHRAs Centre for Biological Reference Materials on an AVF5090 filling line (Bausch & Stroebel, Ilshofen, Germany) in August 2025 with on-line check-weighing of a proportion of the filled ampoules. Filled ampoules were partially stoppered with halobutyl 13mm diameter igloo closures and lyophilised in a CS100 freezer dryer (Serail, Le Coudray Saint Germer, France). Freezing was performed at -50°C for 2 hours then held for a further 2 hours before applying vacuum. Primary drying was performed at -35°C for 41 hours at 100 μ bar vacuum, followed by a ramp to 25°C for 10 hours, then 30 hours of secondary drying at 25°C and 30 μ bar vacuum. Ampoules were back filled with dry nitrogen to atmospheric pressure, stoppered in the dryer before removal and flame sealed. The sealed ampoules were then stored at -20°C under continuous temperature monitoring. A total of 4,412 ampoules were filled. The candidate standard was coded 25/160

and stored at -20°C. The 2 serum samples with low titres across all 4 serotypes were pooled to prepare a low-positive control preparation which was prepared, filled and lyophilised as described for 25/160, but with a lower target fill weight of 0.25 g per ampoule. A total of 483 ampoules were filled and the low-positive preparation was coded 25/170.

3.1 Quality control testing of the candidate standard

The freeze-dried product, 25/160, was successfully lyophilised forming a robust, off-white cake. Fill precision was assessed by weighing ampoules that were sampled throughout the filling run. A total of 165 ampoules were weighed, with a mean fill mass of 0.52 g and a coefficient of variation of 0.53%. Residual water activity and oxygen content were measured after sealing as indicators of successful freeze-drying and ampoule integrity, respectively. Water activity was measured non-invasively by frequency modulated spectroscopy (FMS) using a FMS-1400H Water Activity Headspace Analyzer (Lighthouse Instruments, Charlottesville, VA, USA), calibrated at ambient temperature against a saturated solution of NaCl to give the equilibrium relative humidity (% ERH). The mean ERH measured in 12 ampoules of 25/160 was 1.33%. Oxygen content was measured non-invasively by frequency modulated infra-red spectroscopy using an FMS-760 Oxygen Headspace Analyzer (Lighthouse Instruments, Charlottesville, VA, USA). The mean oxygen headspace measured in 12 ampoules of 25/160 was 0.16%. Microbial bioburden testing for bacterial and mould/yeast contamination was performed on pre-fill, post-fill and lyophilised samples and no bioburden was detected (0 cfu/mL) for all tested samples. A full summary of the quality control test results is shown in Table 1. The summary of the quality control test results for the low-positive preparation 25/170 is also shown in Table 1.

3.2 Assessment of homogeneity for 25/160

The precision of fill provides good evidence for the homogeneity of the batch in terms of the amount filled per ampoule. For 25/160, the precision of fill (CV of the mean filling mass) was 0.53%, which is considered to be acceptable for a pooled serum standard with a fill volume of 0.5 mL per ampoule.

To provide further assurance regarding homogeneity of the batch, anti-LPS IgG titres for all 4 serotypes were measured in 9 ampoules that were taken from the beginning (n=3), middle (n=3) and end (n=3) of the production run. Each set of 3 ampoules was tested on a separate ELISA plate using a randomised plate layout. The pre-fill mini-pool was included on each plate as a positive control and potency estimates for all ampoules were calculated relative to this sample using EDQM software CombiStats™ v1.2.2 [11]. Geometric mean potency estimates based on fill position are summarised in Table 2. For all antigens, further analysis of these results gave negative estimates of the uncertainty of measurement component for ampoule heterogeneity, indicating that no heterogeneity could be detected in these assays, further supporting the homogeneity of the batch.

4. Stability Studies

An accelerated thermal degradation study was conducted to predict the long-term stability of 25/160 and to inform decisions related to shipment of the product at ambient temperature. Stability assessment was conducted using antibodies against one serotype as a marker for

stability of the product overall. Ampoules of the candidate standard were stored at elevated temperatures of +4°C, +20°C, +37°C, +45°C and +56°C. Ampoules stored at -70°C served as the baseline for comparison. Ampoules were retrieved after 6 months of storage and held at -20°C until the day of testing. The levels of anti-*S. flexneri* 6 LPS IgG were then measured using the WRAIR ELISA.

Potencies relative to the -70°C sample were calculated by sigmoid curve analysis using CombiStats™ and are shown in Table 3. Long-term stability was predicted using the Arrhenius model for accelerated degradation studies [12] and the predicted loss for samples stored at -20°C was negligible (0.015% per year).

Furthermore, the results from this study showed no significant loss in binding activity up to +45°C after 6 months, supporting the conclusion that the candidate standard will be sufficiently stable during shipment at ambient temperatures. Stability after reconstitution was not assessed but can be assessed by end users of the standard.

5. Collaborative Study

A multi-laboratory collaborative study was run under the MHRA study code CS763. The aims of the study were to:

- Assess performance of the candidate standard, 25/160, in immunoassays that are currently being used by expert laboratories who are working on *Shigella*
- Assess commutability of 25/160 using a panel of clinical samples
- Confirm suitability of product coded 25/170 to serve as a low-positive control for *Shigella* immunoassays

5.1 Collaborative Study Participants

Laboratories were invited to participate based on their expertise and experience in performing immunoassays measuring *Shigella* antibody levels in human serum/plasma. Twenty-four laboratories agreed to participate. However, three laboratories withdrew from the study citing lack of resources and/or reagents to complete the required testing. One laboratory did not submit results to MHRA. The remaining participating laboratories were from 12 different countries representing 5 WHO regions: Austria (1 lab), Bangladesh (1), China (2), France (1), India (2), Israel (1), Italy (2), Kenya (2), Malawi (1), South Korea (1), UK (1), USA (5). One laboratory submitted results to MHRA late (caused by significant delays obtaining an import permit for study samples). This laboratory is included as a study participant, but their data was not received in time to be incorporated into the results and analysis in this report. All participating laboratories are referred to in this report by randomly allocated code number that is not related to the order of listing in Appendix 1. The participating laboratories included academic labs and research institutes, vaccine developers and manufacturers, national control / regulatory labs. A number of the participating labs are actively involved in the analysis of samples from *Shigella* vaccine clinical trials.

5.2 Collaborative Study Samples

In addition to the candidate standard (25/160), participants received a study sample panel consisting of 34 blinded samples. The panel was selected based on screening results obtained at MHRA and was intended to provide samples spanning a range from low to high IgG levels against the 4 serotypes of interest. The final panel included the following types of sample:

- 19 individual serum donations from the Phase 2b trial using the tetravalent *Shigella* vaccine, 4 of which were not included in the pool used to prepare the candidate standard and 15 of which were in that pool
- 6 WHO International Standards (human immunoglobulin or pooled serum or plasma) provided as reconstituted aliquots that had medium to high responses to at least 1 serotype of interest (based on pre-study screening done by MHRA)
- 5 serum samples from a Phase 2b challenge study that were collected from individuals who were challenged orally (*S. flexneri* 2a, strain 2457T) after receiving two doses of a monovalent synthetic carbohydrate-based *S. flexneri* 2a conjugate vaccine (NCT04078022)
- 1 pool of serum that was prepared using 20 individual serum samples collected from individuals in North Africa (Algeria, Morocco, Libya, Tunisia) provided by a

commercial supplier (Cerba Research); this pooled sample had medium to high responses against all 4 serotypes

- 1 duplicate of the candidate standard provided as a reconstituted sample to be included on every plate as a positive control
- 1 immunoglobulins-depleted human serum to be included on every plate as an analytical negative control
- 1 reconstituted sample of the low positive control (lyophilised product 25/170)

The full list of study samples with traceability for the identifiers used in this report is shown in Table 4. Due to limited volumes available for the 5 serum samples obtained from the *S. flexneri* 2a challenge trial (samples 28, 29, 30, 31, 32), only participants who were performing an assay for *flexneri* 2a and/or *sonnei* were provided with these samples resulting in a slightly smaller study panel for *flexneri* 3a and 6.

5.3 Collaborative Study Design

The study protocol is provided in Appendix 2 and was conducted under MHRA study code CS763. For each method contributed by a participating laboratory, participants were asked to perform three independent runs, using a fresh ampoule of the candidate standard for each test. Participants were provided with an additional ampoule of the candidate standard to allow for a preliminary assay, if desired. For the panel of test samples, a single aliquot of each serum sample was provided to use across all independent runs.

Participants were requested to run all samples in the same assay and were provided with a recommended plate layout and starting dilutions for ELISA. This recommended assay design required 4 x 96-well ELISA plates to complete 1 run. Participants were asked to perform serial titrations (or multiple dilutions) of all samples to enable the appropriate analysis to be performed by MHRA.

Participants were required to include a serial titration of the candidate standard, in duplicate, on every plate as well as sample 33 (the reconstituted duplicate of the candidate standard serving as a positive control) and sample 34 (serving as an analytical negative control). These were mandatory requirements. Participants had the flexibility to use a different plate layout or modified starting dilutions for samples in line with in-house methods if required.

A results-reporting sheet was provided for participants to record all essential information including the raw data from each run of each assay method they performed.

5.4 Collaborative Study Assay Methods

Assays used by the participants are summarized in Table 5. ELISA, detecting IgG antibodies, was the most commonly used method and a small number of laboratories performed a multiplex bead based assay (Luminex). The majority of laboratories performing ELISA used LPS as the coating antigen, and a small number of labs reported using the O-antigen for coating plates. The *Shigella* strains from which coating antigens were obtained are listed in Table 6a, and the key features of the ELISA methods used are summarised in Tables 6b and 6c.

Where laboratories performed more than one assay method, laboratory codes are followed by a letter indicating the different methods (e.g. Lab 24a, 24b).

5.5 Statistical Analysis

With the exception of laboratories 12 and 24a, an analysis of dose-response curves was performed for all assays in order to calculate potencies of study samples relative to the candidate standard. For most assays a sigmoid curve model with a $\log_{10}$ transformation of the assay response was applied. In a small number of cases where there were insufficient responses to fit a full curve, a parallel line model with log transformation of assay response using a linear section of the response range was used. Calculations were performed using CombiStats™.

Again, with exception of the ELISAs performed by laboratories 12 and 24a, samples were considered to give a valid estimate of relative potency if they showed a significant regression ($p < 0.05$), a visual assessment of the dose-response plot was satisfactory, and they gave a slope-ratio (relative to the candidate standard) within the range 0.70 – 1.43. If the positive control sample 33 did not give a valid estimate, or if it gave a relative potency estimate outside the range 0.70 – 1.43, then the entire plate was excluded and no estimates from that plate were calculated. In addition, for ELISA data, the negative control sample 34 was confirmed to give OD values below 0.2 before the plate was accepted for analysis. It should be noted that these validity criteria were intended for use in the analysis of data from this study only, in order to apply consistent criteria to all laboratories and obtain a set of relative potency estimates suitable for further evaluation. They should not be interpreted as suitable for routine use in assessing assay validity and may be overly stringent or lenient for some laboratories.

Laboratories 12 and 24a used an alternative assay design that did not test a full dilution series of the study samples on each plate and they reported results in EU/mL for all study samples from three assay runs. These EU/mL estimates were used to calculate the relative values shown in this report by expressing each sample result relative to that for the candidate standard. For laboratory 12, as EU/mL results were reported for individual dilutions of the candidate standard, these data were used to fit a dose-response curve for the standard ($\log$ EU/mL vs $\log$ dilution) and estimates for the study samples interpolated from the fitted curve. For laboratory 24a, EU/mL study sample estimates were simply divided by the corresponding estimate for the candidate standard, as estimates at individual dilutions were not available from the data returned. An assay run was considered valid if the positive control sample 33 gave a relative estimate within the range 0.70 – 1.43. No additional validity criteria, beyond those routinely applied by these labs as part of the assay SOP, were applied to individual samples.

Relative potency estimates were combined to generate an unweighted geometric mean (GM) potency for each laboratory, and these laboratory GMs were used to calculate overall unweighted geometric mean potencies. Variability between laboratories has been expressed using geometric coefficients of variation ($GCV = \{10^s - 1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates). Any log-transformed laboratory GM estimates identified as significant ($p < 0.05$) outliers using Grubbs' test are indicated in the results tables and were excluded from further analysis.

Due to the sensitivity of these summary statistics to outliers and anomalous results, overall estimates were also calculated as medians, and inter-laboratory variability was quantified as interquartile ranges (IQR), expressed as the fold-change UQ/LQ where UQ and LQ denote the upper and lower quartiles respectively. Calculations of median, LQ and UQ were performed on a log scale before being back-transformed to the original scale of measurement.

5.6 Collaborative Study Results

Most laboratories performed the three independent tests using the four-plate layout suggested in the study protocol. Laboratory 10 used seven plates per independent test, and Laboratory 17 used seven or eight plates (depending on serotype) per independent test to accommodate their routine control samples. Laboratory 12 and 24a followed their in-house assay design in which each test sample was tested at three dilutions (1:100, 1:4000, 1:160000). Each dilution was tested on a separate plate, with each dilution plate run in triplicate (a total of 9 plates per run). In addition, every plate included an internal standard serum dilution curve. In total, data from 1000 plates, with 6322 sample results, were returned to MHRA for independent analysis.

5.6.1 Assay validity

The assay validity breakdown by laboratory and serotype is shown in Table 7 and Table 8, and the overall summary is shown in Table 9. Data submitted by lab 16 were not suitable for dose-response curve analysis due to a lack of significant regression and/or non-parallelism with the candidate standard, and no further calculations were performed for this lab.

Across all laboratories, methods and serotypes 95% of plates were valid, and 88% of all sample results were valid. Almost all invalid exclusions were due to the results obtained for the positive control sample 33 (i.e. sample 33 was non-parallel or its relative potency was outside the acceptance criteria of 0.70 - 1.43), or to non-parallelism between the test sample and the candidate standard.

The vast majority of plates had a mean OD <0.2 for the analytical negative control sample 34. An OD value exceeding 0.2 was observed for sample 34 on 27 ELISA plates (4%). Only one of these plates (lab 10 Flex6 assay 1 plate 1) showed a significant regression for sample 34 and this plate was therefore excluded; the remaining 26 plates were accepted for further analysis. Laboratories 12 and 24a, who reported results in EU/mL, also found sample 34 to be below the limit of quantification for their assays. No significant regression was observed for sample 34 in the Luminex assays by laboratories 5, 7 and 14b.

5.6.2 Intra-laboratory variability

For each laboratory and sample where two or more valid estimates of relative potency were obtained, intra-laboratory variability was expressed as the ratio of maximum to minimum estimate obtained for the sample. A small number of cases (1.4%) where this value exceeded 8.0 were noted, and results for the corresponding laboratory and sample were excluded from further analysis. All other values are shown in Figure 1, with median values by laboratory and serotype shown in Table 10.

An acceptable level of intra-laboratory variability was evident from the calculated values. For Flex2a assays, 90% of calculated ratios (Figure 1) were less than 2.0, with similar outcomes for Flex3a and Flex6 assays (89% and 93% respectively). A higher level of intra-lab variability was noted for Sonnei assays, with 80% of ratios below 2.0 and higher median values as shown in Table 10. It was noted that a higher proportion of study samples had a relatively low potency in Sonnei assays compared with the other strains, which contributed to the higher levels of variability observed in the study.

5.6.3 Results from Flex 2a assays

Laboratory geometric mean relative potency estimates are summarised in Table 11 and Figure 2. Samples are ordered by increasing study median potency. Empty cells indicate that no valid estimates were obtained in that laboratory. Overall summary statistics including inter-laboratory variability are shown in Table 12. Eleven individual sample results were identified as outliers by Grubbs' test and were excluded from further analysis. Median relative potency estimates ranged from 0.038 (sample 3) to 6.419 (sample 12), and five samples had a study median relative potency <0.1 (i.e. the anti-*S. flexneri* 2a LPS IgG response was less than 10% of the response observed for the candidate standard). These five samples are highlighted in Figure 2 and were not included in the assessment of commutability (see below).

94% of the Interquartile Range (IQR) values were ≤ 4 and 69% of the IQR values were ≤ 2 . For samples with a study median relative potency >0.1 , the sample with the highest variability across laboratories was sample 16. No trends or grouping of results according to the type of sample (Table 4) were observed.

To provide an assessment of commutability using a 'calibration effectiveness' approach [13], laboratory geometric mean relative potency estimates were expressed as a percentage of overall study median values for each sample, as shown in Table 13 and Figure 3. Samples with a low median relative potency below 0.1 (i.e. the anti-*S. flexneri* 2a LPS IgG response was less than 10% of the response observed for the candidate standard) were excluded from this assessment. Across all samples, the laboratory geometric mean relative potency estimate was within 50-200% of the study median value in 89% of cases, as highlighted in Table 13. For this serotype, more than half of the laboratories obtained estimates within 50-200% for at least 90% of the samples for which a valid estimate was obtained. Results obtained by laboratory 12 showed fewer than 70% of samples with estimates within 50-200% of the study median value, most of which were samples with a low response, and it is noted that no validity criteria were applied to individual sample results for this lab.

For laboratories performing ELISAs including a full dilution series of all study samples, endpoint titres were defined as the final dilution giving an OD value greater than 0.2 at the specified absorbance. Summary statistics for these endpoint titres are shown in Table 14, together with summary statistics for relative potency estimates from the same laboratories. 85% of IQR values for relative potency estimates are less than, or equal to, the corresponding values for endpoint titres suggesting that the expression of results relative to the candidate standard improves agreement between laboratories. The study median end point titre for the candidate standard 25/160 was 8000. The study median end point titre for the low-positive 25/170 was 800.

5.6.4 Results from Flex 3a assays

Laboratory geometric mean relative potency estimates are summarised in Table 15 and Figure 4. Samples are ordered by increasing study median potency. Empty cells indicate that no valid estimates were obtained in that laboratory. Overall summary statistics including inter-laboratory variability are shown in Table 16. One individual sample result was identified as an outlier by Grubbs' test and was excluded from further analysis. Median relative potency estimates ranged from 0.065 (sample 5) to 4.909 (sample 14), and five samples had a study median relative potency <0.1 (i.e. the anti-*S. flexneri* 3a LPS IgG response was less than 10% of the response observed for the candidate standard). These five samples are highlighted in Figure 4 and were not included in the assessment of commutability (see below).

85% of Interquartile Range (IQR) values were ≤ 4 and 44% of the IQR values were ≤ 2 and the interlaboratory variability was generally higher than the variability observed for Flex 2a assays. For samples with a study median relative potency >0.1 , the samples with the highest variability across laboratories were sample 9 and 13. No trends or grouping of results according to the type of sample (Table 4) were observed.

To provide an assessment of commutability using a ‘calibration effectiveness’ approach, laboratory geometric mean relative potency estimates were expressed as a percentage of overall study median values for each sample, as shown in Table 17 and Figure 5. Samples with a low median relative potency below 0.1 were excluded from this assessment. Across all samples, the laboratory geometric mean relative potency estimate was within 50-200% of the study median value in 74% of cases as highlighted in Table 17. For this serotype, only three laboratories (lab 5, 17, 20) obtained estimates within 50-200% for at least 90% of the samples for which a valid estimate was obtained. Results obtained by six laboratories (lab 1, 7, 10, 12, 18, 19) showed less than 70% of samples with an estimate within 50-200% of the study median value.

For laboratories performing ELISAs including a full dilution series of all study samples, endpoint titres were defined as the final dilution giving an OD value greater than 0.2. Summary statistics for these endpoint titres are shown in Table 18, together with summary statistics for relative potency estimates from the same laboratories. 91% of IQR values for relative potency estimates are less than, or equal to, the corresponding values for endpoint titres suggesting that the expression of results relative to the candidate standard improves agreement between laboratories. The study median end point titre for the candidate standard 25/160 was 3200. The study median end point titre for the low-positive 25/170 was 400.

5.6.5 Results from Flex 6 assays

Laboratory geometric mean relative potency estimates are summarised in Table 19 and Figure 6. Samples are ordered by increasing study median potency. Empty cells indicate that no valid estimates were obtained in that laboratory. Overall summary statistics including interlaboratory variability are shown in Table 20. Four individual sample results were identified as outliers by Grubbs’ test and were excluded from further analysis. Median relative potency estimates ranged from 0.015 (sample 3) to 3.635 (sample 13) and six samples had a study median relative potency <0.1 (i.e. the anti-*S. flexneri* 6 LPS IgG response was less than 10% of the response observed for the candidate standard). These six samples are highlighted in Figure 6 and were not included in the assessment of commutability (see below).

81% of Interquartile Range (IQR) values were ≤ 4 and 48% of the IQR values were ≤ 2 suggesting similar levels of interlaboratory variability as observed for the Flex 3a assays. For samples with a study median relative potency >0.1 , the samples with the highest variability across laboratories were sample 4 and 9. No trends or grouping of results according to the type of sample (Table 4) were observed.

To provide an assessment of commutability using a ‘calibration effectiveness’ approach, laboratory geometric mean relative potency estimates were expressed as a percentage of overall study median values for each sample, as shown in Table 21 and Figure 7. Samples with a low median relative potency below 0.1 were excluded from this assessment. Across all samples, the laboratory geometric mean relative potency estimate was within 50-200% of the study

median value in 77% of cases as highlighted in Table 21. For this serotype, four laboratories (labs 1, 10, 17, 19) obtained estimates within 50-200% for at least 90% of the samples for which a valid estimate was obtained. Results obtained by five laboratories (labs 6, 9, 13, 14b, 18,) showed fewer than 70% of samples with an estimate within 50-200% of the study median value.

For laboratories performing ELISAs including a full dilution series of all study samples, endpoint titres were defined as the final dilution giving an OD value greater than 0.2. Summary statistics for these endpoint titres are shown in Table 22, together with summary statistics for relative potency estimates from the same laboratories. 81% of IQR values for relative potency estimates are less than, or equal to, the corresponding values for endpoint titres suggesting that the expression of results relative to the candidate standard improves agreement between laboratories. The study median end point titre for the candidate standard 25/160 was 16000. The study median end point titre for the low-positive 25/170 was 400.

5.6.6 Results from Sonnei assays

Laboratory geometric mean relative potency estimates are summarised in Table 23 and Figure 8. Samples are ordered by increasing study median potency. Empty cells indicate that no valid estimates were obtained in that laboratory. Overall summary statistics including inter-laboratory variability are shown in Table 24. Five individual sample results were identified as outliers by Grubbs' test and were excluded from further analysis. Median relative potency estimates ranged from 0.014 (sample 5) to 4.830 (sample 27), and twenty samples had a study median relative potency <0.1 (i.e. the anti-sonnei LPS IgG response was less than 10% of the response observed for the candidate standard) – a much higher proportion compared to the other serotypes. These twenty samples are highlighted in Figure 8 and were not included in the assessment of commutability (see below).

44% of Interquartile Range (IQR) values were ≤ 4 and 22% were ≤ 2 , suggesting much higher levels of interlaboratory variability compared to those observed for the other serotypes. For samples with a study median relative potency >0.1 , the samples with the highest variability across laboratories were samples 17 and 29. No trends or grouping of results according to the type of sample (Table 4) were observed.

To provide an assessment of commutability using a 'calibration effectiveness' approach, laboratory geometric mean relative potency estimates were expressed as a percentage of overall study median values for each sample, as shown in Table 25 and Figure 9. Samples with a low median relative potency below 0.1 were excluded from this assessment. Across all samples, the laboratory geometric mean relative potency estimate was within 50-200% of the study median value in 72% of cases, as highlighted in Table 25. For this serotype, five laboratories (lab 1, 5, 10, 14b, 24a) obtained estimates within 50-200% for at least 90% of the samples for which a valid estimate was obtained. Nearly all other labs had less than 70% of samples with an estimate within 50-200% of the study median value.

For laboratories performing ELISAs including a full dilution series of all study samples, endpoint titres were defined as the final dilution giving an OD value greater than 0.2. Summary statistics for these endpoint titres are shown in Table 26, together with summary statistics for relative potency estimates from the same laboratories. 75% of IQR values for relative potency estimates are less than or equal to the corresponding values for endpoint titres suggesting that the expression of results relative to the candidate standard improves agreement between

laboratories – although the improvement is slightly lower compared to that seen for the other serotypes. The study median end point titre for the candidate standard 25/160 was 13500. The study median end point titre for the low-positive 25/170 was 283.

5.6.7 Other data returned by study participants

Lab 5 returned results from an ELISA using IpaB protein as the coating antigen. The candidate standard, 25/160, performed well, giving a full dose-response curve, and all assays from this lab were valid (based on the outcomes for coded duplicate sample 33). Approximately 50% of the study samples gave valid estimates but the remaining samples had very low relative potencies causing significant non-parallelism. No further analysis was performed for this assay.

Labs 12 and 24a returned results (in EU/mL) from an ELISA for the *S. flexneri* 1b serotype and obtained results above the LOQ for nearly all study samples and the candidate standard 25/160. No further analysis was performed for this assay.

6. Discussion, Conclusions and Proposal

The candidate standard (25/160) was successfully lyophilised and is a homogeneous batch of ampoules with low moisture and oxygen content and a good stability profile. The product can be shipped at ambient temperature, reducing the costs for users of the standard. Stability assessment was conducted by measuring anti-LPS IgG responses to a single serotype because there is no expectation that antibodies specific to one serotype in a polyclonal serum sample will exhibit a different stability profile to antibodies against a different serotype. A further test will be conducted (to also include one additional serotype) at the 12-month time point, and this data will be available at the time of review of the project by the WHO ECBS. Product coded 25/170 was also successfully lyophilised. No assessment of stability or homogeneity was performed for 25/170 because of its intended use as a qualitative control material rather than a primary standard.

The results obtained from the multi-laboratory collaborative study showed that the candidate standard (25/160) gave a good dose response, for all 4 serotypes, in both ELISA and Luminex immunoassays. For the study samples, valid estimates of relative potency were obtained for 88% of the results obtained across all samples and assay runs. The validity criteria that were applied appear to be appropriate since 7 labs were able to achieve $\geq 95\%$ validity across all serotypes that they tested. The intra-laboratory variability, expressed as a median ratio of maximum to minimum relative potency estimates for each lab, was no greater than 1.5 in most laboratories for Flex 2a, 3a and 6 assays. However, for sonnei assays, the ratio was notably higher than 1.5 in half of the labs. The inter-laboratory variability was also notably higher for sonnei assays compared to assays for the other serotypes. A much higher proportion of study samples had a relatively low potency in sonnei assays when compared to the other serotypes which is likely to be a major contributing factor to the higher levels of variability observed in the study for this serotype. Indeed, if we excluded the low potency samples, the inter-lab variability for sonnei assays was comparable to that seen for the other serotypes, with 83% of the IQR values ≤ 4 .

The variability between labs was notably high for specific samples for some serotypes although, except for sample 9, which showed higher variability in assays for all 4 serotypes, there was no sample-related trend observed. Sample 9 is one of the serum donations included in the serum pool used to prepare the candidate standard 25/160, and we have no explanation for why this sample showed high variability across all 4 serotypes. In general, we did not observe any differences in outcomes (validity or variability) for ELISA vs. Luminex methods, (although we acknowledge that only 3 labs performed Luminex methods in this study).

Comparison of the IQR for relative potency estimates and end point titres suggest that expression of results relative to the candidate standard 25/160 improves the agreement between laboratories. The conclusions are less robust for sonnei compared to the other serotypes because fewer study samples were included in the analysis for sonnei. However, the same overall pattern was observed. Our conclusions are based on the testing of a large study sample panel which had samples covering a range of responses (low, medium and high) against all 4 serotypes. Some samples from the Phase 2b study with the tetravalent *Shigella* vaccine were included in the panel and, because of the number of trial participants that were in each arm, we can be certain that at least some of these samples were from individuals who received the vaccine. Some of these samples were also included in the candidate pool, so are not strictly independent samples, but our sample panel did include a small number of samples from individuals who participated in an earlier challenge study and who had been challenged with

S. flexneri 2a after immunisation with a monovalent *S. flexneri* 2a vaccine. Vaccinee samples from other studies covering other serotypes were not available to us for this project.

Three laboratories returned results from assays using antigens that are not present in the tetravalent vaccine that was used in the clinical trial from which the source material used to produce the candidate standard was obtained. This included measurement of anti-IpaB IgG levels from one laboratory and anti-*S. flexneri* 1b IgG levels from two laboratories. Both antigens are components of other vaccines that are in clinical development [4], and a response to both antigens was observed in the candidate standard and in 25/170. This may reflect naturally acquired antibody following exposure to *Shigella* spp. even in areas that may not have a high burden of disease, although in the case of antibody responses to *S. flexneri*, some cross reactivity of antibodies to different *S. flexneri* serotypes cannot be ruled out [14] and has not been further assessed in this study. These data suggest that the candidate standard 25/160 may be suitable for use in immunoassays that target *Shigella* antigens other than *S. flexneri* 2a, 3a, 6 and *sonnei*, although it is important to note that the results we have presented here that demonstrate commutability of the candidate standard have been obtained only from the *S. flexneri* 2a, 3a, 6 and *sonnei* immunoassays.

The candidate standard is intended for use in the calibration of immunoassays that are used to measure anti-*Shigella* antibody levels in clinical samples. The data from this collaborative study suggests that calibration of assays so that results are traceable to the standard, and reported in IU, will facilitate assay harmonisation and make it easier to compare data that is generated by different laboratories. Following adoption by WHO, laboratories performing *Shigella* immunoassays should express results in International Units relative to the WHO IS (or a standard calibrated against it) as opposed to end point titres.

This study has focused on binding assays which are routinely used for immunogenicity studies. The use of functional antibody assays, such as the serum bactericidal activity (SBA) assay is also important during vaccine development and evaluation. In our in-house testing at MHRA, our preliminary results suggest that the candidate standard 25/160 has bactericidal activity against *S. flexneri* 2a (2457T), *S. flexneri* 3a (J178) and *S. sonnei* (Moseley) and the standard is therefore likely to be suitable for use as a standard or control in functional assays. SBA assay for *S. flexneri* 6 has not yet been performed at MHRA. We plan to run a future collaborative study that will evaluate the performance of SBA assays currently being used by *Shigella* laboratories and will further evaluate the performance of 25/160 in these assays as part of that study.

6.1 Proposal to WHO

Product coded **25/160** is recommended for establishment as **the 1st WHO International Standard for Antibodies to *Shigella* serotypes *S. flexneri* 2a, 3a, 6 and *S. sonnei***
Assigned arbitrary values, in International Units, are proposed as follows:

- *S. flexneri* 2a to be assigned a value of 50 IU per ampoule
- *S. flexneri* 3a to be assigned a value of 50 IU per ampoule
- *S. flexneri* 6 to be assigned a value of 50 IU per ampoule
- *S. sonnei* to be assigned a value of 50 IU per ampoule

After reconstitution of the ampoule contents with 0.5 mL water, the solution will contain 100 IU/mL for each serotype. Following use of material for product quality control testing and collaborative study evaluation, approximately 4,000 ampoules of 25/160 remain available for adoption by WHO.

Product coded **25/170** is recommended for establishment as a **WHO International Reference Reagent for Antibodies to *Shigella* serotypes *flexneri* 2a, 3a, 6 and *S. sonnei* (low-positive)**; we do not propose any assigned values for 25/170. Following use of material for product quality control testing and collaborative study evaluation, approximately 400 ampoules of 25/170 remain available for adoption by WHO.

Products coded 25/160 and 25/170 will be stored at -20°C and distributed from the MHRAs Centre for Biological Reference Materials, South Mimms, UK.

7. Comments Received from Collaborative Study Participants

Comments were received from 11 laboratories. Most responses concerned editorial corrections and clarifications which have been addressed in this final version of the report. Some participants asked for additional information regarding reasons for invalid results in their data set and the project team provided this additional information where requested. In the draft report circulated to participants for comment, the proposal for assigned values, albeit arbitrary, was based on the study consensus end point titre data for each serotype. One participant queried whether this may lead to an interpretation or expectation from some laboratories that there is a link between end point titres and the International Unit, which is not the intention of the project team. In the final version of the report, we have decoupled the proposed arbitrary value assignment from the end point titre data. We have also added a recommendation in the text for laboratories to report their results from *Shigella* immunoassays in International Units, once the candidate standard has been adopted by WHO.

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10. Tables and figures

Table 1. Profile of 25/160, 25/170 and results from quality control tests

Product code	25/160	25/170
Presentation	Lyophilised	Lyophilised
Container type and size	2.5 mL DIN Ampoule	2.5 mL DIN Ampoule
Appearance	Freeze-dried white cake	Freeze-dried white cake
No. ampoules filled	4412	483
Date of fill	15 th August 2025	29 th August 2025
Recommended storage temp	-20°C or below	-20°C or below
Mean fill mass	0.52g (CV = 0.53%, n=165)	0.27g (CV = 1.15%, n=32)
Mean equilibrium relative humidity	1.33% (CV = 9.30%, n=12)	1.27% (CV = 6.01%, n=12)
Mean of oxygen content	0.16% (CV = 49.87%, n=12)	0.43% (CV = 23.45%, n=12)
Bacterial colony count (CFU/mL)	0 (pre-fill), 0 (post-lyophilisation)	0 (pre-fill), 0 (post-lyophilisation)
Mould/Yeast colony count (CFU/mL)	0 (pre-fill), 0 (post-lyophilisation)	0 (pre-fill), 0 (post-lyophilisation)

CV = coefficient of variation; n = number of samples tested; CFU = colony forming unit

Table 2. Homogeneity assessment of candidate standard 25/160

Serotype	Fill Position	Potency Estimate as % of positive control (95% CI)
<i>S. flexneri</i> 2a	Beginning	99.5910 (89.9969, 110.208)
	Middle	92.6405 (81.6774, 105.075)
	End	90.9921 (78.0637, 106.062)
<i>S. flexneri</i> 3a	Beginning	91.6202 (82.6975, 101.506)
	Middle	95.8848 (81.6085, 112.658)
	End	99.3432 (75.9986, 129.859)
<i>S. flexneri</i> 6	Beginning	92.0535 (76.5036, 110.764)
	Middle	97.9308 (85.5512, 112.102)
	End	91.1894 (74.3583, 111.830)
<i>S. sonnei</i>	Beginning	103.352 (71.5976, 149.189)
	Middle	95.6948 (77.4253, 118.275)
	End	98.7001 (81.6368, 119.330)

Table 3. Accelerated degradation assessment of candidate standard 25/160 after 6 months storage at elevated temperatures (NT = Not Tested; NC = Not Calculated)

Storage temperature	Geometric mean potency relative to –70°C (95% CI)	Predicted % loss per year	Predicted % loss per month
-20°C	NT	0.015	0.001
4°C	1.053 (0.986 – 1.123)	0.218	0.018
20°C	0.913 (0.812 – 1.027)	1.032	0.086
37°C	1.002 (0.871 – 1.153)	4.449	0.378
45°C	0.955 (0.855 – 1.067)	NC	NC

Table 4. Collaborative Study Sample Panel

Study sample ID	Sample description	Sample product code (where applicable)	Included in the candidate standard 25/160
1	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	No
2	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	No
3	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	No
4	3 rd WHO IS for Anti-Rabies Immunoglobulin	19/244	N/A
5	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	No
6	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
7	Low positive control	25/170	N/A
8	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
9	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
10	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
11	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
12	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
13	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
14	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
15	1 st WHO IS for Human Anti-Pneumococcal Reference Serum	007SP	N/A
16	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
17	1 st WHO IS for Tetanus Immunoglobulin, Human	13/240	N/A
18	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
19	1 st WHO IS for Anti-Influenza H5N1, Human Plasma	07/150	N/A
20	1 st WHO IS for Diphtheria Antitoxin, Human	10/262	N/A
21	1 st WHO IS for Anti-Vi IgG Serum, human	16/138	N/A
22	In-house pool of human serum collected from individuals in North Africa	AF78	N/A
23	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes

24	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
25	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
26	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
27	Serum sample from Phase 2b tetravalent <i>Shigella</i> vaccine trial	N/A	Yes
28	Serum sample from Phase 2b monovalent <i>S. flexneri</i> 2a vaccine trial	N/A	No
29	Serum sample from Phase 2b monovalent <i>S. flexneri</i> 2a vaccine trial	N/A	No
30	Serum sample from Phase 2b monovalent <i>S. flexneri</i> 2a vaccine trial	N/A	No
31	Serum sample from Phase 2b monovalent <i>S. flexneri</i> 2a vaccine trial	N/A	No
32	Serum sample from Phase 2b monovalent <i>S. flexneri</i> 2a vaccine trial	N/A	No
33	Reconstituted duplicate of the candidate standard	25/160	N/A
34	Analytical negative control (Immunoglobulins-depleted human serum)	SF505-2 (BBI Solutions)	No

Table 5. Assay methods used by participating laboratories

Lab code	Method(s) used	<i>S. flex</i> 2a	<i>S. flex</i> 3a	<i>S. flex</i> 6	<i>S. sonnei</i>	Other
1	ELISA	✓	✓	✓	✓	N/A
5	Luminex	✓	✓	✓	✓	IpaB
6	ELISA	✓	✓	✓	✓	N/A
7	Luminex	✓	✓	✓	✓	N/A
8	ELISA	✓	NT	NT	✓	N/A
9	ELISA	✓	✓	✓	✓	N/A
10	ELISA	✓	✓	✓	✓	N/A
12	ELISA	✓	✓	NT	✓	<i>S. flex</i> 1b
13	ELISA	✓	NT	✓	✓	N/A
14a	ELISA	✓	✓	✓	✓	N/A
14b	Luminex	✓	✓	✓	✓	N/A
15	ELISA	✓	✓	✓	✓	N/A
16	ELISA	✓	✓	✓	✓	N/A
17	ELISA	✓	✓	✓	✓	N/A
18	ELISA	✓	✓	✓	✓	N/A
19	ELISA	✓	✓	✓	✓	N/A
20	ELISA	✓	✓	✓	✓	N/A
21	ELISA	✓	✓	NT	✓	N/A
22	ELISA	✓	NT	NT	✓	N/A
23	Data not included in report ¹	✓	NT	NT	✓	N/A
24a	ELISA (method 1)	✓	NT	✓	✓	<i>S. flex</i> 1b
24b	ELISA (method 2)	NT	NT	NT	✓	

¹Results were submitted by laboratory 23 but not in time for inclusion in this report; NT = not tested

Table 6a. Detailed information for the coating antigens used by study participants

Lab code	Antigen type, strain and source			
	Flex 2a	Flex 3a	Flex 6	Sonnei
1	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #092313)	LPS, strain SSU2415 WRAIR (Lot #060121)	LPS strain Moseley WRAIR (Lot #062019)
5	LPS, strain 2457T WRAIR	LPS, strain J17B WRAIR	LPS, strain SSU2415 WRAIR	LPS, Moseley WRAIR
6	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #092313)	LPS, strain SSU2415 WRAIR (Lot #060121)	LPS, strain Moseley WRAIR Lot (#092308)
7	O-antigen Inventprise	O-antigen Inventprise	O-antigen Inventprise	O-antigen Inventprise
8	LPS WRAIR	N/A	N/A	LPS WRAIR
9	LPS, strain 2457T UMSD	LPS, strain B5510 UMSD	LPS, strain B5691 UMSD	LPS, strain 53G UMSD
10	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #112718)	LPS, strain SSU2415 WRAIR Lot (#060121)	LPS, strain Moseley WRAIR Lot (#062019)
12	O-antigen, strain 2457T GVGH	O-antigen, strain 6885 GVGH	N/A	LPS, strain 53G GVGH
13	LPS, strain 454 (Institut Pasteur)	N/A	LPS, strain SC544 (Nils Carlin, Etvax, Sweden)	LPS, strain NVGH 1859 (GVGH) derived from strain 53G
14	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #112718)	LPS, strain SSU2415 WRAIR Lot (#060121)	LPS, strain Moseley WRAIR Lot (#062019)
15	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #112718)	LPS, strain SSU2415 WRAIR Lot (#060121)	LPS, strain Moseley WRAIR Lot (#062019)
16	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #112718)	LPS, strain SSU2415 WRAIR Lot (#060121)	LPS, strain Moseley WRAIR Lot (#062019)
17	LPS, strain 2457T WRAIR (Lot #110619)	LPS, strain J17B WRAIR (Lot #112718)	LPS, strain SSU2415 WRAIR Lot (#060121)	LPS, strain Moseley WRAIR Lot (#062019)
18	LPS Mass. Gen. Hosp.	LPS Mass. Gen. Hosp.	LPS Mass. Gen. Hosp.	LPS Mass. Gen. Hosp.
19	O-antigen, strain 2457T In-house	O-antigen, strain J17B In-house	O-antigen, strain CCH060 In-house	O-antigen, strain Moseley In-house
20	O-Antigen Inventprise	O-Antigen Inventprise	O-Antigen Inventprise	O-Antigen Inventprise
21	O-antigen, strain 2457T GVGH	O-antigen, strain 6885 GVGH	N/A	LPS, strain 53G GVGH
22	O-antigen, strain CMCC51062	N/A	N/A	O-antigen, strain CMCC10709
24a	O-antigen GVGH	N/A	LPS GVGH	LPS GVGH
24b	N/A	N/A	N/A	LPS, strain Moseley WRAIR

Table 6b. Key features of the ELISA assays used by study participants – Part 1 of 2.

Lab code	Plates	Coating conditions				Blocking conditions		
	Supplier/ Lot No. and description	Conc.	Coating buffer	Inc. time	Inc. temp.	Buffer	Inc. time	Inc. temp.
1	Thermo #3555 Immulon 1B U-bottom	10 ug/mL	Carbonate	16-18 hrs	5°C (± 3°C)	2% Casein buffer	0.5 hr	22°C (± 2°C)
6	Thermo #442404 MaxiSorp Flat bottom	1 µg/mL	Carbonate	16-18 hrs	4°C	5% milk in PBS with 0.05% Tween	1 hr	37°C
8	Corning #9018 Polystyrene Flat bottom	10 µg/mL	Carbonate	17 hrs	4°C	5% milk in PBS	0.5-2 hrs	22°C
9	Thermo #3455 Immulon 2HB Flat bottom	2.5 µg/mL	PBS	1.5 hrs	37°C	10% milk in PBS	Overnight	4°C
10	Thermo #5086098 Immulon 1B U-bottom	10 µg/mL	Carbonate	16-18 hrs	4°C	2% Casein buffer	0.5-1 hr	22°C
12	Thermo #449824 MaxiSorp Round bottom	0.5-5 µg/mL	Carbonate (2a), PBS (3a, Sonnei)	Overnight	2-8 °C	5% milk in PBS	1 hr	25°C
13	Corning #3590 Polystyrene Flat-bottom	0.5-10 µg/mL	Carbonate	1 hr	37°C	0.5% Casein buffer with 0.5% BSA	1 hr	37°C
14	Thermo #442404 MaxiSorp Flat bottom	5 µg/mL	Carbonate	Overnight	4°C	5% milk in TBS with 0.05% Tween	1 hr	37°C
15	Thermo #3455 Immulon 2HB Flat bottom	10 ug/mL	Carbonate	16-18 hrs	4°C (± 2°C)	2% Casein buffer	At least 1 hr	22°C (± 2°C)
16	Thermo #439545 MaxiSorp Flat bottom	1 µL/mL	PBS	16-20 hrs	4 °C	1% BSA in PBS	0.5 hr	37 °C
17	Thermo #439545 MaxiSorp Flat bottom	1 µg/mL Sf2a 5 µg/mL Sf3a, Sf6, Ss	DPBS	Overnight (up to 72 h)	2 to 8°C	3% milk in DPBS with 0.2% Tween 20	1.5-2 hrs	Room temp.
18	Thermo #269620 Non-treated Flat bottom	1 µg/mL	PBS	16-18 hrs	Room temp.	0.1% BSA in PBS	0.5 hr	37°C
19	Thermo #439545 MaxiSorp Flat bottom	1 µg/mL	Carbonate	Overnight (18 hrs)	4 °C	1% BSA in PBS with 0.05% Tween-20	1 hr	Room temp.
20	Thermo #442404 MaxiSorp Flat bottom	1.25 µg/mL	PBS	Overnight (12-18 hrs)	5± 3°C	5% skimmed milk in PBS	1 hr (± 5 mins)	35°C (± 2°C)
21	Thermo #442404 MaxiSorp Flat bottom	0.5-5 µg/mL	PBS	Overnight	2-8°C	3% Skimmed milk	1 hr	37°C
22	JINCANHUA #1041-007 Flat bottom	10 ug/mL	PBS	Overnight	2-8°C	4% BSA in PBS with 0.05% Tween 20	1.5 hrs	37°C
24a	Thermo #449824 MaxiSorp Round bottom	0.5-15 µg/mL	Carbonate (2a) or PBS (3a, 6, Sonnei)	Overnight	2-8°C	5% skimmed milk in PBS	1 hr	Room temp.
24b	Thermo #3555 Immulon 1B U-bottom	10 ug/mL	Carbonate	17 hrs	4-8°C	2% Casein buffer	1 hr	Room temp.

Table 6c. Key features of the ELISA assays used by study participants – Part 2 of 2.

Lab code	Samples			Conjugate					Substrate		
	Buffer	Inc. time	Inc. temp.	Description	Supplier and Lot No.	Dilution	Inc. time	Inc. temp.	Type	Inc. time	Inc. temp.
1	2% Casein buffer	2 hrs (± 2 min)	22°C (± 2°C)	Reverse AP Labelled Goat Anti-Human IgG(γ)	SeraCare #5220- 0348	1 µg/mL	1 hr (± 2min)	22°C (± 2°C)	pNPP	30 min	22°C (± 2°C)
6	5% milk in PBS with Tween 0.05%	1 hr	37°C	HRP AffiniPure Goat Anti-Human IgG, Fcγ-specific	JIR #109-035-008	1:5000	1 hr	37°C	TMB	15 min	Room temp.
8	5% milk in PBS	2 hrs	22°C	Reverse AP Labelled Goat Anti-Human IgG(γ)	SeraCare #5220- 0348	1:250	1 hr	22°C	pNPP	30 min	22°C
9	10% non-fat milk in PBS with tween	1 hr	37°C	Goat anti-Human IgG (H+L) Secondary Antibody, HRP	Invitrogen #31410	1:2000	1 hr	37°C	TMB	15 min	Room temp.
10	2% Casein buffer	2 hrs	22°C	Reserve AP labeled goat-anti-human IgG (γ)	SeraCare #5220-0348	1 µg/mL	1 hr	22°C	pNPP	30 min	22°C
12	5% milk in PBS	2 hrs	25°C	Goat Anti-Human IgG (γ-chain specific) AP antibody	Sigma #A3187	1:8000 (2a) 1:5000 (3a) 1:4000 (sonnei)	1 hr	25°C	pNPP	1 hr	25°C
13	0.5% Casein buffer with BSA 0.5%	16-18 hrs	22°C (± 2°C)	Reserve AP labeled goat-anti-human IgG (γ)	KPL #5220-0348	1:5000	16-18 hrs	22°C (± 2°C)	pNPP	15 min	22°C (± 2°C)
14	5% milk in TBS with 0.05% Tween	1 hr	37°C	Goat anti-Human IgG-Fc Fragment Antibody, HRP	Bethyl #A80-104P	1:100000	1 hr	37°C	TMB	20 min	Room temp.
15	2% Casein buffer	2 hrs (± 2 min)	22°C (± 2°C)	Reserve AP labeled goat-anti-human IgG (γ)	SeraCare #5220-0348	1/1000	1 hr (± 5 min)	22°C (± 2°C)	pNPP	30 min (±2 min)	22°C (± 2°C)
16	PBS + 0.05% BSA	1 hr	37 °C	HRP AffiniPure Goat Anti-Human IgG, Fcγ-specific	JIR #109-035-008	1/5000	1 hr	37°C	TMB	5 min	Room temp.
17	3% milk in DPBS with 0.2% Tween 20	1.5-2 hrs	Room temp.	Polyclonal Rabbit anti-human IgG-HRP conjugate	Dako #P0214	1:10000 (Sf2a, Sf3a) 1:20000 (Sf6, Ss)	1.5-2 hrs	Room temp.	TMB	10 min	Room temp.
18	0.1% BSA in PBS with 0.05% Tween-20	1.5 hrs	Room temp.	HRP AffiniPure Rabbit Anti-Human IgG, F(ab') ₂ -specific	JIR #309-035-006	1/1000	1.5 hrs	Room temp.	OPD	20 min	Room temp.
19	1% BSA in PBS with 0.05% Tween-20	2 hrs	Room temp.	Goat Anti-Human IgG-HRP	SB #2040-05	1:5000	1 hr	Room temp.	TMB	10 min	Room temp.
20	1% skimmed milk in PBS	1 hr (± 5 mins)	35°C (± 2°C)	Anti-Human IgG HRP produced in rabbit	Sigma #A8792	1:10000	1 hr (± 5 min)	35°C (± 2°C)	TMB	15 min	23± 2°C
21	0.3% Skimmed milk	1 hr	37°C	Anti-Human IgG HRP Conjugate	Sigma #A8667	1:40000	1 hr	37°C	TMB	10 min	Room temp.
22	1% BSA in PBS with Tween 0.05%	1.5 hrs	37°C	Goat Anti-Human IgG (γ-chain specific) AP antibody	Sigma #A3187	1:60000	1.5 hrs	37°C	pNPP	1.5 hrs	37°C
24a	5% skimmed milk in PBS	2 hrs	Room temp.	Goat Anti-Human IgG (γ-chain specific) AP antibody	Sigma #A3187	Not reported	1 hr	Room temp.	pNPP	1 hr	Room temp.

24b	2% Casein buffer	2 hrs	Room temp.	Reserve AP labeled goat-anti-human IgG (γ)	SeraCare #5220-0348	1 μ g/mL	1 hr	Room temp.	Not reported	30 min	Room temp.
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Table 7. Assay validity summary for serotypes *S. flexneri* 2a and 3a

	Lab	<i>S. flexneri</i> 2a						<i>S. flexneri</i> 3a					
		Plates	Valid	As %	Samples	Valid	As %	Plates	Valid	As %	Samples	Valid	As %
ELISA	1	12	11	92%	96	87	91%	12	11	92%	81	73	90%
	6	12	12	100%	96	96	100%	12	12	100%	81	81	100%
	8	12	12	100%	95	93	98%	NT					
	9	12	12	100%	96	94	98%	12	12	100%	81	79	98%
	10	22	20	91%	96	87	91%	19	16	84%	81	67	83%
	12	27	n/a	n/a	96	n/a	n/a	27	n/a	n/a	96	n/a	n/a
	13	12	12	100%	96	96	100%	NT					
	14a	12	8	67%	96	63	66%	12	12	100%	81	77	95%
	15	12	12	100%	96	96	100%	12	12	100%	81	80	99%
	17	24	24	100%	96	96	100%	21	21	100%	81	80	99%
	18	12	11	92%	96	88	92%	12	11	92%	81	73	90%
	19	16	15	94%	128	116	91%	16	12	75%	108	71	66%
	20	12	12	100%	96	91	95%	12	12	100%	81	75	93%
	21	12	11	92%	96	84	88%	12	4	33%	81	27	33%
	22	12	12	100%	95	84	88	NT					
	24a	27	n/a	n/a	96	n/a	n/a	NT					
	24b	NT						NT					
Luminex	5	12	12	100%	96	96	100%	12	12	100%	96	96	100%
	7	12	12	100%	96	96	100%	12	12	100%	96	95	99%
	14b	12	12	100%	96	83	86%	12	12	100%	96	84	88%

Note: Lab 12 and 24a ELISAs were valid according to requirements of the standard operating procedure for those assays; NT = not tested

Table 8. Assay validity summary for serotypes *S. flexneri* 6 and *S. sonnei*

	Lab	<i>S. flexneri</i> 6						<i>S. sonnei</i>					
		Plates	Valid	As %	Samples	Valid	As %	Plates	Valid	As %	Samples	Valid	As %
ELISA	1	12	12	100%	81	78	96%	12	11	92%	96	79	82%
	6	12	12	100%	81	80	99%	12	12	100%	96	95	99%
	8	NT						12	11	92%	95	75	79%
	9	12	9	75%	81	52	64%	12	11	92%	96	56	58%
	10	19	13	68%	81	52	64%	23	21	91%	96	88	92%
	12	NT						27	n/a	n/a	96	n/a	n/a
	13	12	11	92%	81	78	96%	12	12	100%	96	96	100%
	14a	12	12	100%	81	79	98%	12	12	100%	96	75	78%
	15	12	12	100%	81	81	100%	12	12	100%	96	96	100%
	17	21	19	90%	81	75	93%	24	24	100%	96	94	98%
	18	12	12	100%	81	81	100%	12	12	100%	96	96	100%
	19	12	8	67%	63	40	63%	12	8	67%	96	48	50%
	20	12	11	92%	81	70	86%	12	12	100%	96	87	91%
	21	NT						12	11	92%	96	75	78%
	22	NT						12	12	100%	95	45	47%
	24a	27	n/a	n/a	96	n/a	n/a	27	n/a	n/a	96	n/a	n/a
	24b	NT						21	20	95%	87	73	84%
Luminex	5	12	12	100%	96	96	100%	12	12	100%	96	96	100%
	7	12	12	100%	96	96	100%	12	12	100%	96	96	100%
	14b	12	12	100%	96	71	74%	12	12	100%	96	72	75%

Note: Lab 12 and 24a ELISAs were valid according to requirements of the standard operating procedure for those assays; NT = not tested

Table 9. Overall assay validity summary for all serotypes

	Lab	All					
		Plates	Valid	As %	Samples	Valid	As %
ELISA	1	48	45	94%	354	317	90%
	6	48	48	100%	354	352	99%
	8	12	23	92%	190	168	88%
	9	48	44	92%	354	281	79%
	10	83	70	84%	354	294	83%
	12	81	n/a	n/a	288	n/a	n/a
	13	36	35	97%	273	270	99%
	14a	48	44	92%	354	294	83%
	15	48	48	100%	354	353	100%
	17	90	88	98%	354	345	97%
	18	48	46	96%	354	338	95%
	19	56	43	77%	395	275	70%
	20	48	47	98%	354	323	91%
	21	36	26	72%	273	186	68%
	22	24	24	100%	190	129	68%
	24a	81	n/a	n/a	288	n/a	n/a
	24b	21	20	95%	87	73	84%
Luminex	5	48	48	100%	384	384	100%
	7	48	48	100%	384	383	100%
	14b	48	48	100%	384	310	81%

Note: Lab 12 and 24a ELISAs were valid according to requirements of the standard operating procedure for those assays

Table 10. Intra-laboratory variability expressed as median ratio of maximum to minimum relative potency estimates for each laboratory; shading indicates ratio ≤ 1.5

	Lab	<i>S. flexneri</i> 2a	<i>S. flexneri</i> 3a	<i>S. flexneri</i> 6	<i>S. sonnei</i>
ELISA	1	1.19	1.26	1.21	1.30
	6	1.17	1.36	1.33	1.36
	8	1.29	NT	NT	1.79
	9	1.38	1.23	1.20	1.17
	10	1.62	1.42	1.68	1.66
	12	1.26	2.07	NT	2.27
	13	1.19	NT	1.20	1.61
	14a	1.15	1.34	1.39	1.42
	15	1.30	1.31	1.33	1.80
	17	1.18	1.29	1.42	1.64
	18	1.45	1.31	1.40	1.56
	19	1.39	1.61	1.26	1.20
	20	1.31	1.54	1.33	1.38
	21	1.83	1.01	NT	1.42
	22	1.20	NT	NT	1.19
	24a	1.33	NT		
	24b	NT	NT	NT	1.63
Luminex	5	1.16	1.10	1.09	1.14
	7	1.10	1.12	1.13	1.12
	14b	1.52	1.50	1.46	2.02

NT = not tested by that laboratory; empty cells indicate cases where there was insufficient data to allow intra-laboratory variability to be calculated

Figure 1. Intra-laboratory variability expressed as ratio of maximum to minimum relative potency estimates for each laboratory and sample

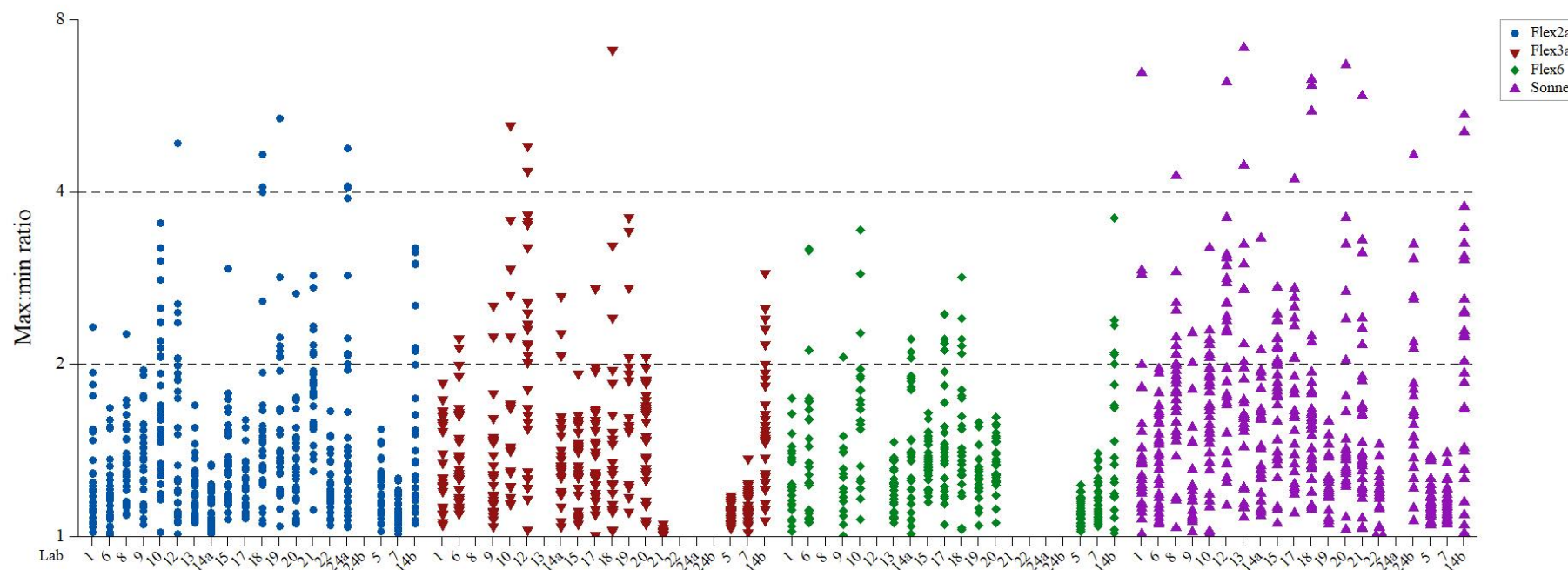


Table 11. Lab geometric mean relative potency estimates (*S. flexneri* 2a); samples ordered by increasing study median potency; outliers identified by Grubbs' test highlighted and excluded from further analysis; empty cells indicate no valid estimates obtained

Sample	ELISA																Luminex		
	Lab																		
	1	6	8	9	10	12	13	14a	15	17	18	19	20	21	22	24a	5	7	14b
3	0.038	0.016	0.054	0.033	0.040	0.014	0.035	0.024	0.053	0.016	0.229	0.125	0.057	0.031		0.038	0.071	0.012	0.058
9	0.157	0.038	0.102	0.015	0.143	<LoQ	0.079	0.044	0.189	0.009	0.104	0.132	0.032	0.017		0.020	0.052	0.003	0.124
7	0.058	0.063	0.075	0.068	0.068	0.026	0.068	0.060	0.074	0.045	0.174	0.089	0.094	0.067	0.025	0.080	0.158	0.043	0.086
15	0.134	0.047	0.146	0.048	0.136	0.024	0.081	0.093	0.156	0.038	0.284	0.075	0.085	0.079	0.030	0.074	0.127	0.031	0.091
2	0.106	0.061	0.112	0.027	0.136	0.041	0.101	0.101	0.123	0.057	0.134		0.028	0.030	0.044	0.031	0.163	0.072	0.105
21	0.119	0.082	0.166	0.065	0.138	0.048	0.103	0.105	0.149	0.075	0.264	0.135	0.193	0.085	0.077	0.165	0.166	0.054	0.110
1	0.642*	0.144	0.174	0.107	0.162	0.047	0.138	0.108	0.157	0.118	0.144	0.080	0.123	0.109	0.096	0.231	0.225	0.159	0.071
22	0.274	0.122	0.329	0.134	0.314	0.036	0.165	0.252	0.325	0.124	0.395	0.251	0.161	0.104	0.122	0.141	0.295	0.071	0.202
19	0.167	0.144	0.238	0.114	0.262	0.145	0.172	0.197	0.234	0.133	0.220	0.220	0.153	0.091	0.107	0.174	0.260	0.141	0.179
6	0.240	0.094	0.201	0.057	0.300	0.080	0.234	0.204	0.259	0.121	0.203	0.098	0.119	0.075	0.056	0.140	0.239	0.193	0.278
17	0.348	0.169	0.495	0.122	0.459	0.062	0.331	0.283	0.468	0.137	0.515	0.382	0.911	0.411	0.109	0.198	0.330	0.127	
20	0.365	0.270	0.535	0.204	0.427	0.085	0.421	0.392	0.490	0.225	0.445	0.372	0.811	0.161	0.217	0.300	0.442	0.202	0.539
5	0.264	0.364	0.263	0.445	0.392	0.462	0.339	0.467	0.289	0.398	0.486	0.253	0.488	0.329	0.541	0.475	0.767	0.252	0.507
23	0.404	0.532	0.461	0.444	0.531	0.441	0.488	0.349	0.434	0.432	1.840*	0.998*	0.603	0.472	0.206*	0.544	0.472	0.501	0.440
18	0.320	0.714	0.489	0.522	0.405	0.407	0.481	0.457	0.380	0.505	0.533	0.825	1.360	0.551	0.534	0.718	0.932	0.723	0.512
24	0.935	0.539	0.832	0.259	0.993	0.528	0.655	0.777	0.835	0.586	2.773*	0.572	0.290	0.362	0.375	0.317	0.576	0.678	0.578
4	0.844	0.419	1.808	0.457	0.603	0.092	0.697	0.519	1.002	0.396	0.821	0.165	0.606		0.291	0.859	0.707	0.288	
16	2.097	0.304	1.416	0.124	1.962	0.563	0.858	1.296	1.812	0.696	1.391	0.120	0.146	0.250	0.286	0.205	0.518	0.807	1.436
13	0.596	0.819	0.755	0.541	0.742	0.692	0.879	0.633	0.650	0.664	0.732	0.751	1.280*	0.689	0.607	0.819	0.567	0.752	0.942
11	0.739	1.032	0.820	0.796	0.867	0.811	0.941	0.778	0.703	0.872	0.687	1.256	1.936*	0.683	0.735	0.989	0.904	1.040	0.960
25	1.572	0.945	1.465	0.726	1.443	0.944	1.240	1.020	1.465	0.979	17.963*	0.603	0.966	0.740	0.772	0.684	0.766	1.296	1.452
14	0.772	1.428	0.879	1.232	0.852	1.142	1.019	0.580	0.822	1.163	0.886	2.153	2.997	1.925	1.061	1.488	1.041	1.208	0.512
30	0.757	1.482	0.847	1.800	0.691	0.988	1.063	0.740	0.785	1.067		1.450	0.853	0.973	1.074	1.131	2.075	1.592	2.625
31	0.998	1.964	1.246	2.545	1.656	1.626	1.628	1.092	1.051	1.428	2.539	1.828	1.741	1.371	1.954	1.047	2.086	1.490	4.508*
27	0.878	2.501	1.086	3.877	0.999	2.226	1.510	1.385	0.880	1.650	5.867	1.347	2.031	3.114	2.876	4.401	2.768	2.185	1.571
10	2.309	2.179	2.239	1.750	2.207	2.811	2.465	1.629	2.015	2.212	1.782	2.231	3.298	1.852	1.874	1.833	1.358	2.187	2.251
28	0.615*	2.934	1.826	3.103	1.476	2.950	1.971	1.204	1.260	2.590	1.624	4.467	2.711	2.164	2.295	2.146	1.989	2.447	2.804
32	1.730	3.047	1.600	2.876	2.255	2.683	2.446	1.171	1.451	2.908	1.887	4.947	2.582	2.426	1.967	2.991	2.485	3.238	4.753
8	4.971	2.439	2.269	2.883	2.432	6.376	2.339	3.007	2.343	2.963	2.721	1.600	2.920	3.243	4.417	2.325	2.717	2.705	4.702
26	2.926	4.093	4.366	5.064	4.401	2.915	3.624	2.885	2.804	4.123	3.072	3.545	5.581	5.204	4.279	3.774	2.072	4.546	3.011
29	4.283	4.581	5.265	3.260	6.498	3.220	4.772	3.781	3.848	4.979		4.532	3.813	2.016	4.347	2.423	2.279	5.726	7.064
12	7.489	6.920	6.591	5.656	7.359	6.707	6.508	5.573	6.112	8.025	5.198	12.941	5.676	5.246	5.161	8.143	3.248	6.332	1.764*

Figure 2. Boxplot of laboratory geometric mean relative potency estimates for *S. flexneri* 2a

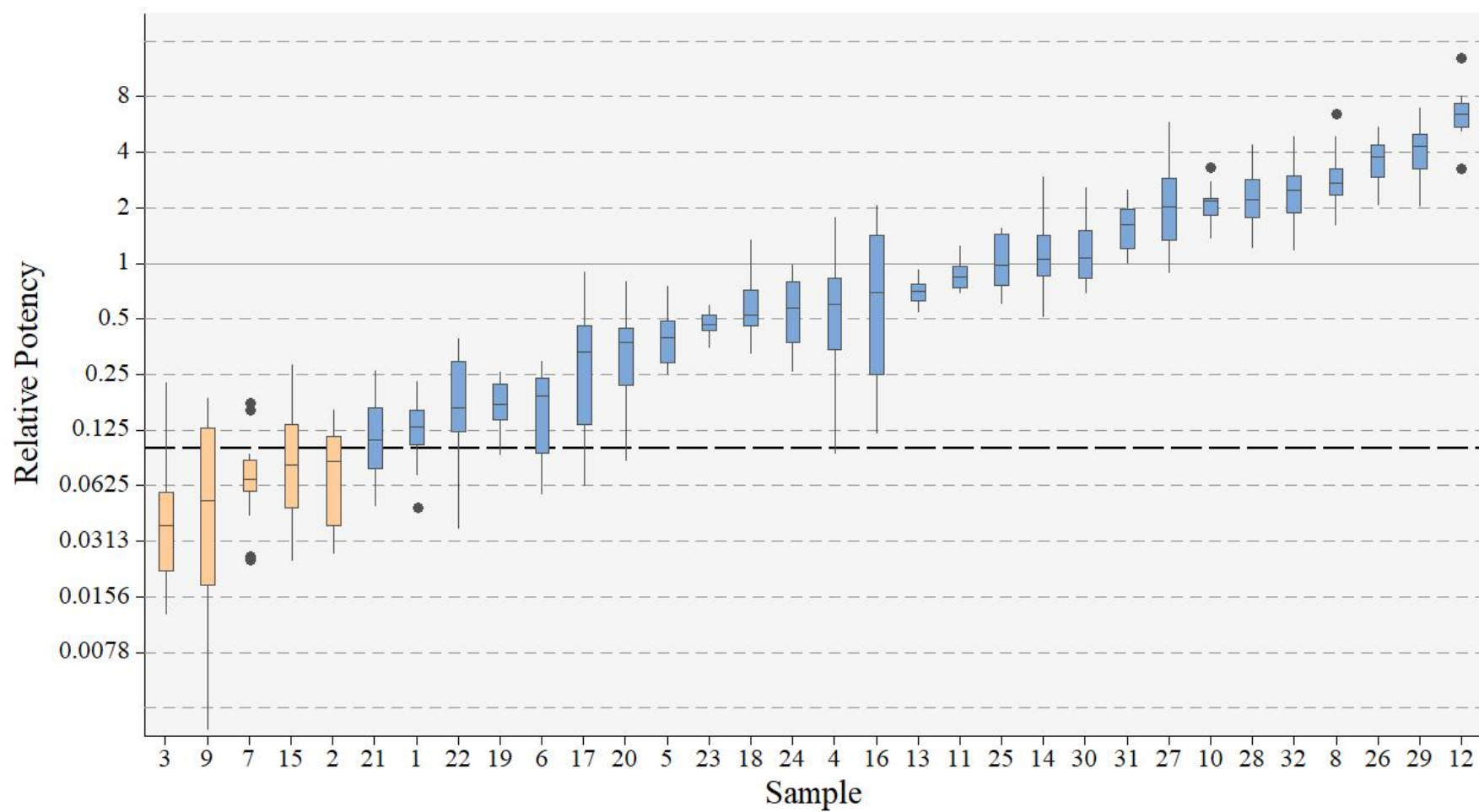


Table 12. Summary statistics for laboratory geometric mean relative potency estimates for *S. flexneri* 2a

Sample	GM	Median	GCV	UQ/LQ	N
3	0.039	0.038	113%	2.2	18
9	0.046	0.052	221%	6.3	17
7	0.067	0.068	63%	1.4	19
15	0.077	0.081	91%	2.7	19
2	0.069	0.085	86%	2.7	18
21	0.110	0.110	57%	2.0	19
1	0.124	0.130	49%	1.5	18
22	0.173	0.165	83%	2.3	19
19	0.169	0.172	36%	1.5	19
6	0.148	0.193	73%	2.5	19
17	0.265	0.330	101%	3.1	18
20	0.323	0.372	69%	2.0	19
5	0.392	0.398	36%	1.6	19
23	0.468	0.466	14%	1.2	16
18	0.562	0.522	41%	1.5	19
24	0.552	0.577	50%	1.8	18
4	0.507	0.603	103%	2.1	17
16	0.584	0.696	166%	5.2	19
13	0.705	0.712	16%	1.2	18
11	0.856	0.843	18%	1.3	18
25	1.014	0.973	36%	1.8	18
14	1.111	1.061	54%	1.5	19
30	1.135	1.065	46%	1.7	18
31	1.564	1.627	34%	1.5	18
27	1.965	2.031	73%	2.1	19
10	2.091	2.187	22%	1.2	19
28	2.212	2.229	40%	1.5	18
32	2.442	2.485	44%	1.5	19
8	2.956	2.721	39%	1.3	19
26	3.690	3.774	29%	1.5	19
29	4.040	4.314	41%	1.5	18
12	6.365	6.419	32%	1.3	18

Table 13. Lab geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 2a; samples with study median relative potency <0.1 excluded; values within 50-200% highlighted; empty cells indicate no valid estimates obtained

Sample	ELISA																Luminex		
	Lab																		
	1	6	8	9	10	12	13	14a	15	17	18	19	20	21	22	24a	5	7	14b
21	108.7	74.4	151.3	59.5	125.9	43.6	94.0	95.7	136.0	68.4	240.4	122.8	176.0	77.0	70.4	149.8	151.1	49.4	100.0
1		110.9	133.3	82.2	124.3	36.4	105.8	83.3	120.9	90.8	110.3	61.3	94.5	83.5	74.0	177.3	172.8	122.4	54.2
22	165.7	73.9	198.9	80.9	189.9	21.9	100.0	152.4	196.4	74.7	239.1	151.5	97.5	63.1	74.0	85.0	178.6	43.1	122.3
19	97.2	84.1	138.8	66.7	152.8	84.3	100.0	114.8	136.6	77.8	128.0	128.5	89.0	52.9	62.6	101.5	151.7	82.3	104.2
6	124.5	48.9	104.3	29.6	155.8	41.4	121.5	105.9	134.5	62.8	105.4	50.7	61.9	38.8	28.9	72.8	123.7	100.0	144.4
17	105.3	51.2	149.9	37.0	138.8	18.7	100.3	85.6	141.6	41.3	156.0	115.6	275.6	124.5	32.9	60.0	99.7	38.6	
20	98.0	72.6	143.7	54.8	114.8	22.8	113.3	105.5	131.6	60.5	119.6	100.0	218.0	43.2	58.4	80.7	118.7	54.4	144.8
5	66.3	91.5	66.0	111.7	98.6	116.0	85.2	117.3	72.6	100.0	122.0	63.5	122.7	82.7	136.0	119.3	192.6	63.2	127.3
23	86.7	114.1	98.9	95.2	113.8	94.6	104.7	74.7	93.1	92.6			129.2	101.2		116.7	101.1	107.4	94.4
18	61.3	136.9	93.8	100.0	77.6	78.0	92.2	87.7	72.8	96.7	102.1	158.2	260.7	105.7	102.4	137.6	178.7	138.7	98.1
24	162.0	93.3	144.2	44.8	172.1	91.6	113.6	134.6	144.6	101.5		99.0	50.2	62.7	65.0	54.9	99.8	117.5	100.2
4	140.0	69.5	299.8	75.7	100.0	15.3	115.6	86.0	166.2	65.7	136.2	27.4	100.5		48.3	142.4	117.2	47.7	
16	301.3	43.7	203.4	17.8	281.9	80.9	123.3	186.2	260.3	100.0	199.9	17.2	21.0	35.9	41.1	29.4	74.4	115.9	206.3
13	83.8	115.1	106.2	76.0	104.3	97.2	123.5	88.9	91.4	93.4	102.8	105.6		96.8	85.3	115.1	79.7	105.8	132.3
11	87.7	122.4	97.3	94.4	102.8	96.2	111.6	92.3	83.4	103.4	81.5	149.0		81.0	87.2	117.3	107.2	123.3	113.8
25	161.6	97.1	150.6	74.6	148.4	97.1	127.5	104.8	150.6	100.7		62.0	99.3	76.1	79.4	70.3	78.8	133.2	149.3
14	72.8	134.6	82.9	116.1	80.3	107.6	96.1	54.7	77.5	109.7	83.6	203.0	282.5	181.5	100.0	140.3	98.2	113.9	48.3
30	71.1	139.1	79.6	169.0	64.9	92.8	99.8	69.5	73.7	100.2		136.2	80.1	91.4	100.8	106.2	194.8	149.5	246.4
31	61.3	120.8	76.6	156.4	101.8	99.9	100.1	67.1	64.6	87.8	156.1	112.4	107.0	84.3	120.1	64.4	128.2	91.6	
27	43.2	123.1	53.5	190.9	49.2	109.6	74.4	68.2	43.3	81.2	288.9	66.3	100.0	153.3	141.6	216.7	136.3	107.6	77.3
10	105.5	99.6	102.4	80.0	100.9	128.5	112.7	74.5	92.1	101.1	81.5	102.0	150.8	84.7	85.7	83.8	62.1	100.0	102.9
28		131.7	81.9	139.2	66.2	132.4	88.4	54.0	56.5	116.2	72.9	200.4	121.6	97.1	103.0	96.3	89.2	109.8	125.8
32	69.6	122.6	64.4	115.7	90.7	108.0	98.4	47.1	58.4	117.0	76.0	199.1	103.9	97.6	79.2	120.4	100.0	130.3	191.3
8	182.7	89.6	83.4	105.9	89.4	234.3	86.0	110.5	86.1	108.9	100.0	58.8	107.3	119.2	162.3	85.4	99.8	99.4	172.8
26	77.5	108.4	115.7	134.2	116.6	77.2	96.0	76.5	74.3	109.3	81.4	93.9	147.9	137.9	113.4	100.0	54.9	120.5	79.8
29	99.3	106.2	122.0	75.6	150.6	74.6	110.6	87.6	89.2	115.4		105.0	88.4	46.7	100.7	56.2	52.8	132.7	163.7
12	116.7	107.8	102.7	88.1	114.6	104.5	101.4	86.8	95.2	125.0	81.0	201.6	88.4	81.7	80.4	126.9	50.6	98.6	

Figure 3. Laboratory geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 2a; samples with study median relative potency <0.1 excluded

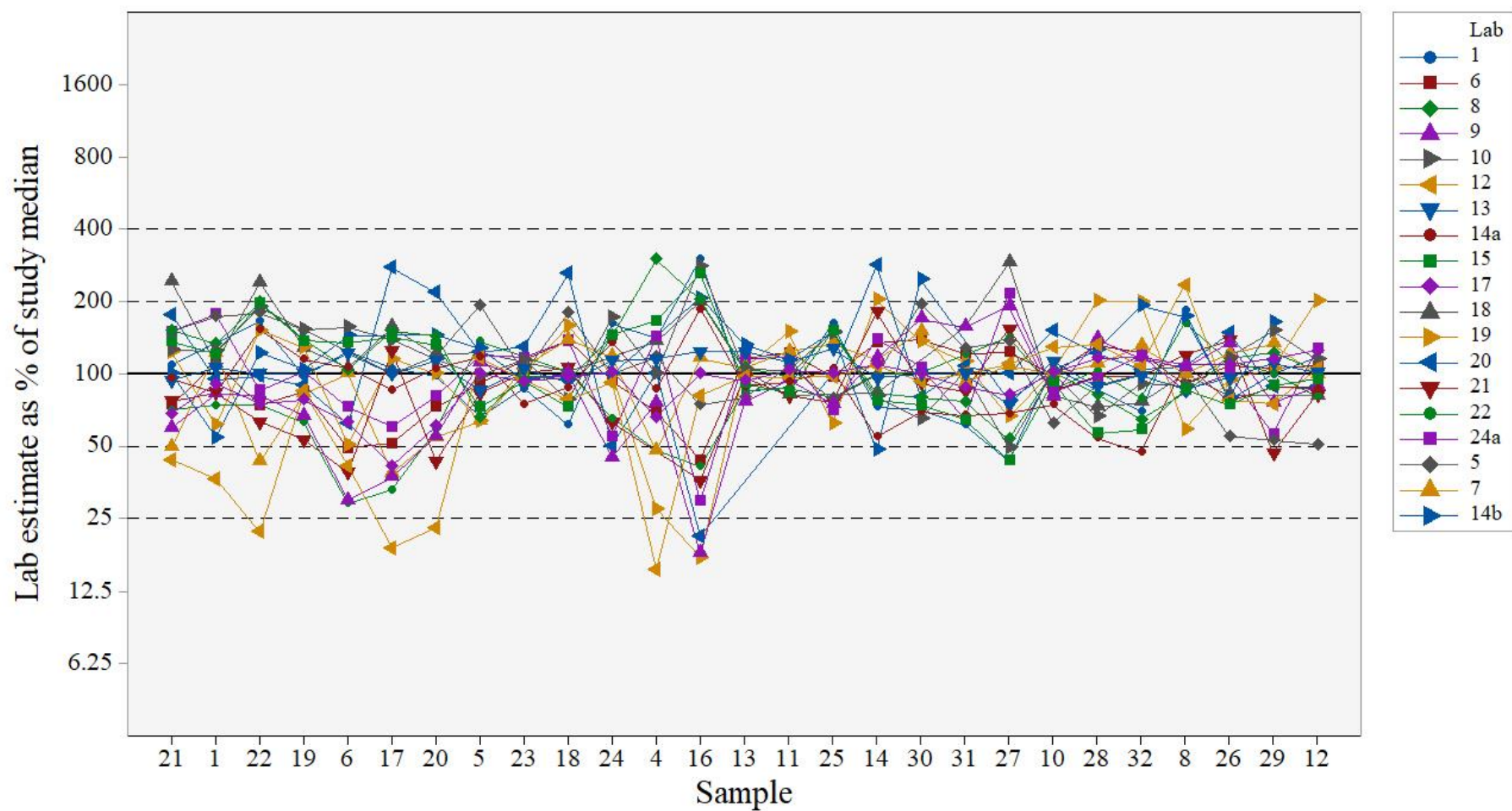


Table 14. Summary of endpoint titres and relative potency estimates for *S. flexneri* 2a; samples with study median relative potency <0.1 excluded; shading indicates where the IQR for relative potencies is less than or equal to the corresponding value for endpoint titres

Sample	Endpoint titres				Relative Potencies				N
	GM	Median	GCV	UQ/LQ	GM	Median	GCV	UQ/LQ	
21	1097	800	195%	4.0	0.115	0.105	52%	1.8	13
1	1532	1600	118%	1.3	0.130	0.130	21%	1.3	12
22	2035	1600	130%	2.0	0.196	0.165	60%	2.5	13
19	1681	1600	99%	1.7	0.163	0.167	39%	1.6	13
6	1464	1600	183%	4.0	0.144	0.201	80%	2.5	13
17	3345	3200	231%	2.4	0.306	0.348	92%	2.8	13
20	3528	3200	177%	3.0	0.347	0.392	58%	2.0	13
5	4199	3200	78%	2.0	0.380	0.392	27%	1.4	13
23	3706	4000	229%	4.9	0.464	0.461	16%	1.2	11
18	5378	6400	136%	2.0	0.522	0.505	42%	1.2	13
24	4604	4000	130%	2.8	0.565	0.620	60%	2.2	12
4	6801	8000	140%	1.8	0.627	0.604	63%	1.8	12
16	6054	8000	226%	4.8	0.660	0.858	178%	4.9	13
13	6188	6400	116%	1.1	0.686	0.677	15%	1.1	12
11	6946	6400	115%	1.4	0.798	0.787	14%	1.2	12
25	11600	11314	179%	2.4	1.071	0.999	33%	1.6	12
14	13897	16000	140%	4.0	1.091	1.019	53%	1.4	13
30	10690	11314	160%	2.8	0.970	0.911	34%	1.4	12
31	12108	13500	204%	7.9	1.560	1.628	36%	1.6	13
27	17746	16000	206%	4.0	1.851	1.650	83%	2.6	13
10	18964	16000	135%	2.0	2.104	2.179	20%	1.2	13
28	22332	20785	183%	2.4	2.002	2.065	38%	1.7	12
32	23168	32000	146%	2.1	2.099	2.255	34%	1.5	13
8	31615	32000	131%	4.0	2.910	2.883	27%	1.2	13
26	43318	32000	142%	2.0	3.932	4.123	27%	1.4	13
29	40143	45255	113%	2.8	4.139	4.314	34%	1.3	12
12	56552	64000	127%	1.0	6.205	6.112	16%	1.2	13
25/160	9482	8000	90%	2.0					13

Table 15. Lab geometric mean relative potency estimates for *S. flexneri* 3a; samples ordered by increasing study median potency; outliers identified by Grubbs' test highlighted and excluded from further analysis; empty cells indicate no valid estimates obtained

Sample	ELISA												Luminex		
	Lab														
	1	6	9	10	12	14a	15	17	18	19	20	21	5	7	14b
5	0.054	0.028	0.096	0.066	<LoQ	0.013	0.105	0.029	0.265		0.118		0.064	0.007	0.082
1	0.073	0.031	0.043	0.097	<LoQ	0.015	0.119	0.039	0.143	0.054	0.071		0.109	0.301	0.123
7	0.054	0.088	0.129	0.107	0.207	0.017	0.095	0.031	0.351	0.052	0.241		0.064	0.036	0.114
2	0.093	0.027	0.046	0.109	0.019	0.020	0.116	0.052	0.176		0.146		0.098	0.024	0.163
3	0.053	0.036	0.076	0.073	<LoQ	0.019	0.105	0.071	0.465	0.136	0.132		0.129	0.098	0.165
11	0.078	0.317	0.097	0.181	0.041	0.048	0.157	0.091	0.336	0.073	0.178		0.146	0.480	0.115
16	0.141	0.115	0.214	0.275	0.059	0.059	0.194	0.104	0.408	0.161	0.197		0.255	0.068	0.157
9	0.540	0.169	0.049	0.988	0.041	0.094	0.659	0.113	0.199		0.121		0.187	0.037	0.418
15	0.198	0.143	0.146	0.440	0.050	0.062	0.260	0.105	0.484	0.084	0.176		0.225	0.187	0.199
6	0.300	0.140	0.094	0.345	0.034	0.084	0.314	0.190	0.306	0.147	0.191		0.213	0.071	0.205
8	0.496	0.059	0.237	0.707	0.169	0.165	0.456	0.352	0.826	0.061	0.363		0.248	0.041	0.224
21	0.315	0.152	0.167	0.473	0.097	0.127	0.344	0.204	0.486	0.264	0.369	0.251	0.290	0.609	0.288
19	0.334	0.325	0.282	0.392	0.371	0.196	0.391	0.278	0.356	0.390	0.293	0.241	0.304	0.431	0.233
10	0.330	0.459	0.702	0.529	0.245	0.219	0.418	0.292	0.374	0.384	0.744		0.484	1.123	0.342
13	0.470	0.127	0.121	0.987	0.060		1.044	0.183	0.599		0.127		0.405	0.615	0.841
23	0.645	0.337	0.317	1.079	1.071	0.400	0.787	0.424	0.698	0.947	0.657	0.389	0.476	0.380	0.434
24	1.073	0.792	0.280	2.590	0.368	0.490	1.274	0.618	0.630	1.101	0.365	0.179	0.296	0.148	0.428
17	1.151	0.678	0.428	1.756	0.224	0.355	1.530	0.605	0.531	0.750	0.902		0.675	1.199	
18	0.630	0.901	0.666	1.008	0.555	1.032	1.401	0.707	0.736	0.471	0.794	0.972	0.756	0.489	0.667
20	1.302	0.784	0.552	2.382	0.324	0.554	1.164	0.817	1.090	0.875	0.806	0.483	0.765	1.201	1.249
26	0.360	1.298	1.183	0.585	0.878	0.544	0.485	0.715	1.955	1.082	1.203	0.584	0.873	3.012	0.573
27	2.386	0.887	0.649		1.534	1.289	2.372	1.117	8.010*	0.400	0.538	1.072	0.716	1.049	1.307
25	2.215	1.551	0.589		1.715	1.425	1.589	1.334	8.320	0.761	3.031	0.528	1.059	1.781	
4	2.667	1.784	1.470	2.885	1.030	1.116	2.475	1.662	1.572		1.985		1.430	1.878	4.379
22	0.438	1.672	1.117		3.277	3.053	2.135	3.300	1.526	4.870	2.905	0.252	1.480	1.215	3.017
12	0.931	3.752	0.442		8.941	6.824	2.699	8.137	2.026		5.067		3.279	4.791	1.601
14	1.112	5.188	5.778		4.246	1.128	0.732	2.241	0.878	4.576	4.090		2.781	7.377	4.165

Figure 4. Boxplot of laboratory geometric mean relative potency estimates for *S. flexneri* 3a

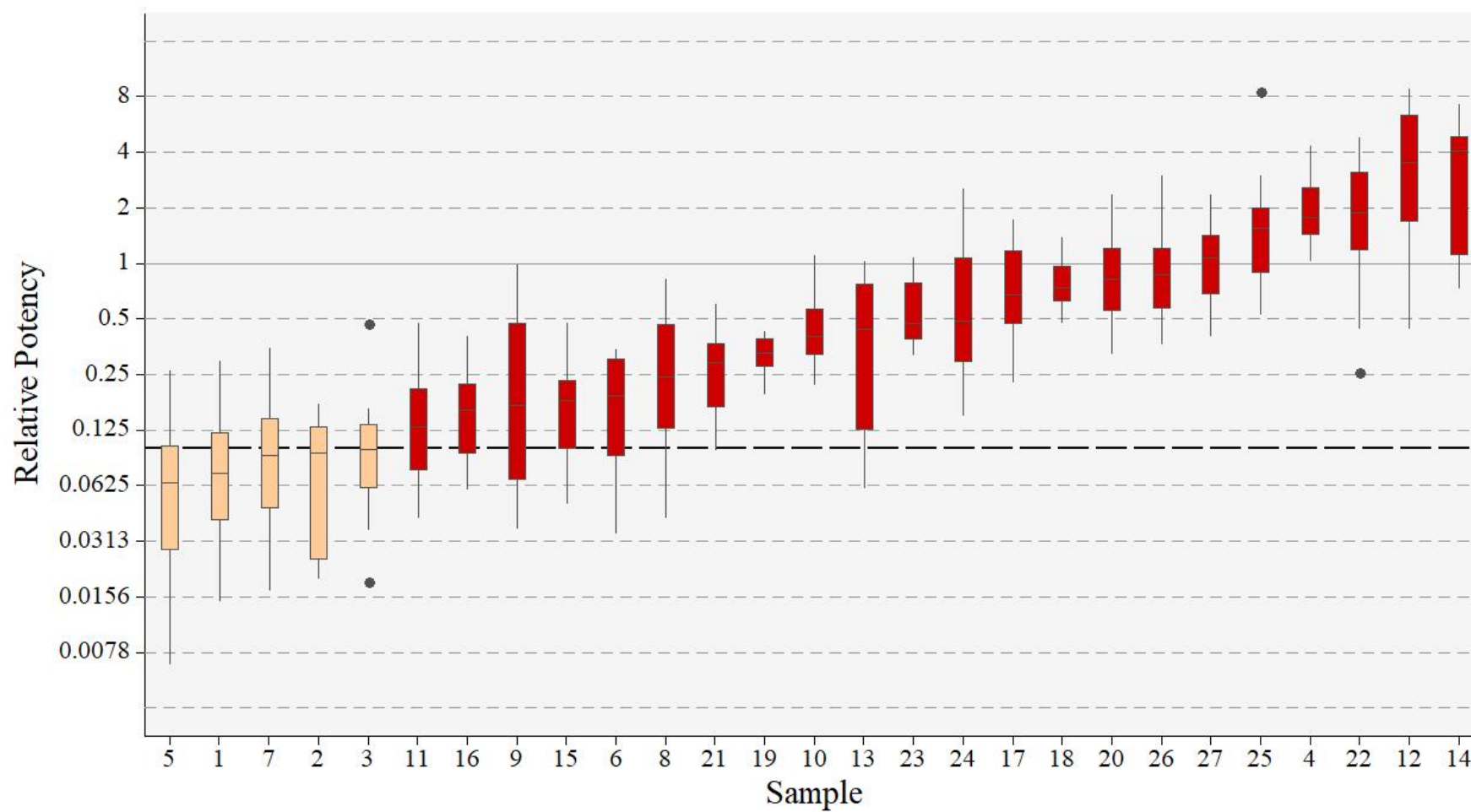


Table 16. Summary statistics for laboratory geometric mean relative potency estimates for *S. flexneri* 3a

Sample	GM	Median	GCV	UQ/LQ	N
5	0.052	0.065	177%	3.4	12
1	0.072	0.073	118%	2.8	13
7	0.083	0.091	131%	2.4	14
2	0.063	0.093	130%	4.4	13
3	0.090	0.098	118%	1.9	13
11	0.131	0.130	106%	2.2	14
16	0.148	0.159	80%	2.0	14
9	0.168	0.169	191%	4.5	13
15	0.163	0.181	93%	1.9	14
6	0.158	0.190	96%	2.7	14
8	0.226	0.242	152%	2.6	14
21	0.263	0.288	68%	1.9	15
19	0.314	0.325	25%	1.4	15
10	0.430	0.401	56%	1.6	14
13	0.324	0.436	164%	5.2	12
23	0.554	0.476	52%	1.9	15
24	0.531	0.490	117%	2.8	15
17	0.714	0.678	80%	2.2	13
18	0.753	0.736	35%	1.4	15
20	0.856	0.817	63%	1.8	15
26	0.868	0.873	76%	2.1	15
27	1.038	1.072	70%	1.8	13
25	1.503	1.551	105%	1.7	13
4	1.873	1.784	49%	1.7	13
22	1.701	1.890	128%	2.4	14
12	3.001	3.507	147%	2.9	12
14	2.680	4.090	119%	4.1	13

Table 17. Lab geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 3a; samples with study median relative potency <0.1 excluded; values within 50-200% highlighted; empty cells indicate no valid estimates obtained

Sample	ELISA												Luminex		
	Lab														
	1	6	9	10	12	14a	15	17	18	19	20	21	5	7	14b
11	59.9	244.5	74.7	139.7	31.8	37.1	121.0	70.3	259.7	56.6	137.1		112.9	370.3	88.6
16	88.5	72.4	134.3	172.5	37.3	37.2	121.5	65.3	255.8	101.3	123.7		159.9	42.8	98.7
9	318.9	100.0	28.9	582.8	24.3	55.3	388.9	66.9	117.5		71.2		110.2	21.6	246.4
15	109.3	78.7	80.6	242.7	27.4	34.2	143.5	58.1	266.6	46.4	97.0		124.2	103.1	109.7
6	157.9	73.6	49.3	181.5	17.9	44.2	164.9	99.9	160.6	77.3	100.1		112.2	37.1	108.0
8	204.5	24.2	97.9	291.4	69.6	68.0	187.9	145.0	340.7	25.0	149.6		102.1	17.1	92.3
21	109.2	52.7	58.0	163.9	33.5	44.2	119.5	70.8	168.6	91.7	127.9	87.0	100.4	211.2	100.0
19	102.8	100.0	86.7	120.6	114.3	60.2	120.3	85.5	109.4	119.9	90.1	74.1	93.6	132.7	71.8
10	82.3	114.6	175.3	132.0	61.1	54.8	104.3	72.9	93.3	95.9	185.8		120.9	280.4	85.4
13	107.8	29.1	27.8	226.2	13.9		239.1	41.9	137.2		29.2		92.8	140.9	192.6
23	135.7	70.9	66.6	226.9	225.2	84.0	165.5	89.3	146.9	199.2	138.1	81.7	100.0	79.9	91.3
24	219.0	161.7	57.1	528.6	75.2	100.0	260.1	126.1	128.6	224.9	74.6	36.5	60.5	30.3	87.4
17	169.8	100.0	63.2	259.2	33.0	52.5	225.8	89.3	78.4	110.7	133.1		99.7	176.9	
18	85.7	122.5	90.5	137.0	75.4	140.3	190.4	96.0	100.0	64.0	107.9	132.1	102.8	66.5	90.6
20	159.3	95.9	67.5	291.4	39.6	67.7	142.4	100.0	133.4	107.1	98.6	59.1	93.7	146.9	152.9
26	41.2	148.6	135.5	67.0	100.5	62.3	55.6	81.9	223.9	123.9	137.7	66.9	100.0	344.9	65.6
27	222.7	82.8	60.5		143.1	120.3	221.3	104.3		37.3	50.2	100.0	66.8	97.9	121.9
25	142.9	100.0	38.0		110.6	91.9	102.5	86.0	536.6	49.1	195.5	34.1	68.3	114.9	
4	149.5	100.0	82.4	161.8	57.8	62.6	138.8	93.2	88.2		111.3		80.2	105.3	245.5
22	23.2	88.5	59.1		173.4	161.6	113.0	174.6	80.8	257.7	153.8	13.3	78.3	64.3	159.7
12	26.5	107.0	12.6		254.9	194.6	77.0	232.0	57.8		144.5		93.5	136.6	45.6
14	27.2	126.8	141.3		103.8	27.6	17.9	54.8	21.5	111.9	100.0		68.0	180.4	101.8

Figure 5. Laboratory geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 3a; samples with study median relative potency <0.1 excluded

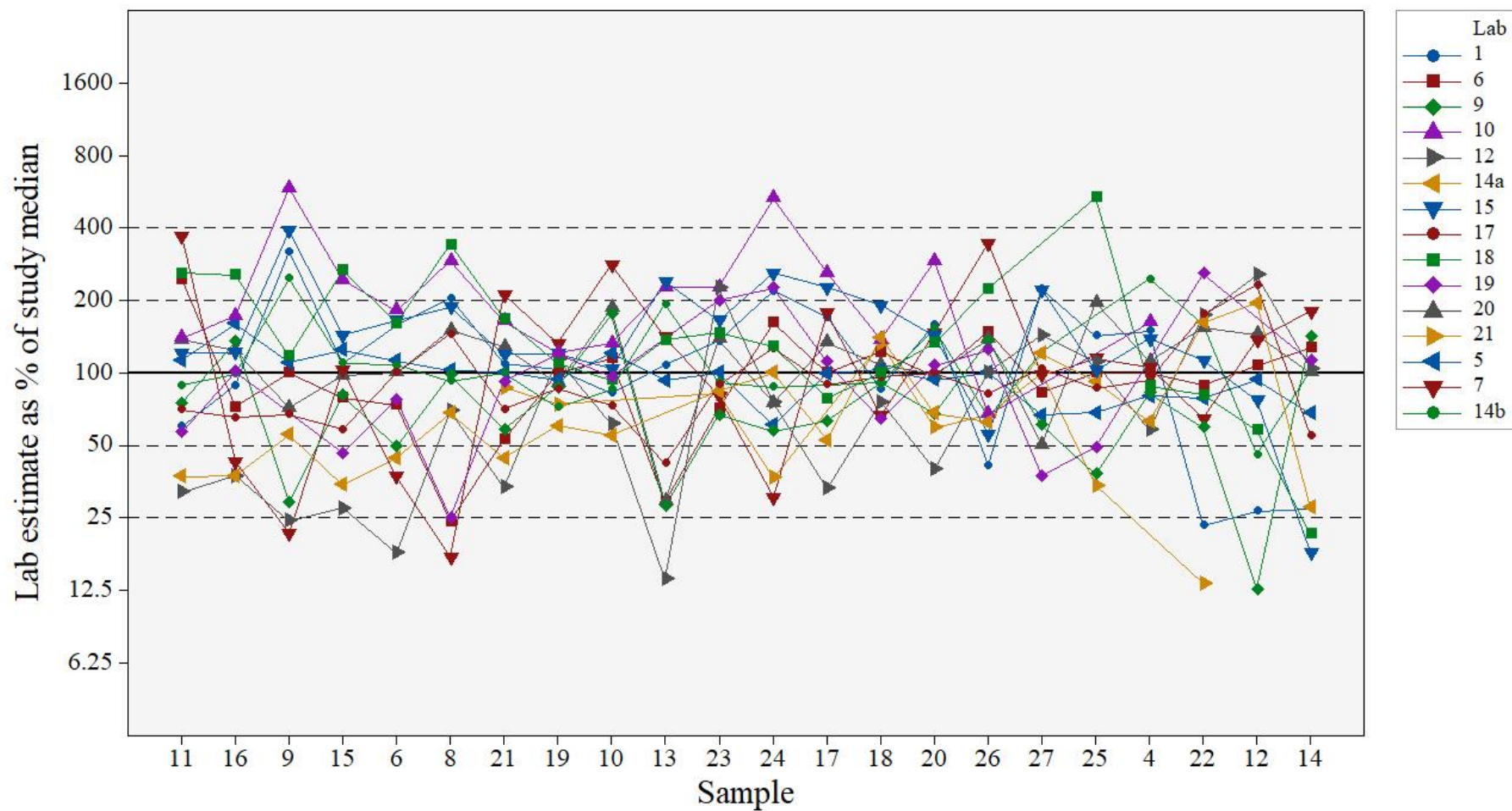


Table 18. Summary of endpoint titres and relative potency estimates for *S. flexneri* 3a; samples with study median relative potency <0.1 excluded; shading indicates where the IQR for relative potencies is less than or equal to the corresponding value for endpoint titres

Sample	Endpoint titres				Relative Potencies				N
	GM	Median	GCV	UQ/LQ	GM	Median	GCV	UQ/LQ	
11	534	400	362%	8.0	0.138	0.157	92%	2.0	9
16	631	400	232%	2.0	0.165	0.194	77%	1.9	9
9	821	1600	248%	4.0	0.209	0.169	174%	4.8	9
15	704	800	316%	4.0	0.186	0.176	93%	1.8	9
6	821	800	149%	4.0	0.194	0.191	71%	2.2	9
8	1541	1600	149%	4.0	0.323	0.363	125%	2.1	9
21	914	800	207%	2.8	0.262	0.281	61%	2.1	10
19	941	800	147%	2.0	0.302	0.309	24%	1.3	10
10	1761	1600	138%	4.0	0.421	0.418	49%	1.6	9
13	1156	1265	240%	2.8	0.318	0.293	156%	5.3	8
23	1777	1600	149%	2.8	0.531	0.523	51%	1.8	10
24	2344	2263	201%	5.7	0.629	0.624	118%	2.5	10
17	2853	3200	179%	4.0	0.767	0.678	75%	2.2	9
18	3633	3200	161%	2.4	0.860	0.846	28%	1.4	10
20	2693	3200	139%	3.4	0.886	0.811	62%	1.9	10
26	3530	1600	363%	5.7	0.781	0.647	71%	2.2	10
27	6075	6400	243%	3.3	1.133	1.094	72%	1.8	8
25	6864	6400	321%	10.0	1.630	1.551	129%	1.7	9
4	8474	12800	154%	2.5	1.877	1.784	36%	1.6	9
22	4704	6400	230%	8.0	1.392	1.672	145%	2.6	9
12	8234	9051	187%	4.7	2.642	3.182	173%	3.3	8
14	5737	4525	239%	4.2	1.964	1.590	131%	4.1	8
25/160	3982	3200	97%	2.4					10

Table 19. Lab geometric mean relative potency estimates for *S. flexneri* 6; samples ordered by increasing study median potency; outliers identified by Grubbs' test highlighted and excluded from further analysis; empty cells indicate no valid estimates obtained

Sample	ELISA											Luminex		
	Lab													
	1	6	9	10	13	14a	15	17	18	19	20	5	7	14b
3	0.011	0.003	0.007	0.012	0.085	0.015	0.076	0.010	0.235		0.037	0.038	0.000	0.117
7	0.024	0.018	0.012	0.033	0.211	0.013	0.070	0.019	0.160	0.065	0.034	0.106	0.012	0.111
1	0.038	0.007	0.008	0.035	0.139	0.029	0.125	0.037	0.133	0.046	0.173	0.083	0.001	0.101
21	0.051	0.026	0.020	0.057	0.342	0.059	0.178	0.067	0.277	0.081	0.876	0.156	0.020	0.231
15	0.066	0.019	0.017	0.084	0.251	0.081	0.258	0.063	0.312	0.072	0.034	0.132	0.005	0.194
19	0.074	0.043	0.054	0.085	0.341	0.059	0.166	0.098	0.236	0.097	0.056	0.220	0.050	0.265
6	0.093	0.040	0.038	0.112	0.448	0.061	0.195	0.087	0.236	0.165	0.045	0.269	0.057	0.486
14	0.078	0.035	0.078	0.111	0.251	0.055	0.115	0.065	0.456	0.173	0.116	0.280	0.083	0.209
5	0.108	0.123	0.126	0.138	0.139	0.135	0.114	0.086	0.170	0.138	0.116	0.286*	0.122	0.114
9	0.117	0.036	0.012	0.184	0.813	0.046	0.669	0.097	0.259	0.182	0.039	0.333	0.025	0.792
24	0.708	0.124	0.061	0.111	0.192	0.043	0.250	0.206	0.338	0.480	0.110	0.209	0.067	0.330
17	0.171	0.105	0.059	0.262	1.190	0.119	0.483	0.203	0.807	0.371	0.151	0.326	0.032	0.659
22	0.144	0.062	0.081	0.145	0.354	0.259	0.633	0.246	0.343	0.185	0.865	0.221	0.022	0.386
20	0.218	0.115	0.080	0.211	1.309	0.202	0.634	0.263	0.539	0.379	0.153	0.392	0.047	0.851
18	0.164	0.194	0.192	0.260	0.421	0.284	0.250	0.217	0.589	0.452	0.173	0.503	0.189	0.263
2	0.369	0.511	0.383		0.274	0.586	0.298	0.298	0.246	0.637	0.232	0.626	0.368	0.341
16	0.529	0.244	0.371	0.375	0.406	0.415	0.331	0.255	0.666	0.385	0.350	0.749	0.372	0.371
10	0.512	0.236	0.337	0.583	1.149	0.485	0.689	0.478	0.394	0.319	0.238	0.675	0.451	0.783
23	0.058*	0.515	0.420	0.593	0.763	0.618	0.606	0.479	0.600	0.883	0.376	0.728	0.434	0.715
4	0.812	0.239	0.163	0.681	3.273	0.510	1.519	0.586	1.189	1.114	0.250	0.724	0.060	2.436
12	2.145	1.279	1.200	1.816	2.311	1.944	1.736	2.231	0.980		2.179	1.521	1.815	
25	2.274		1.818	2.405	1.690	4.370*	1.683	2.240	2.369		2.038	1.858	2.677	
26	2.752	2.825	2.707	2.279	1.576	6.472*	2.358	2.760	2.687		3.287	1.892	3.218	3.492
11	4.207	3.909		2.796	1.455	5.122	2.393	3.561	1.924		3.049	2.058	3.189	1.290
8	4.384	3.691	2.724	2.934	1.676	6.847	2.737	3.536	1.122		2.737	2.088	3.323	3.273
27	4.307	3.634	3.504	3.519	2.357	6.712	2.627	4.026	5.490		3.110	2.449	3.932	
13	4.228	3.011	2.276	3.836	3.635		4.504	3.679	1.461	2.528	3.444	1.785	3.899	9.835

Figure 6. Boxplot of laboratory geometric mean relative potency estimates for *S. flexneri* 6

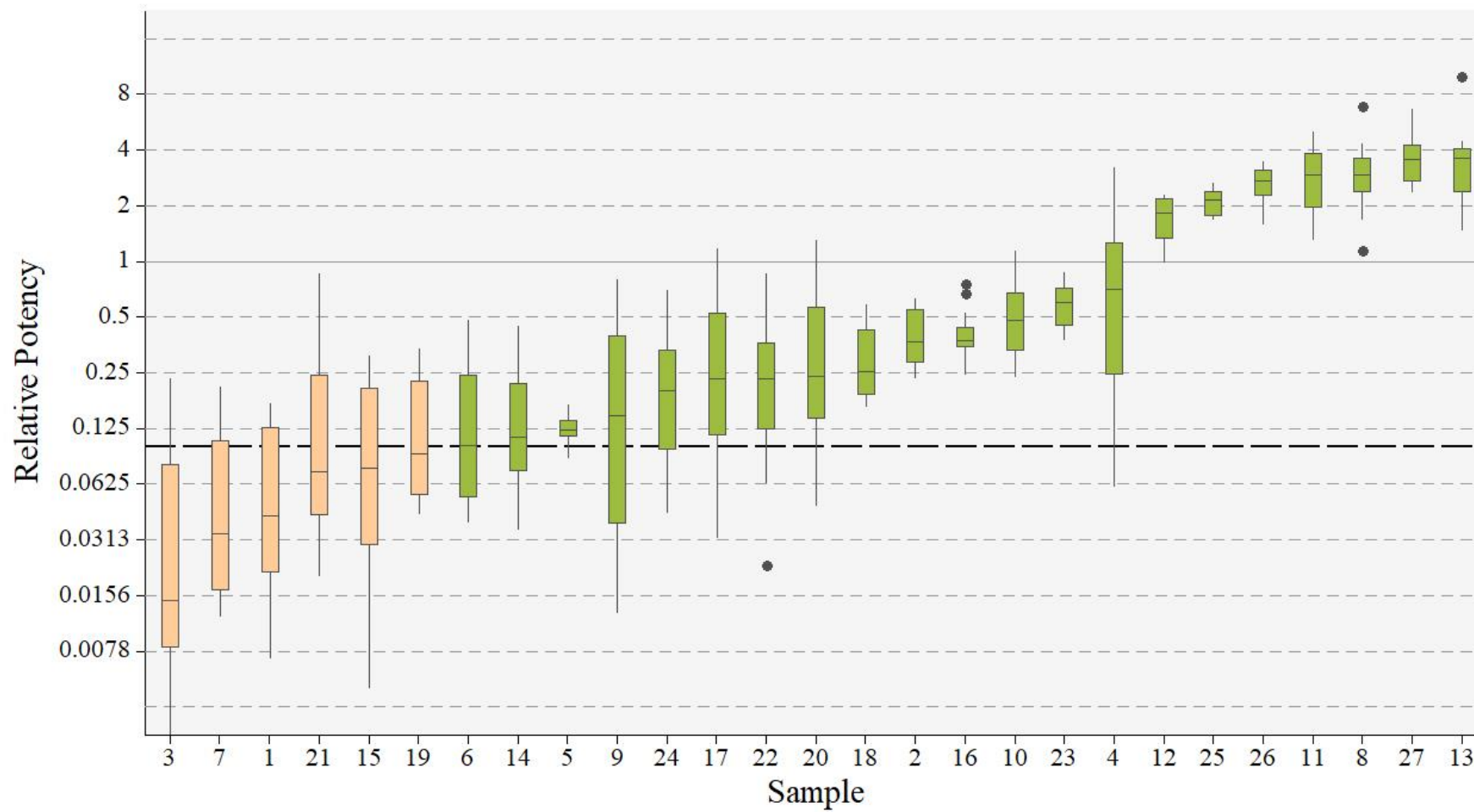


Table 20. Summary statistics for laboratory geometric mean relative potency estimates for *S. flexneri* 6

Sample	GM	Median	GCV	UQ/LQ	N
3	0.019	0.015	478%	7.3	13
7	0.040	0.033	171%	5.2	14
1	0.038	0.042	306%	3.9	14
21	0.095	0.073	211%	4.1	14
15	0.069	0.077	228%	4.4	14
19	0.104	0.091	101%	3.6	14
6	0.117	0.102	138%	3.9	14
14	0.119	0.113	103%	2.6	14
5	0.124	0.123	18%	1.2	13
9	0.127	0.146	282%	7.7	14
24	0.173	0.199	125%	2.8	14
17	0.230	0.231	176%	3.6	14
22	0.199	0.233	160%	2.4	14
20	0.267	0.240	151%	3.0	14
18	0.271	0.255	53%	2.0	14
2	0.375	0.368	42%	1.7	13
16	0.396	0.374	36%	1.2	14
10	0.476	0.482	57%	1.9	14
23	0.577	0.600	29%	1.5	13
4	0.616	0.702	196%	3.9	14
12	1.707	1.815	32%	1.5	12
25	2.081	2.136	18%	1.3	10
26	2.593	2.729	26%	1.2	12
11	2.692	2.920	53%	1.8	12
8	2.895	2.934	56%	1.3	13
27	3.634	3.576	37%	1.4	12
13	3.315	3.635	60%	1.5	13

Table 21. Lab geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 6; samples with study median relative potency <0.1 excluded; values within 50-200% highlighted; empty cells indicate no valid estimates obtained

Sample	ELISA											Luminex		
	Lab													
	1	6	9	10	13	14a	15	17	18	19	20	5	7	14b
6	90.9	38.9	37.8	110.0	439.9	59.6	191.9	85.2	232.4	162.6	44.5	264.0	55.8	478.1
14	69.2	31.1	69.0	98.4	222.6	48.5	101.7	57.6	403.9	153.8	102.9	248.0	73.7	185.5
5	87.2	100.0	102.3	111.7	112.7	109.1	92.5	69.9	137.7	112.1	93.6		98.6	92.2
9	80.2	24.4	8.5	126.2	556.4	31.8	457.9	66.4	177.4	124.7	26.8	228.1	17.3	542.2
24	356.2	62.5	30.8	56.0	96.4	21.7	125.7	103.8	170.0	241.6	55.6	105.1	33.5	166.2
17	74.1	45.7	25.5	113.5	515.7	51.7	209.1	88.1	349.7	160.8	65.3	141.3	13.7	285.6
22	61.6	26.8	34.7	62.0	151.8	111.2	271.7	105.6	147.0	79.4	371.2	94.7	9.6	165.7
20	91.1	48.1	33.4	88.0	546.3	84.4	264.7	109.7	225.1	158.1	64.0	163.5	19.7	355.2
18	64.2	76.2	75.2	102.1	165.1	111.5	98.0	85.1	231.0	177.2	67.7	197.3	74.1	103.1
2	100.0	138.7	103.9		74.3	159.1	81.0	80.8	66.7	172.9	62.9	169.9	100.0	92.5
16	141.6	65.2	99.4	100.4	108.8	111.1	88.6	68.1	178.2	103.0	93.7	200.6	99.6	99.3
10	106.3	49.0	70.0	121.1	238.7	100.8	143.1	99.2	81.8	66.2	49.4	140.1	93.6	162.6
23		85.8	70.0	98.8	127.2	103.0	101.0	79.9	100.0	147.2	62.7	121.5	72.4	119.2
4	115.6	34.1	23.3	97.0	466.2	72.7	216.4	83.5	169.4	158.7	35.6	103.1	8.5	347.0
12	118.2	70.4	66.1	100.0	127.3	107.1	95.7	122.9	54.0		120.0	83.8	100.0	
25	106.4		85.1	112.6	79.1		78.8	104.8	110.9		95.4	87.0	125.3	
26	100.8	103.5	99.2	83.5	57.7		86.4	101.1	98.4		120.4	69.3	117.9	127.9
11	144.1	133.9		95.8	49.8	175.4	82.0	121.9	65.9		104.4	70.5	109.2	44.2
8	149.5	125.8	92.9	100.0	57.1	233.4	93.3	120.5	38.2		93.3	71.2	113.3	111.6
27	120.4	101.6	98.0	98.4	65.9	187.7	73.5	112.6	153.5		87.0	68.5	110.0	
13	116.3	82.8	62.6	105.5	100.0		123.9	101.2	40.2	69.5	94.7	49.1	107.2	270.5

Figure 7. Laboratory geometric mean relative potency estimates expressed as % of study median value for *S. flexneri* 6; samples with study median relative potency <0.1 excluded

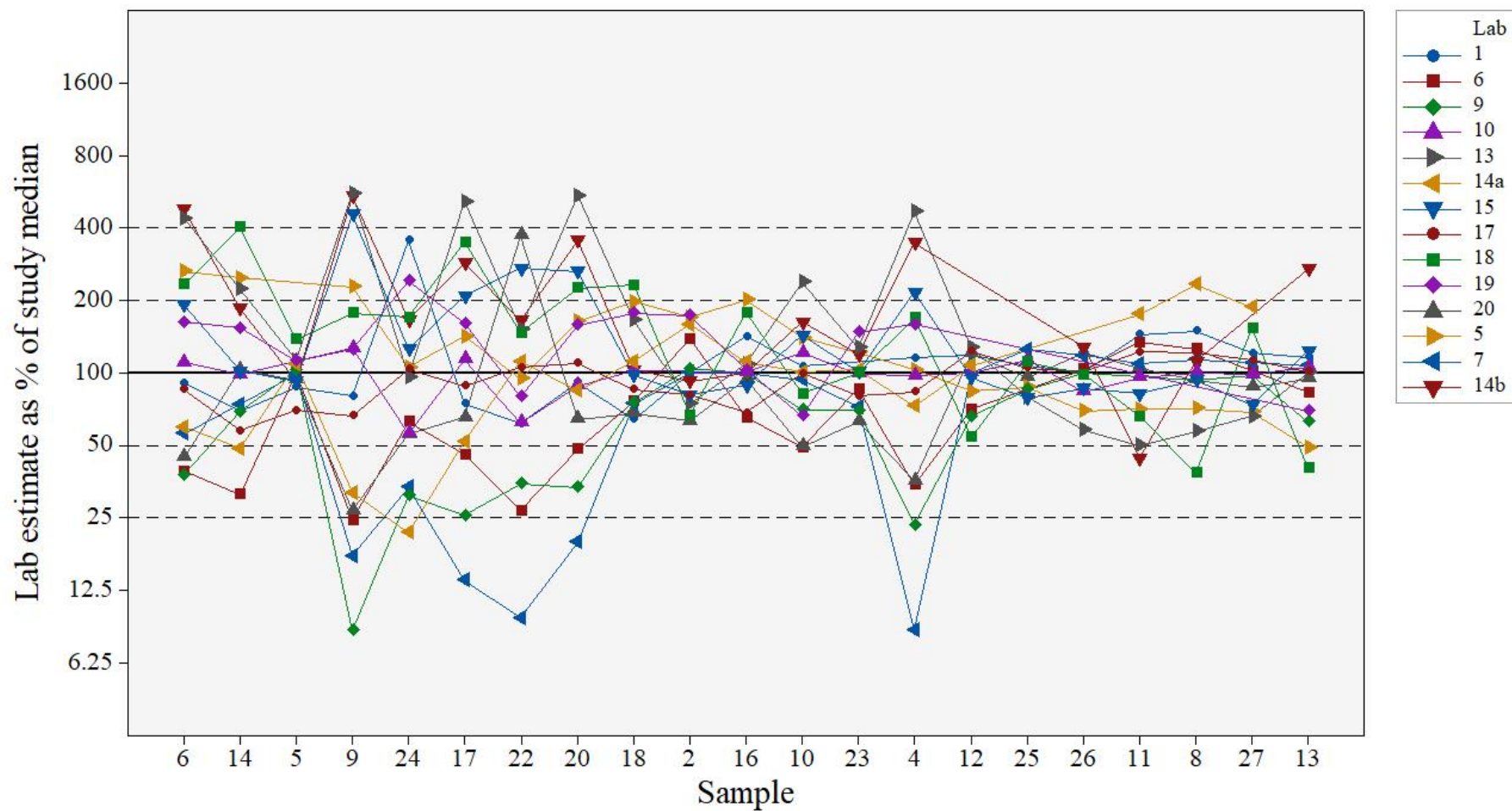


Table 22. Summary of endpoint titres and relative potency estimates for *S. flexneri* 6; samples with study median relative potency <0.1 excluded; shading indicates where the IQR for relative potencies is less than or equal to the corresponding value for endpoint titres

Sample	Endpoint titres				Relative Potencies				N
	GM	Median	GCV	UQ/LQ	GM	Median	GCV	UQ/LQ	
6	1485	1600	121%	1.0	0.097	0.090	128%	3.5	10
14	1233	1600	216%	3.3	0.103	0.093	110%	1.7	10
5	2100	2078	133%	2.0	0.124	0.125	20%	1.2	10
9	1592	1600	202%	5.0	0.109	0.107	282%	5.8	10
24	2041	1600	259%	3.4	0.158	0.154	127%	2.2	10
17	2951	3200	189%	2.8	0.233	0.187	157%	3.3	10
22	3390	3200	208%	2.8	0.232	0.253	132%	2.4	10
20	3234	3578	198%	3.4	0.262	0.214	132%	2.7	10
18	3163	3200	149%	3.4	0.252	0.233	50%	1.4	10
2	3711	6400	172%	2.4	0.339	0.298	38%	1.4	9
16	4902	6400	194%	2.0	0.378	0.373	35%	1.2	10
10	6736	7200	127%	3.4	0.458	0.482	62%	1.6	10
23	7903	12800	165%	5.0	0.541	0.593	24%	1.3	9
4	9256	12800	160%	2.4	0.628	0.632	151%	3.6	10
12	26599	32000	143%	3.4	1.717	1.879	35%	1.6	10
25	22582	27885	141%	2.4	2.045	2.136	16%	1.3	8
26	36996	51200	145%	4.0	2.536	2.707	23%	1.2	9
11	35207	25600	147%	4.0	2.954	3.049	49%	1.6	9
8	41985	40477	167%	5.8	2.914	2.834	64%	1.3	10
27	47918	64000	142%	3.5	3.751	3.576	37%	1.3	10
13	60911	64000	152%	4.0	3.185	3.635	42%	1.3	9
25/160	12285	16000	124%	1.8					10

Table 23. Lab geometric mean relative potency estimates for *S. sonnei*; samples ordered by increasing study median potency; outliers identified by Grubbs' test highlighted and excluded from further analysis; empty cells indicate no valid estimate obtained

Sample	ELISA																	Luminex		
	Lab																			
	1	6	8	9	10	12	13	14a	15	17	18	19	20	21	22	24a	24b	5	7	14b
5	0.005	0.004		0.006	0.007	0.008	0.026		0.026	0.005	0.096	0.036	0.049	0.010		0.050	0.014	0.034	0.004	0.044
2		0.002		0.005	0.005	0.007	0.026		0.020		0.050	0.050	0.031	0.010		0.022	0.014	0.022	0.002	0.073
14	0.019	0.003	0.039	0.012	0.018	0.009	0.082	0.004	0.065	0.008	0.194	0.414	0.203	0.013	0.011	0.013	0.025	0.044	0.023	0.108
31	0.021	0.004	0.096	0.017	0.013	0.009	0.044	0.004	0.071	0.005	0.350	0.061	0.105	0.011		0.037	0.022	0.047	0.013	0.079
30	0.044	0.006	0.090	0.012	0.036	0.008	0.022	0.004	0.135	0.009	0.176	0.147	0.200	0.007		0.009		0.028	0.008	0.079
3	0.035	0.002	0.042	0.008	0.034	0.008	0.025		0.171	0.004	0.171		0.095	0.015		0.015	0.053	0.020	0.006	0.096
7	0.025	0.012	0.047	0.010	0.020	0.006	0.057		0.103	0.005	0.104	0.139				0.016	0.035	0.030	0.002	0.059
8	0.061	0.002		0.004	0.054	0.008	0.026	0.012	0.197			0.041	0.069	0.011		0.004	0.078	0.036	0.003	0.051
12	0.032	0.004	0.128	0.015	0.038	0.008		0.007	0.154		0.124	0.157	0.213	0.032		0.004	0.053	0.027	0.021	0.008
1	0.057	0.005	0.109	0.010	0.049	0.009	0.042	0.007	0.228	0.008	0.067	0.139	0.044	0.012		0.027	0.076	0.037	0.003	
28	0.048	0.034	0.078	0.041	0.039	0.021	0.055	0.024	0.118	0.029	0.249	0.100	0.101	0.026	0.025	0.018		0.103	0.028	0.071
11	0.031	0.037	0.067	0.016	0.035	0.015		0.012	0.109		0.148	0.332	0.103	0.016		0.305	0.050	0.044	0.016	0.064
6	0.043	0.011	0.097	0.008	0.043	0.012	0.118	0.007	0.181	0.011	0.125	0.556	0.052	0.019		0.057	0.077	0.175	0.005	0.206
10	0.066	0.009	0.134	0.021	0.055	0.026		0.030	0.194	0.032	0.182	0.085	0.197	0.024		0.145	0.098	0.129	0.053	0.244
15	0.124	0.013	0.346	0.023	0.123	0.014	0.112	0.027	0.415	0.026	0.175	0.415	0.143	0.030		0.064	0.190	0.059	0.028	0.079
32	0.073	0.032	0.155	0.066	0.066	0.065	0.282	0.027	0.185	0.040	0.359	0.303	0.328	0.046	0.019	0.185	0.110	0.169	0.087	0.058
9	0.238		0.336	0.014	0.327	0.007		0.020	0.270	0.023	0.135		0.094	0.020		0.019	0.475	0.070	0.025	0.113
19	0.082	0.051	0.129	0.040	0.079	0.052	0.209	0.051	0.161	0.052	0.233	0.337	0.098	0.049	0.043	0.123	0.181	0.163	0.038	0.221
21	0.074	0.024	0.129	0.023	0.093	0.027	0.650	0.028	0.263	0.029	0.185	0.360	0.179	0.041	0.018	0.093	0.091	0.122	0.017	0.160
16	0.078	0.007*	0.139	0.042	0.091	0.094	0.192	0.043	0.178	0.091	0.253	0.128	0.216	0.122	0.096	0.075	0.069	0.187	0.053	0.171
29	0.109	0.012	0.148	0.015	0.098	0.017	0.122	0.019	0.460	0.020	0.357	0.292	0.188			0.160		0.111	0.015	0.090
17	0.182	0.065	0.261	0.051	0.945	0.018	0.189	0.039	0.600	0.047	0.367	2.699	0.730	0.067	0.026	0.113	0.387	0.130	0.068	
22	0.341	0.118	0.404	0.118	0.368	0.040	0.198	0.169	1.071	0.116	0.302	1.119	0.179	0.107	0.085	0.113	0.449	0.259	0.088	0.269
26	0.240	0.171	1.509	0.170	0.189	0.163	0.308	0.188	0.375	0.249	0.710	0.466	1.080	0.127	0.254	0.209	0.288	0.379	0.255	0.287
20	0.273	0.189	0.444	0.129	0.341	0.069	0.478	0.193	0.873	0.168	0.449	2.665	0.494	0.169	0.101	0.248	0.723	0.335	0.225	
23	0.428	0.306	0.554	0.267	0.438	0.666	0.568	0.711	0.653	0.488	0.263	1.208	0.594	0.714	0.553	0.729	2.327*	0.700	0.501	0.760
24	0.537	0.521	0.665	0.375	0.591	0.679	0.710	0.579	0.734	0.512	0.288*	5.016*	0.670	0.571	0.144	0.575	0.603	0.675	0.508	0.608
4	0.650	0.222	2.734	0.263	0.789	0.094	1.069	0.049	2.988	0.262	0.967		0.882	0.313	0.938	0.645	2.116	0.510	0.308	2.049
25	4.891*	0.422	0.844	0.303	0.489	0.977	0.776	0.719	1.060	0.788	0.524		1.285	0.791	0.615	0.596	0.757	0.812	0.664	0.502
13	2.319		2.733	2.450	3.466	2.460	2.494	3.141	1.457	2.978	2.412	3.571	1.919	0.702	2.422	9.304	2.974	1.911	2.533	3.032
18	4.448	5.932	4.497		5.819	3.723	3.412	4.686	3.140	4.097	6.308	1.956	4.106	8.228	3.539	5.362	3.470	2.525	4.687	
27	9.775	1.891	0.748		2.899	5.598	4.853	3.003	4.807	5.402	10.609		3.230	5.739	5.236	4.901	0.943	2.542	4.094	8.015

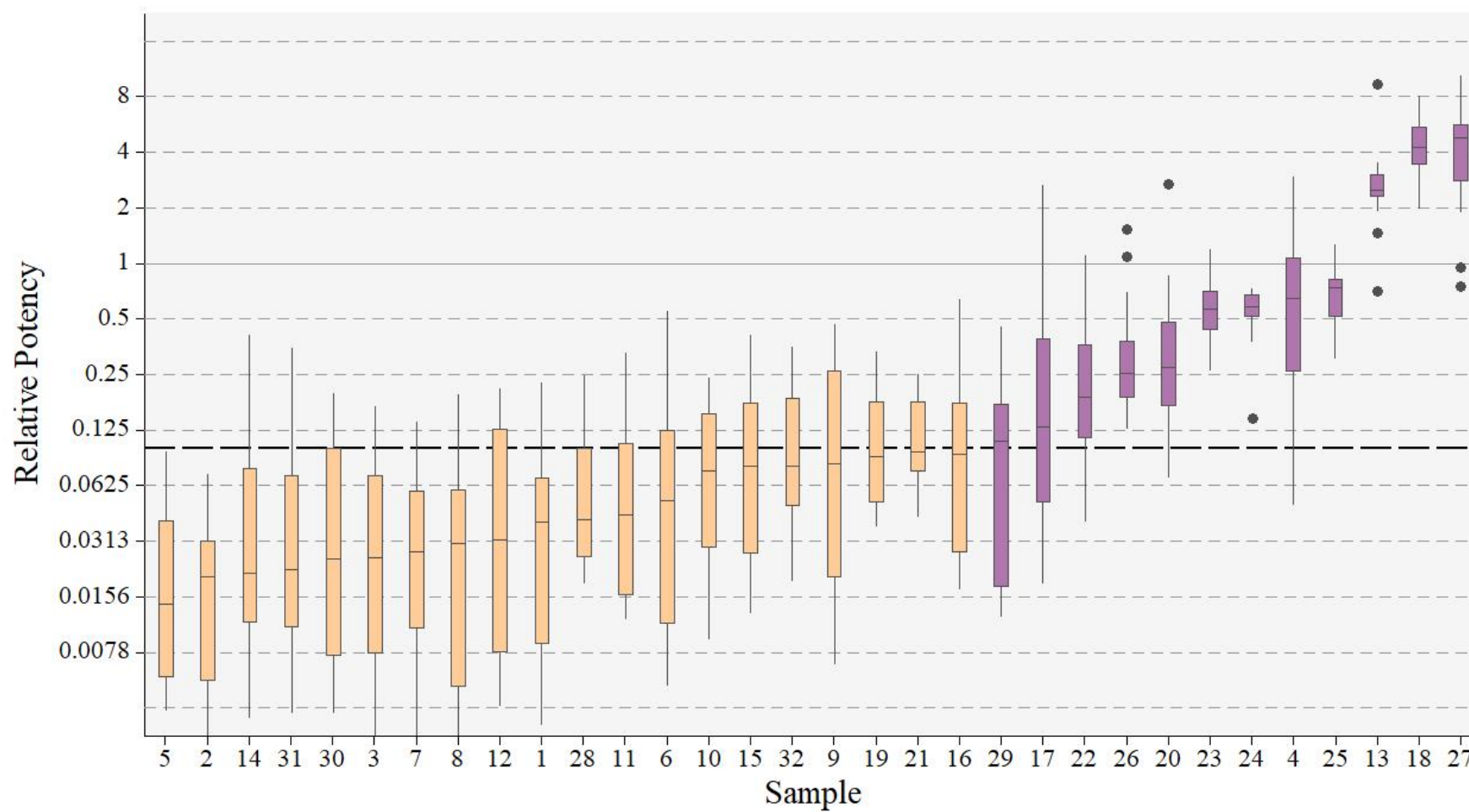
Figure 8. Boxplot of laboratory geometric mean relative potency estimates for *S. sonnei*

Table 24. Summary statistics for laboratory geometric mean relative potency estimates for *S. sonnei*

Sample	GM	Median	GCV	UQ/LQ	N
5	0.015	0.014	185%	5.8	17
2	0.014	0.020	196%	4.6	15
14	0.028	0.021	275%	5.8	20
31	0.026	0.022	246%	5.5	19
30	0.027	0.025	280%	11.1	18
3	0.024	0.025	268%	6.7	17
7	0.024	0.027	235%	4.9	16
8	0.020	0.030	286%	8.3	16
12	0.030	0.032	274%	15.5	17
1	0.028	0.040	244%	6.9	18
28	0.049	0.041	105%	3.3	19
11	0.048	0.044	182%	6.3	17
6	0.044	0.052	285%	10.4	19
10	0.067	0.075	158%	4.6	18
15	0.074	0.079	204%	5.8	19
32	0.095	0.080	141%	3.4	20
9	0.066	0.082	289%	12.2	16
19	0.095	0.090	100%	3.3	20
21	0.076	0.092	192%	5.9	20
16	0.107	0.096	71%	2.3	19
29	0.073	0.109	244%	8.4	17
17	0.152	0.130	281%	6.6	19
22	0.207	0.188	132%	3.0	20
26	0.299	0.255	89%	2.0	20
20	0.302	0.273	132%	2.6	19
23	0.547	0.568	46%	1.5	19
24	0.544	0.585	45%	1.3	18
4	0.582	0.650	201%	3.6	19
25	0.680	0.738	42%	1.5	18
13	2.546	2.494	62%	1.3	19
18	4.209	4.274	41%	1.5	18
27	3.845	4.830	104%	1.9	18

Table 25. Laboratory geometric mean relative potency estimates expressed as % of study median value for *S. sonnei*; samples with study median relative potency <0.1 excluded; values within 50-200% highlighted; empty cells indicate no valid estimates obtained

Sample	ELISA																	Luminex		
	Lab																			
	1	6	8	9	10	12	13	14a	15	17	18	19	20	21	22	24a	24b	5	7	14b
29	100.0	11.1	135.0	13.7	89.2	15.1	111.5	17.4	420.0	18.6	325.7	267.1	171.8			145.8		101.3	13.6	82.6
17	139.8	49.7	200.6	39.0	724.9	14.1	145.1	29.6	460.7	36.4	281.9	2071.2	560.2	51.7	19.6	86.8	297.3	100.0	51.9	
22	181.2	62.6	214.8	62.7	195.7	21.2	105.1	89.6	569.4	61.6	160.8	595.1	95.1	56.8	45.2	60.1	238.9	137.9	46.9	143.0
26	94.1	66.9	592.3	66.9	74.1	64.0	120.8	73.7	147.1	97.6	278.7	182.8	423.9	49.7	99.8	82.2	112.9	148.6	100.2	112.6
20	100.0	69.2	162.8	47.2	124.9	25.2	175.2	70.7	319.7	61.7	164.5	976.7	180.9	61.8	37.0	90.8	265.0	122.9	82.4	
23	75.5	53.9	97.6	47.0	77.2	117.2	100.0	125.2	115.0	85.9	46.3	212.9	104.7	125.7	97.5	128.5		123.3	88.3	133.9
24	91.7	89.1	113.6	64.0	101.0	116.1	121.4	99.0	125.4	87.6			114.5	97.5	24.5	98.3	103.1	115.4	86.9	104.0
4	100.0	34.2	420.7	40.4	121.4	14.5	164.5	7.5	459.6	40.3	148.8		135.7	48.1	144.3	99.2	325.5	78.4	47.4	315.2
25		57.3	114.4	41.1	66.3	132.4	105.2	97.5	143.6	106.8	71.1		174.2	107.2	83.3	80.8	102.6	110.0	90.0	68.1
13	93.0		109.6	98.2	138.9	98.6	100.0	125.9	58.4	119.4	96.7	143.1	76.9	28.1	97.1	373.0	119.2	76.6	101.5	121.5
18	104.1	138.8	105.2		136.2	87.1	79.8	109.6	73.5	95.9	147.6	45.8	96.1	192.5	82.8	125.5	81.2	59.1	109.7	
27	202.4	39.2	15.5		60.0	115.9	100.5	62.2	99.5	111.8	219.6		66.9	118.8	108.4	101.5	19.5	52.6	84.8	165.9

Figure 9. Laboratory geometric mean relative potency estimates expressed as % of study median value for *S. sonnei*; samples with study median relative potency <0.1 excluded

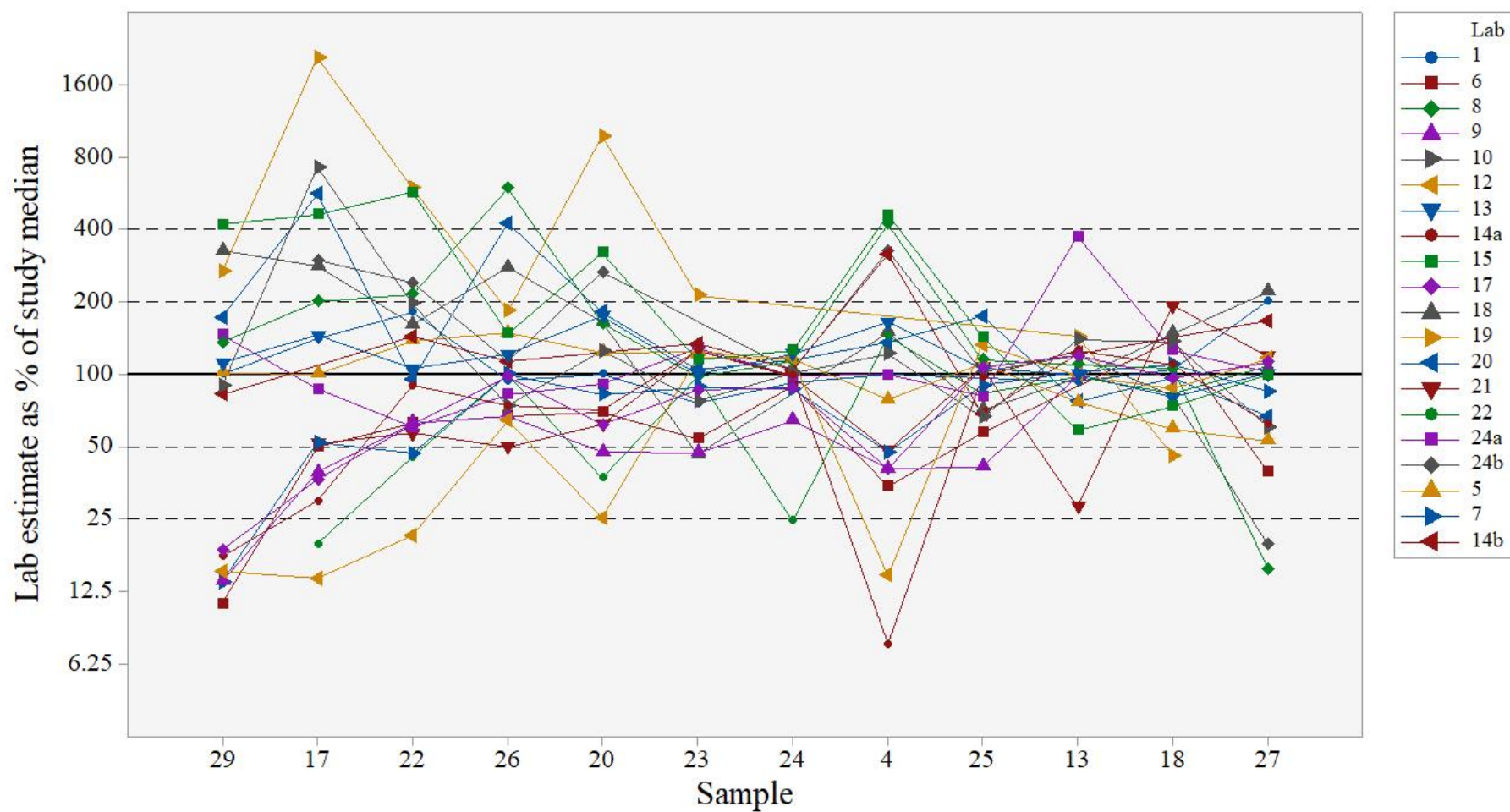


Table 26. Summary of endpoint titres and relative potency estimates for *S. sonnei*; samples with study median relative potency <0.1 excluded; shading indicates where the IQR for relative potencies is less than or equal to the corresponding value for endpoint titres

Sample	Endpoint titres				Relative Potencies				N
	GM	Median	GCV	UQ/LQ	GM	Median	GCV	UQ/LQ	
29	816	800	379%	3.4	0.075	0.109	268%	8.5	11
17	2108	3200	341%	4.0	0.157	0.186	232%	7.1	14
22	3123	3200	130%	4.0	0.221	0.188	105%	3.1	14
26	3665	3200	198%	4.7	0.310	0.251	108%	1.9	14
20	3682	3200	193%	4.0	0.295	0.305	94%	2.7	14
23	5701	6400	126%	2.0	0.478	0.553	42%	1.4	13
24	6198	6400	138%	2.0	0.521	0.579	53%	1.3	13
4	7198	8000	191%	2.0	0.636	0.834	208%	3.8	14
25	7641	6400	93%	2.2	0.675	0.757	47%	1.5	13
13	25336	32000	117%	2.7	2.271	2.450	52%	1.3	13
18	44550	32000	218%	4.0	4.563	4.448	33%	1.6	13
27	40610	32000	199%	2.5	3.560	4.807	122%	1.9	13
25/160	10868	14697	143%	3.4					14

Appendix 1. Collaborative Study Participants (in alphabetical order by country)

Participant	Organisation	Country
Dr. Romana Hochreiter	Valneva Austria GmbH	AUSTRIA
Dr. Firdausi Qadri	International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b)	BANGLADESH
Xiaohua Hu, Hongbin Fu and Guodong Wang	Beijing Zhifei Lvzhu Biopharmaceutical Co., Ltd	CHINA
Dr. Li Bin and Dr. Bin Wang	National Institutes for Food and Drug Control, Beijing	CHINA
Laurence Mulard and Marion Bouchet	Institut Pasteur	FRANCE
Dr. Brunda Ganneru	Bharat Biotech International Ltd.	INDIA
Dr Kapil Maithal and Dr Harish Chandra	Zydus Lifesciences Limited	INDIA
Prof. Dani Cohen, Dr. Valeria Asato and Ms. Anya Bialik	Tel Aviv University, School of Public Health	ISRAEL
Marta Benincasa, Dr Francesca Mancini and Dr Omar Rossi	GSK Vaccines Institute for Global health	ITALY
Alessandro Manenti and Eleonora Molesti	VisMederi srl	ITALY
Elizabeth Odundo	Kenya Medical Research Institute (KEMRI)/Walter Reed Army Institute of Research (WRAIR) – Africa Microbiology Hub, Kericho (MHK)	KENYA
Melissa Kapulu	KEMRI - Wellcome Trust Research Programme	KENYA
Prof Khuzwayo Jeren, Dr Prisca Matambo, Dr Jonathan Mandolo and Amidah Luhanga	Kamuzu University of Health Sciences and Malawi Liverpool Wellcome Research Programme	MALAWI
Jae-Ouk Kim and Dongwook Lee	International Vaccine Institute	SOUTH KOREA
Khadija Boumdian	Medicines and Healthcare products Regulatory Agency	UNITED KINGDOM
Dr Manoj Kumar and Dr Florence Seal	Inventprise, Inc.	USA
Subhra Chakraborty and Zachary Mazanek	Johns Hopkins University	USA
Marcela Pasetti and Esther Ndungo	University of Maryland School of Medicine	USA
Ti Lu	University of Missouri - Columbia	USA
Dr Renee Laird	Walter Reed Army Institute of Research (WRAIR)	USA

Appendix 2. Study protocol that was provided to all study participants

EVALUATION OF THE CANDIDATE 1st WHO INTERNATIONAL STANDARD FOR ANTI-*SHIGELLA* SERUM (HUMAN)

1. INTRODUCTION

The aim of this collaborative study is to evaluate the candidate standard for its suitability to serve as WHO International Standard for *Shigella* immunoassays. The commutability of the candidate material will be assessed using a panel of representative clinical samples across the range of different immunoassays included in the study.

2. MATERIAL

2.1. Material provided

- CS763 Sample A (Candidate 1st WHO IS for Anti-*Shigella* Serum (Human)). Supplied freeze-dried. One ampoule should be reconstituted with 0.5 ml sterile water on the day of first use and the contents transferred to a suitable tube. Where multiple assays are being performed for different serotypes, the ampoule should be stored at +2°C to +8°C degrees. **A new ampoule should be used for each independent run (refer to section 4 for more details). One spare ampoule has also been provided.**
- CS763 panel: Coded CS763 Sample 1 to CS673 Sample 34 inclusive. Supplied as frozen liquid samples in 0.5 ml Sarstedt tubes. All serum samples included in the panel have tested negative for blood-borne virus markers (HIV antibodies, Hepatitis B antibody, and Hepatitis C RNA). Samples should be thawed on the day of first use and kept at +2°C to +8°C.

Important notes:

1. **Samples 1 to sample 27 inclusive are to be tested against all target serotypes.**
2. Samples 28 to sample 32 inclusive are **to be tested against *S. flexneri* 2a and *S. sonnei* only by ELISA** due to limited volume. Note that for multiplex assays where all serotypes are assayed together, results can be reported for ALL serotypes.
3. Sample 33 and sample 34 are to be tested against all target serotypes and should be **included on every plate.**

3. OPENING OF AMPOULES

The candidate standard, CS763 Sample A, is presented in 2.5 ml DIN ampoules. 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, using suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. A new disposable ampoule breaker is provided with each DIN ampoule.

4. TESTS AND METHODOLOGY

Participants are requested to perform three independent runs (on different days) for each method being performed. **One independent run** is defined as the **complete set of assays required to test all target serotypes that your laboratory can test for.** A new ampoule of the candidate standard should be used for each independent run.

For the panel of clinical samples, a single aliquot of each serum sample is provided. This aliquot is to be used for all assays across all independent runs. The samples are shipped frozen and should be thawed on the day of first use. Once thawed, the sample should be stored at +2°C to +8°C. **Do not re-freeze.**

If any sample is received in a thawed condition, please store it at +2°C to +8°C and make a note on the reporting data sheet. The sample can still be used.

Important note: Plan your assays carefully to ensure there is enough volume to perform all required tests across all runs (see table 1 below)

Table 1: Sample volumes

Sample	Allocated volume per independent run (µl)	Total volume per tube (µl)
1-27	50	150
28-32	25	75
33	50	150
34	100	300

Participants should use their existing in-house method and provide all relevant assay details (see section 5). However, to allow for reliable comparison across laboratories, the following requirements apply to every assay:

- The candidate standard CS763 **Sample A** must be tested **in duplicate on every plate**. A serial titration of the candidate standard must be performed to obtain a suitable dose response curve.
- A serial titration (or multiple dilutions) should also be performed for each of the test samples. Recommended starting dilutions for ELISA are shown in Table 2 below. An initial assay can be run to optimise the starting dilution if preferred.
- CS763 **Sample 33** and CS763 **Sample 34** must be **included on every plate** (single column).

The following plate layouts are suggested to test all samples:

Plate layouts for assays against *S. flexneri* 2a and *S. sonnei*:

Plate 1

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	A	33	1	2	3	4	A	5	6	7	8	34

Plate 2

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	A	33	9	10	11	12	A	13	14	15	16	34

Plate 3

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	17	18	19	20	21	33	A	22	23	24	A	34

Plate 4

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	25	26	27	28	29	33	A	30	31	32	A	34

Plate layouts for assays against *S. flexneri* 3a and *S. flexneri* 6 (sample 28 – sample 32 not tested in ELISA):

Plate 1

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	A	33	1	2	3	4	A	5	6	7	8	34

Plate 2

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	A	33	9	10	11	12	A	13	14	15	16	34

Plate 3

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	17	18	19	20	21	33	A	22	23	24	A	34

Plate 4

Column	1	2	3	4	5	6	7	8	9	10	11	12
Sample	25	26	27	blank	blank	33	A	blank	blank	blank	A	34

Table 2. Recommended starting dilutions of the test samples for ELISA. Dilutions greater than 1/100 are shaded in grey. Not to be tested indicates that samples should not be tested separately in ELISA but may be tested by multiplex.

Sample	<i>S. flexneri</i> 2a	<i>S. flexneri</i> 3a	<i>S. flexneri</i> 6	<i>S. sonnei</i>
Sample A	1/500	1/100	1/500	1/500
1	1/100	1/100	1/100	1/100
2	1/100	1/100	1/100	1/100
3	1/100	1/100	1/100	1/100
4	1/500	1/100	1/100	1/500
5	1/100	1/100	1/100	1/100
6	1/100	1/100	1/100	1/100
7	1/100	1/100	1/100	1/100
8	1/2000	1/100	1/100	1/100
9	1/100	1/100	1/100	1/500
10	1/2000	1/100	1/100	1/100
11	1/100	1/100	1/100	1/100
12	1/2000	1/100	1/100	1/100
13	1/100	1/100	1/1000	1/500
14	1/500	1/100	1/100	1/100
15	1/100	1/100	1/100	1/100
16	1/500	1/100	1/100	1/100
17	1/100	1/100	1/100	1/100
18	1/100	1/100	1/100	1/500
19	1/100	1/100	1/100	1/100
20	1/100	1/100	1/100	1/100
21	1/100	1/100	1/100	1/100
22	1/100	1/100	1/100	1/100
23	1/500	1/100	1/100	1/100
24	1/500	1/100	1/100	1/100
25	1/500	1/100	1/100	1/100
26	1/2000	1/100	1/100	1/100
27	1/500	1/100	1/100	1/500
28	1/1000	Not to be tested	Not to be tested	1/100
29	1/500	Not to be tested	Not to be tested	1/100
30	1/500	Not to be tested	Not to be tested	1/100
31	1/500	Not to be tested	Not to be tested	1/100
32	1/500	Not to be tested	Not to be tested	1/100
33	1/500	1/100	1/500	1/500
34	1/100	1/100	1/100	1/100

Appendix 3. Draft Instructions for Use for 25/160



WHO International Standard
1st WHO International Standard for antibodies to Shigella
serotypes S. flexneri 2a, 3a, 6 and S. sonnei
NIBSC code: 25/160
Instructions for use
(Version 2.4, Dated 30/09/2025)

5

1. INTENDED USE

This material is intended for the standardisation of immunoassays used to measure the levels of anti-*flexneri* 2a LPS / O-Ag IgG, anti-*flexneri* 3a LPS / O-Ag IgG, anti-*flexneri* 6 LPS / O-Ag IgG and anti-*sonnei* LPS O-Ag IgG.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

S. flexneri 2a antibodies = 50 IU per ampoule
 S. flexneri 3a antibodies = 50 IU per ampoule
 S. flexneri 6 antibodies = 50 IU per ampoule
 S. sonnei antibodies = 50 IU per ampoule

Reactivity for S. flexneri 1b and lpaB were also confirmed by some participants in the WHO collaborative study but no values have been assigned for antibodies to these antigens.

4. CONTENTS

Country of origin of biological material: USA.

Ampoules of 25/160 contain 0.5 mL of freeze dried pooled, filtered human serum. The pool consists of sera from 28 healthy adults who were participants in a randomized double-blind placebo controlled study to evaluate the efficacy of a tetravalent shigella vaccine.

5. STORAGE

On receipt, store ampoules at -20°C.

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

6. DIRECTIONS FOR OPENING

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

7. USE OF MATERIAL

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



The contents of each ampoule should be reconstituted with 0.5 mL sterile distilled water to give 100 IU/mL for each of the 4 antibodies. Ensure that all material is resuspended, including any splashes around the neck of the ampoule

8. STABILITY

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

Data from an accelerated thermal degradation study provided evidence that 25/160 will be stable during transport at ambient temperature.

Stability of the reconstituted material should be determined by the end user. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

N/A

10. ACKNOWLEDGEMENTS

N/A

11. FURTHER INFORMATION

Further information can be obtained as follows;

This material: enquiries@nibsc.org

WHO Biological Standards:

<http://www.who.int/biologicals/en/>

JCTLM Higher order reference materials:

<http://www.bipm.org/en/committees/jc/jctlm/>

Derivation of International Units:

http://www.nibsc.org/standardisation/international_standards.aspx

Ordering standards from NIBSC:

<http://www.nibsc.org/products/ordering.aspx>

NIBSC Terms & Conditions:

http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

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

13. CITATION

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

14. MATERIAL SAFETY SHEET

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

Physical and Chemical properties	
Physical appearance: off white dry powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains human sera	
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.	

characterization and establishment of international and other biological reference standards
[https://www.who.int/publications/m/item/annex2-trs932\(revised2004\)](https://www.who.int/publications/m/item/annex2-trs932(revised2004)). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

15. LIABILITY AND LOSS

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

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: USA
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.044g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

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

Appendix 4. Draft Instructions for Use for 25/170



WHO International Reference Reagent
Antibodies to Shigella serotypes *S. flexneri* 2a, 3a, 6 and *S. sonnei*
(low-positive)
NIBSC code: 25/170
Instructions for use
(Version 2.0, Dated 30/09/2025)

§

1. INTENDED USE

This material is intended for use as a low positive control in immunoassays that measure antibodies to *Shigella* serotypes *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6 and *S. sonnei*. This material is not intended for calibration of assays or secondary standards and users are directed to use product coded 25/160 for that purpose.

2. CAUTION

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

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

This reference reagent does not have an assigned value.

4. CONTENTS

Country of origin of biological material: USA.

Ampoules of 25/170 contain 0.25 mL of freeze dried pooled, filtered human serum. The pool consists of sera from 2 healthy adults who were participants in a randomized double-blind placebo controlled study to evaluate the efficacy of a tetravalent shigella vaccine.

5. STORAGE

On receipt, store ampoules at -20°C or below.

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

6. DIRECTIONS FOR OPENING

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

7. USE OF MATERIAL

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

The contents of each ampoule should be reconstituted with 0.25 mL sterile distilled water. Ensure that all material is resuspended, including any splashes around the neck of the ampoule. When tested in a multi-laboratory collaborative study, the consensus median end point titre in ELISA assays was 800 (*S. flexneri* 2a), 400 (*S. flexneri* 3a), 400 (*S. flexneri* 6) and 283 (*S. sonnei*) [1].

8. STABILITY

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

Stability of the reconstituted material should be determined by the end user. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

N/A

10. ACKNOWLEDGEMENTS

N/A

11. FURTHER INFORMATION

Further information can be obtained as follows:

This material: enquiries@nibsc.org

WHO Biological Standards:

<http://www.who.int/biologicals/en/>

JCTLM Higher order reference materials:

<http://www.bipm.org/en/committees/jc/jctlm/>

Derivation of International Units:

http://www.nibsc.org/standardisation/international_standards.aspx

Ordering standards from NIBSC:

<http://www.nibsc.org/products/ordering.aspx>

NIBSC Terms & Conditions:

http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

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

13. CITATION

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

14. MATERIAL SAFETY SHEET

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

Physical and Chemical properties	
Physical appearance: off white dry powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains human sera	
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.	

characterization and establishment of international and other biological reference standards

[https://www.who.int/publications/m/item/annex2-trs932\(revised2004\)](https://www.who.int/publications/m/item/annex2-trs932(revised2004)). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

15. LIABILITY AND LOSS

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

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: USA
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.022g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

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