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Development, production and characterisation of the proposed Third WHO International Standard for antibodies to hepatitis A virus (human)

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposal(s) contained therein. Written comments on the proposal(s) MUST be received in English by **23 March 2026** and should be addressed to:

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World Health Organization
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Comments may also be submitted electronically to **Dr Ivana Knezevic** at email: knezevici@who.int.

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

Summary

The 2nd International Standard for Hepatitis A Immunoglobulin, coded 97/646, was established by WHO in 1998 and has been widely used for the calibration of assays intended to measure anti-Hepatitis A antibody levels in purified immunoglobulin products and clinical samples. Due to depletion of stocks of 97/646, a candidate replacement standard has been produced by MHRA. The candidate standard, coded 23/260, is a lyophilised preparation of 8% human immunoglobulin that was prepared using the same source material used to produce 97/646. Satisfactory stability and homogeneity of the batch was confirmed following testing conducted by MHRA. Results from an international collaborative study involving 19 laboratories from 10 countries provide good evidence that the candidate standard is fit for purpose and that the candidate standard is commutable with clinical samples across the majority of quantitative methods that were used in the study. Product coded 23/260 is therefore recommended for establishment as the 3rd International Standard for antibodies to hepatitis A virus (human) , with an assigned value of 20 IU/ampoule.

Introduction

An International Reference Preparation for Hepatitis A Immunoglobulin was first established by WHO in 1981 [1] and was subsequently replaced with the Second International Standard for Hepatitis A Immunoglobulin (product coded 97/646) in 1998 [2,3]. The standard is intended to be used for the calibration of immunoassays (and secondary standards) that are used to measure anti-hepatitis A antibody levels with results reported in International Units (IU). These assays are used to measure potency of purified immunoglobulin preparations which are used for pre- and post-exposure prophylaxis against hepatitis A with minimum requirements for potency expressed in IU in at least one regional Pharmacopoeia [4]. These immunoassays are also used with clinical samples (human serum or plasma) to assess immunity as part of epidemiological studies or to assess the response to vaccination with hepatitis A vaccines. Thresholds for seroprotection, expressed in mIU/mL, have been proposed although there is no single cut-off level that defines protection against disease [5].

Since its establishment, the 2nd IS has been used by blood product manufacturers, vaccine manufacturers, clinical diagnostics manufacturers and public health, academic and regulatory laboratories. Stocks of the 2nd IS are now close to exhaustion and a proposal from MHRA to develop a replacement IS was endorsed by the WHO Expert Committee on Biological Standardisation at its 77th meeting in March 2023 [6]. A candidate replacement standard was prepared using a purified immunoglobulin product kindly donated by a blood product manufacturer and was evaluated in an international collaborative study involving 19 laboratories in 10 countries, representing 3 WHO regions. The aim of the collaborative study was to assess performance of the candidate standard in commonly used immunoassays and to estimate a potency in IU relative to the 2nd IS. A small number of purified immunoglobulin samples (from different commercial products) were included in the study for an assessment of continuity, and a larger number of representative clinical samples (plasma and serum) were included to assess commutability of the candidate standard. We report here the results of this multi-lab evaluation, together with the results from stability and homogeneity assessment conducted by MHRA.

Bulk material and processing

The source material used to prepare the candidate standard was taken from the same source material that was used to prepare the Second IS (97/646) and was supplied by the Central Laboratory of the Netherlands Red Cross, Amsterdam. This material was tested and shown to be negative for antibodies to HIV, HBsAg and HCV RNA (re-confirming the results from the same testing that was performed on the material prior to preparation of the 2nd IS). The potency of the stored bulk material was evaluated alongside the current Second WHO IS prior to formulation and was not found to be significantly different.

To prepare the candidate replacement standard, the 16% immunoglobulin source material was diluted with an equal volume of sterile water to produce an 8% immunoglobulin bulk solution which 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 January 2024 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

drier (Serail, Le Coudray Saint Germer, France). Freezing was performed to -50˚C over 2 hours then held for a further 2 hours before applying vacuum. Primary drying was performed at -35˚C for 40 hours at 100 µbar vacuum followed by a ramp to 25˚C over 10 hours, then 30 hours secondary drying at 25°C and 30 µbar 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 9,019 ampoules were filled. The candidate standard was coded 23/260 and stored at -20°C.

The freeze-dried product, 23/260, 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 320 ampoules were weighed, with a mean fill mass of 0.52 g and a coefficient of variation of 0.39%. Residual moisture and oxygen content were measured after sealing as indicators of successful freeze-drying and ampoule integrity respectively. Residual moisture was measured destructively using coulometric Karl Fischer titration (CA-300 coulometer, A1-Environsciences Ltd, Blyth, UK) operated within a dry box and checking performance before analysis using an Aquamicron Check P water standard (A1 Environsciences) to give % w/w moisture readings. The mean residual moisture measured in 12 ampoules of 23/260 was 0.31%. 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 23/260 was 0.28%. Microbial bioburden testing for bacterial and mould/yeast contamination were performed on pre-fill, post-fill and lyophilised samples and revealed a low-level of bioburden (<10 cfu/mL) for all tested samples. A full summary of the quality control test results is shown in Table 1.

Assessment of stability of 23/260

An accelerated thermal degradation study was conducted to predict the long-term stability of 23/260 and to inform decisions related to shipment of the product at ambient temperature. Ampoules of the candidate standard were stored at +4°C, +20°C, +37°C and +45°C, in addition to the normal storage temperature of -20°C which served as the baseline for comparison. Ampoules were retrieved after 1 year and testing for anti-hepatitis A antibodies was performed at MHRA using the DiaSorin LIAISON® Anti-HAV assay. Estimates for each sample, relative to the -20°C sample, were calculated by sigmoid curve analysis using European Directorate for the Quality of Medicines & Healthcare (EDQM) software CombiStats™ [7] and are shown in Table 2. Prediction of long-term stability was done using the Arrhenius model for accelerated degradation studies [8] and the predicted loss for samples stored at -20°C was shown to be negligible (<0.001% per year). Furthermore, the results from this study showed no significant loss in activity up to +37°C after 1 year, supporting a 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.

Assessment of homogeneity of 23/260

The precision of fill provides good evidence for the homogeneity of the batch in terms of the amount filled per ampoule. For 23/260, the precision of fill (CV% of the mean filling mass) was 0.39%, which is acceptable for an immunoglobulin sample with a fill volume of 0.5 mL per ampoule.

To provide further assurance regarding homogeneity of the batch, anti-hepatitis A antibody levels were measured in 10 different ampoules that were randomly selected from across the entire batch. This testing was done using the DiaSorin LIAISON® Anti-HAV assay with all

ampoules tested together (without technical replicates) in two independent assay runs. Data were analysed by parallel line analysis using CombiStats™ and results are shown in Table 3. Quantification of homogeneity was done by performing a two-way analysis of variance (ANOVA) of $\log_{10}$-transformed results in order to estimate the standard uncertainty component for homogeneity, s_{hom}. Potencies expressed relative to ampoule 1 in each assay run ranged from 88.9% to 107.2%, with a low anti-logged s_{hom} estimate of 1.040, corresponding to 4% between-ampoule heterogeneity supporting good homogeneity across the batch of the candidate standard.

Collaborative study design

Participants
Laboratories were invited to participate based on their expertise and experience in performing immunoassays measuring anti-hepatitis A antibodies, and to ensure that a range of assays currently used in the field were represented in the study. Nineteen laboratories agreed to participate, and their details are summarised in Appendix 1. The participants were from 10 countries, representing 3 WHO regions: Austria (1), Belgium (2), China (1), France (3), Germany (4), Italy (2), Japan (1), South Korea (1), UK (1) and USA (3). Participating laboratories were from commercial diagnostics manufacturers, therapeutic product manufacturers, public health and national control laboratories. All participating laboratories are referred to in this report by a code number randomly allocated and not related to the order of listing in Appendix 1.

Collaborative Study Samples
Participants received the 2nd IS 97/646 (study panel code HA-01) and the candidate 3rd IS 23/260 (study panel code HA-02), both lyophilised. They also received (as frozen aliquots) 4 samples each a different commercial purified immunoglobulin product (HA-03 to HA-06), 14 human plasma samples (HA-07 to HA-20) and 10 human serum samples (HA-21 to HA-30). The human serum and plasma samples were from routine blood donations from adult donors and were sourced from the UK NHS Blood and Transport service (NHSBT). All samples were tested at MHRA to confirm negativity for antibodies to HIV, HBsAg and HCV RNA and were pre-screened at MHRA using the DiaSorin LIAISON® Anti-HAV assay to ensure that the study panel contained samples with a range of anti-hepatitis A antibody levels. The human plasma and samples were not pooled (i.e. they donations from a single individual). All plasma and serum samples were filtered sequentially through Nalgene 0.8µM, 0.45µM and finally 0.2µM filters prior to aliquoting and distribution to study participants.

To aid with presentation and interpretation of the study results, purified immunoglobulin samples, plasma samples and serum samples were assigned an additional identifier with the prefix “I” for the immunoglobulin samples, “P” for the plasma samples and “S” for the serum samples. The full list of study samples showing the traceability for the identifiers used in this report is shown in Table 4.

Study Design
The study protocol is provided in Appendix 2 and was conducted under MHRA study code CS723. For each method contributed by a participating laboratory, participants were asked to perform three independent runs, using a fresh set of samples for each test. Participants were provided with an extra set of samples to allow for a preliminary assay where desired.

Participants were asked to test the 2nd IS (97/646), the candidate replacement standard (23/260) and the purified immunoglobulin samples (I-1 to I-4) using serial dilutions to reach the assay end point. Participants were asked to test the plasma (P-1 to P-14) and serum (S-1 to S-10) samples according to their routine practice for testing clinical samples. For quantitative methods, participants were asked to repeat the test for clinical samples where the potency was above the assay quantitative range, using a higher starting dilution for the sample.

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.

Assay Methods
Assays used by the participants are summarized in Table 5. The assay methods used in the study were detecting total Ig or IgG binding antibodies against a whole virus antigen, with all but one assay being commercial. This includes representation from 9 automated platforms and 7 manual kits. The majority of the assays used were a competitive ELISA format, including competitive and direct sandwich formats, with a single direct and indirect ELISA. The assays were based on either absorbance or chemiluminescence detection. Where laboratories performed multiple assay methods, laboratory codes are followed by a letter indicating the different methods (e.g. Lab 1a, 1b).

Collaborative study results and analysis

Summary of data returned by participating laboratories
A total of 24 datasets were returned by participating laboratories, covering 17 methods (Table 5). Where laboratories performed more than one method the results were treated as if from separate laboratories and coded (for example, laboratories 03a and 03b). There were 3 datasets each based on the Elecsys and ACCESS methods, and 2 datasets each based on the Atellica, Liaison and ARCHITECT methods. Of the 24 datasets, 11 were from assays that generate a quantitative result with 10 calibrated against the current WHO IS (97/646), thus expressing this in IU/mL. The remaining 13 datasets provided a qualitative readout. A summary of the qualitative results for the study panel samples from all methods is provided in Table 6. The methods performed by Lab 9, Lab 13 and Lab 14 are designed to estimate sample potency but are not validated to distinguish between a positive and negative sample and qualitative results were therefore not reported by these three labs.

The current IS (97/646) and candidate replacement IS (23/260) along with the immunoglobulin samples I-1 to I-4 were correctly identified as positive by nearly all labs reporting a qualitative result. Lab 15a did not identify any of these 6 samples as positive suggesting a technical issue with the method or its performance (just one replicate of one sample in one assay run was identified as positive).

Lab 15a also failed to identify any of the clinical samples as positive and, excluding the data set from this lab, there was generally very good agreement across labs/methods in terms of the qualitative identification of the plasma and serum samples: plasma sample P-4 was identified as negative in all but one of the data sets (Lab 19 returning a positive identification for this sample); plasma sample P-1 and serum samples S-1 and S-10 were identified as negative in most, but not all data sets reflecting differences in assay sensitivity and cut-off definitions between the different methods used.

Many labs reported issues with reconstitution of the current IS (97/646). This issue was known prior to the study and guidance had been noted within the study protocol. It is considered the high immunoglobulin concentration of the reconstituted sample (16%) is responsible.

Calculation of relative potency estimates

Potencies of candidate standard 23/260 and immunoglobulin samples I-1 to I-4 were calculated relative to the current IS, 97/646. For 4 of the laboratories (04, 07, 16 and 18) a four-parameter logistic (sigmoid curve) model was used, and for all other laboratories a parallel line model was used, to calculate individual assay potency estimates. Calculations were performed using the software, R [9]. Model fit was assessed visually, and non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples. Instances where the ratio of the fitted slopes was outside the range 0.80-1.25 were considered non-parallel and no estimates were reported in such cases.

Results from all valid assays were combined to generate unweighted geometric mean (GM) estimates for each laboratory and these laboratory means were used to calculate overall unweighted geometric means for each sample. Variability between assays and 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). Due to possible outliers and anomalous results, overall median estimates and Huber's robust GM estimates were also calculated using the R package 'WRS2' [10].

Laboratories 12a and 12b were excluded from the analysis as the qualitative data did not allow parallel line analysis to be performed. Laboratory 15a was also excluded due to all samples being reported as negative.

The majority of assays performed gave valid estimates of potency for 23/260 and samples I-1 to I-4 relative to the current IS 97/646. Slope ratios for these samples relative to the current IS, used as a measure of parallelism, ranged from 0.28 to 1.94, with 86% within the range 0.80-1.25, and are shown in Supplementary Figure 1. Analysis of the slope ratio data did not identify any significant trends, supporting the parallelism of 97/646, 23/260 and immunoglobulin samples I-1 to I-4 across the laboratories and assays used in this study.

Potency of candidate standard 23/260

Potency estimates relative to the current IS in IU/mL are summarised in Table 7 and Figure 1. Individual assay relative potency estimates are shown in Supplementary Table 1. Intra-laboratory GCV values, calculated for labs with more than two valid estimates, showed a wide range from 0.85% (lab 07b) to 39.25% (lab 16), with a median value of 4.95% and 12/14 values less than 20% indicating good precision for the majority of participants. .

Laboratory GM estimates for 23/260 ranged from 24.5 IU/mL (lab 01) to 56.3 IU/mL (lab 16), with an overall GM of 38.9 IU/mL (19.5 IU/ampoule), and an inter-laboratory GCV of 22.91%. The distribution of log transformed estimates showed no significant deviations from normality and no laboratories were identified as significant outliers. Calculation of overall estimates as median and robust GM gave values of 39.2 IU/mL (19.6 IU/ampoule) and 39.0 IU/mL (19.5 IU/ampoule) respectively. The results obtained therefore support assignment of 20 IU/ampoule to the candidate standard.

In absence of established parallelism criteria for all assays or study data that could be used to derive such criteria, a slope ratio acceptance range of 0.80-1.25 was chosen as the parallelism acceptance criterion. In order to evaluate the impact of this choice, the data were assessed using various other slope ratio acceptance criteria, ranging from 0.50-2.00 to 0.91-1.10. It was found that the overall potency of the candidate standard relative to the current standard was not affected by the range chosen, with an overall geometric mean estimate of 20 IU/ampoule being obtained in all cases.

Nine laboratories reported results in IU/mL based on the existing calibration of the method used and a summary of these results is shown in Table 8. For six of these laboratories, the geometric mean estimate for 97/646 is within 90-111% of the expected value of 98 IU/mL for this International Standard. For the remaining laboratories, estimates were 31% higher (lab 01), 19% lower (lab 05) and 57% higher (lab 18). The overall GM estimate of 40.7 IU/mL for 23/260 was consistent with the proposed value of 20 IU/ampoule for this candidate standard.

Potencies of immunoglobulin samples I-1 to I-4

Potency estimates relative to the current IS in IU/mL are summarised in Table 7. The immunoglobulin samples showed a similar level of variability to 23/260 when expressed relative to the current standard, with an overall median intra-laboratory GCV of 6.55% and inter-laboratory GCVs ranging from 14.58 to 26.87% for the four samples tested.

The estimated anti-hepatitis A antibody levels in the four immunoglobulin samples were also expressed relative to the candidate standard 23/260, assuming a potency of 20 IU/ampoule. These results are summarised in Table 9. There is a slight improvement in inter-lab GCV for 3 of the 4 immunoglobulin samples (I-2, I-3 and I-4) when expressed relative to the candidate 23/260 compared to the current IS. For sample I-1 a slight increase in inter-laboratory GCV was observed. Table 9 also shows the immunoglobulin samples estimates relative to the candidate IS as a percentage of the potencies calculated relative to the current IS. These percentages range from 69.3% (lab 16) to 159.3% (lab 01), with the majority between 80% and 125%, illustrating the range of possible shifts that may be observed when changing from the current to replacement IS.

Continuity of the IU following introduction of the proposed replacement IS can be directly illustrated using data from the nine laboratories that reported results for the immunoglobulin samples in IU/mL, as shown in Table 10. The overall geometric mean potency estimates for samples I-1 to I-4 relative to the candidate standard were expressed as a percentage of the laboratory reported estimates, ranging from 43.3% (sample I-4, lab 18) to 138.0% (sample I-4, lab 16). The majority (70%) of values were within 80-125%.

Commutability Assessment

Commutability of the current IS, 97/646, and the candidate IS, 23/260, with 4 immunoglobulin samples, 14 plasma samples and 10 serum samples, was assessed by a “calibration effectiveness” approach [11] using data from quantitative assays calibrated against the current WHO IS 97/646 (laboratories 01, 04, 05, 10, 13, 14, 16 and 18). Samples P-1, P-4, S-1 and S-10 were excluded from the analysis as they were reported as negative for the majority of laboratories. Laboratory 08 was excluded as they did not report quantitative estimates for the plasma and serum samples. For the immunoglobulin samples, estimates previously calculated relative to the two standards (Tables 7 and 9) were used. For the plasma and serum samples, estimates were calculated by interpolation from the fitted dose-response curve for 97/646 or

23/260, although no parallelism criteria could be applied as the samples were only tested at single dilutions by most laboratories.

Laboratory geometric mean estimates expressed as a % of the study median estimate for the sample (used as target values for the purpose of this analysis) were used to assess the extent to which laboratories agreed with consensus estimates. A value of 100% indicates complete agreement with the sample target value, so the relative activity of test sample and standard is consistent with other labs and therefore the standard is commutable in such cases. In order to derive an acceptable range (for analysis of this study only) for concluding commutability, the standard deviation of the log transformed values for immunoglobulin samples was calculated within each laboratory, and a pooled value, s_P, was calculated across all laboratories. The acceptable range was then set as $\pm 3s_P$. For this study this gave a range of ±0.21, or 0.623 to 1.605 on the untransformed scale, i.e. the values should be demonstrated to be within the range 62.3% to 160.5% for commutability to be concluded.

Laboratory geometric mean estimates for the immunoglobulin, plasma and serum samples calculated relative to 97/646 and 23/260 are shown in Table 11 and Table 13, assuming potencies of 49 IU/ampoule and 20 IU/ampoule for these samples respectively when used as standard. Overall study values for each sample are shown as GM and median estimates (calculated using log transformed data), with inter-laboratory variation expressed as both GCV and anti-logged MAD (Median Absolute Deviation, calculated using log transformed data) values.

Laboratory geometric mean sample estimates expressed as a % of the study median estimate for the sample are shown in Table 12, Table 14, Figure 2 and Figure 3. Values which fall outside of commutability acceptance range are shaded in Table 12 and Table 14.

Commutability with immunoglobulin samples

Good commutability with immunoglobulin samples was observed for both the existing IS, 97/646, and the candidate IS, 23/260. A median inter-laboratory GCV of 22% was calculated and the maximum 10^{MAD} value was 1.13 (Tables 11 and 13) indicating good harmonisation of results between laboratories through use of either standard. With the exception of samples I-2 and I-4 calculated relative to 97/646 by lab 16 and sample I-4 calculated relative to 23/260 by lab 18, all results were within the acceptable commutability criteria (Tables 12 and 14, Figures 2a and 3a).

Commutability with plasma and serum samples

The degree of commutability of the candidate IS, 23/260, with plasma and serum samples was also observed to be equivalent to that for the existing IS, 97/646. Although inter-laboratory variability was greater than that observed for the immunoglobulin samples, the majority (93%) of 10^{MAD} values were less than 1.50 and it should be noted that higher variability can result from the low potency (<1 IU/mL) of many of the panel samples. For serum samples with potencies exceeding 1 IU/mL, the maximum 10^{MAD} value was 1.19, indicating good harmonisation of results between laboratories through use of either standard (Tables 11 and 13). Excluding lab 13, where poor commutability was observed for both standards, 86% of results were within the commutability criteria for 97/646, and 83% of results for 23/260 (Tables 12 and 14, Figures 2b, 2c, 3b and 3c).

Discussion

The candidate 3rd WHO International Standard for Anti-Hepatitis A Immunoglobulin, Human (product coded 23/260) was successfully produced as a homogeneous batch – the coefficient of variation of the mean filling weight was 0.39%, well below the level considered to be acceptable (CV% <1%) for a product with a 0.5 mL filling volume. No significant heterogeneity was observed from testing 10 randomly selected ampoules for anti-hepatitis A antibody levels. The candidate replacement standard has an excellent stability profile and is sufficiently stable for shipment to users at ambient temperature.

For value assignment of 23/260, valid estimates of potency were obtained from the majority of assays performed by study participants. The inter-laboratory variability (GCV of 22.9%) is comparable to that seen for the current IS where the GCV was reported to be 17.8% and 27.8% for the coded duplicates included in that previous study [3]. For the laboratories whose assays reported results in IU/mL based on the existing calibration of the method, the geometric mean estimate for the current standard 97/646 was within 90-111% of the expected value of 98 IU/mL in 6/9 cases, suggesting that the majority of those assays have been appropriately calibrated. The overall geometric mean estimate for 23/260 (as reported) was 40.7 IU/mL and therefore consistent with the proposed assigned value of 20 IU/ampoule.

Because this standard is used for different purposes, a range of different sample types were included in the collaborative study panel. Samples from 4 different normal human immunoglobulin products were included to evaluate continuity of IU when switching from the current IS to the proposed replacement. A slight improvement in inter-lab GCV was observed for 3 of the 4 immunoglobulin samples when expressed relative to the candidate 23/260 compared to the current IS. A comparison of potency estimates for these 4 samples obtained relative to the current IS and the candidate replacement showed that the estimates obtained with one standard compared to the other were within 80-125% for the majority of laboratories. Similar conclusions were obtained for the subset of laboratories whose assays reported in IU/mL by comparing reported results for the 4 immunoglobulin samples to the potency estimates expressed relative to 23/260.

In addition to the 4 immunoglobulin samples, study participants also tested a large number of human plasma and serum samples to assess commutability. This represents the first formal assessment of commutability for the WHO IS for Anti-Hepatitis A Immunoglobulin. Good commutability with immunoglobulin samples was observed for both the existing IS, 97/646, and the candidate IS, 23/260. With the exception of samples I-2 and I-4 calculated relative to 97/646 by lab 16 and sample I-4 calculated relative to 23/260 by lab 18, all immunoglobulin sample results were within the acceptable commutability criteria. For the human plasma and serum samples inter-laboratory variability was greater than that observed for the immunoglobulin samples but the degree of commutability observed for the candidate replacement IS, 23/260, was equivalent to that for the existing IS, 97/646. The serum and plasma samples were only tested at a single dilution by most laboratories (as per their normal procedure for testing clinical samples) which means that no parallelism criteria could be applied and it should also be noted that higher variability can result from the low potency (<1 IU/mL) of many of the panel samples. Excluding lab 13, where poor commutability was observed for both standards, 86% and 83% of results were within the commutability criteria for 97/646 and 23/260 respectively, demonstrating good commutability of both standards across the laboratories and assay methods included in this study.

Proposal

Product coded **23/260** is recommended for establishment as the 3rd WHO IS for antibodies to hepatitis A virus (human), with an assigned value of **20 IU/ampoule**.

Following use of material for product quality control testing and collaborative study evaluation, 8,672 ampoules remain available for adoption by WHO.

Comments from Participants

Comments were received from 7 participating laboratories. These comments mostly concerned editorial corrections to names and/or affiliations listed in the appendix and/or the report content. One participant asked for clarification of the source and processing of the human serum and plasma samples which has been addressed in the final version of the report. One lab queried why their data had originally been reported as non-parallel and after discussion with the participant and review of the data, the analysis was re-done with exclusion of the highest dilution resulting in valid potency estimates for most assays. All tables of results and figures were updated accordingly and the data from this lab is included in the final potency estimate for the candidate standard (which remained unchanged at 20 IU/ampoule)

Acknowledgments

We gratefully acknowledge the important contributions of the collaborative study participants and the anonymous donors of the immunoglobulin, plasma and serum study samples. We extend our thanks to the MHRA manufacturing, inventory and logistics teams for their expert management in the formulation and production of the candidate material, storage and distribution of the materials to study participants.

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Tables and figures

Table 1. Profile of the candidate standard 23/260 and results from quality control tests

Product code	23/260
Container type and size	2.5 mL DIN Ampoule
Appearance	Freeze-dried white cake
No. ampoules filled	9019
Mean fill mass	0.52 g (CV 0.39 %) (*n* = 320)
Mean residual moisture	0.31 % (CV 26.30 %) (*n* = 12)
Mean of oxygen content	0.28 % (CV 58.93 %) (*n* = 12)
Bacterial colony count (CFU/mL)	<10 cfu/mL
Mould/Yeast colony count (CFU/mL)	<10 cfu/mL

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

Table 2. Accelerated degradation assessment of candidate standard 23/260 after 1 year storage at elevated temperatures

Storage Temperature (°C)	**Potency relative to -20°C reference (n=3)**	**95% Confidence Limits**	
+4	1.01	0.88	1.16
+20	1.02	0.97	1.07
+37	0.96	0.88	1.05
+45	0.90	0.82	0.99

Table 3. Homogeneity assessment of candidate standard 23/260

Ampoule	**Potency as % of ampoule 1**		
	Assay 1	**Assay 2**	**Combined**
1	100.0	100.0	100.0
2	88.9	92.4	90.6
3	92.9	100.3	96.5
4	96.3	97.4	96.9
5	95.1	99.6	97.3
6	98.7	100.5	99.6
7	97.1	107.2	102.1
8	97.9	101.8	99.8
9	88.9	94.1	91.5
10	99.5	105.8	102.6
Min	88.9	92.4	90.6
Max	99.5	107.2	102.6
Two-way ANOVA (log_{10} estimates)	MS_{amps}		0.000663
	MS_{res}		0.000087
	s^2_{hom} (=[MS_{amps} - MS_{res}]/2)		0.000288
	s_{hom}		0.016984

	Anti-log s_{hom}	1.039881

Table 4. Collaborative Study Sample Panel

Sample Study Code	MHRA code for report	Sample Type	Volume (mL)	Container
HA-01	97/646	2nd WHO IS (lyophilised)	0.5	DIN Ampoule
HA-02	23/260	Candidate 3rd WHO IS (lyophilised)		
HA-03	I-1	Human immunoglobulin (liquid frozen)	0.5	Eppendorf tubes
HA-04	I-2			
HA-05	I-3			
HA-06	I-4			
HA-07	P-1	Human plasma (liquid frozen)		
HA-08	P-2			
HA-09	P-3			
HA-10	P-4			
HA-11	P-5			
HA-12	P-6			
HA-13	P-7			
HA-14	P-8			
HA-15	P-9			
HA-16	P-10			
HA-17	P-11			
HA-18	P-12			
HA-19	P-13			
HA-20	P-14			
HA-21	S-1	Human serum (liquid frozen)		
HA-22	S-2			
HA-23	S-3			
HA-24	S-4			
HA-25	S-5			
HA-26	S-6			
HA-27	S-7			
HA-28	S-8			
HA-29	S-9			
HA-30	S-10			

Table 5. Laboratory codes and assay methods used (all methods use whole hepatitis A virus as the antigen)

Lab	Assay	Format	HAV Antibody detected	Readout	Reported Result
1	Bio-Rad MONOLISA anti-HAV EIA kit	Competitive	Total	OD	IU/mL
2	DiaPro AVAB.CE	Competitive	Total	OD & Cutoff Index	+ / -
3a	Elecsys Anti-HAV II (total antibodies) ECLIA - run on cobas e 601	ECLIA	Total	Cutoff Index	+ / -
3b	Elecsys Anti-HAV II (total antibodies) ECLIA - run on cobas e 801	ECLIA	Total	Cutoff Index	+ / -
4	ALPCO Anti-HAV ELISA (RUO)	Direct	IgG	OD & IU/mL	IU/mL
5	Atellica IM Hepatitis A Total (aHAVT)	Competitive CLIA	Total	RLU & mIU/mL (pos/neg)	mIU/mL
6	LIAISON Anti-HAV	Competitive Sandwich CLIA	Total	RLU & Cutoff Index	+ / -
7a	ARCHITECT HAVAb IgG	CMIA, 2-step, Direct Sandwich	IgG	RLU & Cutoff Index	+ / -
7b	Alinity i HAVAb IgG	CMIA, 2-step, Direct Sandwich	IgG	RLU & Cutoff Index	+ / -
8a	ADVIA Centaur HAV Total (aHAVT)	Competitive CLIA	Total	mIU/mL (pos/neg)	mIU/mL
8b	Atellica IM Hepatitis A Total (aHAVT)	Competitive CLIA	Total	mIU/mL (pos/neg)	mIU/mL
9	DiaSource Anti-HAV	Competitive	Not reported	OD	Not reported
10	VIDAS®Anti-HAV	Competitive	Total	mIU/mL	mIU/mL
11	ACCESS anti-HAV	Competitive	Total	RLU & S/CO	+ / -
12a	LIAISON Anti-HAV	Competitive Sandwich CLIA	Total	RLU & Cutoff Index	+ / -

12b	ARCHITECT HAVAb IgG	CMIA, 2-step, Direct Sandwich	IgG	RLU & Cutoff Index	+ / -
13	ACCESS anti-HAV	Competitive	Total	mIU/mL	mIU/mL
14	ACCESS anti-HAV	Competitive	Total	RLU & mIU/mL	IU/mL
15a	ALPHA DIAGNOSTIC Human Anti-Hepatitis A Virus IgG ELISA	Indirect	IgG	OD & U/mL	ELISA unit
15b	Elecsys Anti-HAV II (total antibodies) ECLIA	ECLIA	Total	Cutoff Index	+ / -
16	In-house ELISA	Competitive	Total	OD	IU/mL
17	VITROS Immunodiagnostic Products Anti-HAV Total	Competitive	Total	S/CO	+ / -
18	Beijing Wantai Biotechnology Kit	Competitive	Total	OD	mIU/mL
19	Fortress Diagnostics HAV-IgG ELISA kit	Competitive	IgG	OD & S/CO	+ / -

OD = optical density; S/CO = signal cutoff ratio; IU = International Unit; RLU = relative light unit; ECLIA - electrochemiluminescence immunoassay; CMIA - chemiluminescent microparticle immunoassay; CLIA - chemiluminescent immunoassay

Table 6. Study Sample Panel Qualitative Results Summary (all methods)
Results are indicative of data reported across 3 independent experiments, unless otherwise indicated (*)

	Sample																											
Lab	**97/646**	**23/260**	**I-1**	**I-2**	**I-3**	**I-4**	**P-1**	**P-2**	**P-3**	**P-4**	**P-5**	**P-6**	**P-7**	**P-8**	**P-9**	**P-10**	**P-11**	**P-12**	**P-13**	**P-14**	**S-1**	**S-2**	**S-3**	**S-4**	**S-5**	**S-6**	**S-7**	**S-8**	**S-9**	**S-10**
1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
2	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
3a*	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	-
3b*	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
4#	+	+	+	+	+	+	NT	+	+	NT	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	NT	NT	NT	NT
5	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	-
6	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	Eqv.	+	+	+	-	+	+	+	+	+	+	+	+	-
7a	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
7b	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
8a	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
8b	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
9†																														
10	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
11	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
12a	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	Eqv./+	+	+	+	-	+	+	+	+	+	+	+	+	-
12b	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
13†																														
14†																														
15a*	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+/-
15b	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	-
16	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	Eqv.	+	+	Eqv.	-	+	+	Eqv.	+	Eqv.	+	+	+	-
17	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-
18	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
19	+	+	+	+	+	+/-	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

*Data from 2 independent experiments; #data from a single experiment; †method does not distinguish pos/neg result; +/- = variable result across replicates; Eqv. = equivocal; NT = sample not tested

Table 7. Potency estimates for 23/260 and immunoglobulin samples I-1 to I-4, calculated relative to the current IS, 97/646, in IU/mL

Lab	23/260			I-1			I-2			I-3			I-4		
	GM	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**	**GM**	**GCV**	**N**
01	24.5	n/a	2	33.8	n/a	1	19.8	n/a	2	42.4	n/a	2	31.2	n/a	1
02	28.9	n/a	1	42.0	3.15	3	19.3	n/a	2	32.7	2.60	3	26.4	6.55	3
03a	39.2	n/a	2	49.7	n/a	2	29.8	n/a	2	52.5	n/a	2	43.3	n/a	2
03b	39.2	n/a	2	48.4	n/a	2	28.8	n/a	2	53.3	n/a	2	43.9	n/a	2
04	41.0	3.70	3	40.5	0.64	3	22.9	n/a	2	45.4	0.46	3	30.9	3.51	3
05	35.2	n/a	2	42.8	n/a	2	23.6	8.56	3	43.1	7.84	3	31.9	7.60	3
06	37.7	n/a	2	42.0	n/a	2	23.5	n/a	2	50.1	n/a	2	37.3	n/a	2
07a	31.0	3.59	3	38.3	n/a	2	22.0	1.47	3	42.4	7.17	3	29.1	n/a	2
07b	32.2	0.85	3	41.2	5.20	3	22.9	1.69	3	44.4	6.19	3	30.5	9.77	3
08a	34.9	4.66	3	42.4	3.25	3	23.4	11.30	3	39.9	4.67	3	29.5	6.83	3
08b	35.2	6.49	3	40.8	6.33	3	23.8	5.03	3	40.7	6.27	3	29.5	0.50	3
09	55.9	3.72	3	53.2	5.13	3	38.9	26.98	3	56.5	8.96	3	46.2	9.18	3
10	39.2	3.58	3	38.6	1.36	3	21.4	6.41	3	40.8	13.74	3	32.2	3.60	3
11	37.1	8.26	3	35.4	4.71	3	19.7	10.03	3	38.9	10.26	3	29.3	12.79	3
13	44.4	n/a	2	40.5	n/a	1		NP			NP			NP	
14	38.2	5.24	9	39.4	5.88	3	23.6	2.71	3	46.5	4.05	3	35.5	9.77	3
15b	46.4	2.59	3	47.4	8.24	3	40.5	n/a	1	55.8	3.68	3	42.0	3.62	3
16	56.3	39.25	3	57.9	27.36	3	39.3	32.50	3	64.9	39.91	3	52.1	51.75	3
17	40.1	18.22	3	44.4	23.49	3	24.9	n/a	2	57.1	10.99	3	36.7	15.71	3
18	49.9	29.53	3	35.9	47.11	3	22.6	n/a	2		NP		26.1	39.71	3
19	47.7	11.77	3	49.3	n/a	1	36.5	n/a	2	49.1	n/a	2	38.7	n/a	2
GM		38.9			42.7			25.6			46.6			34.5	
Robust GM		39.0			42.4			24.1			46.6			34.0	
Median		39.2			42.0			23.5			45.4			32.1	
GCV		22.91			14.58			26.87			18.29			21.66	
N		21			21			20			19			20	

GM= Geometric mean, GCV= Geometric coefficient of variation, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample, n/a= not calculated as N < 3

Figure 1. Histogram of geometric mean potency estimates for the candidate standard, 23/260, relative to the current standard, 97/646, in IU/mL

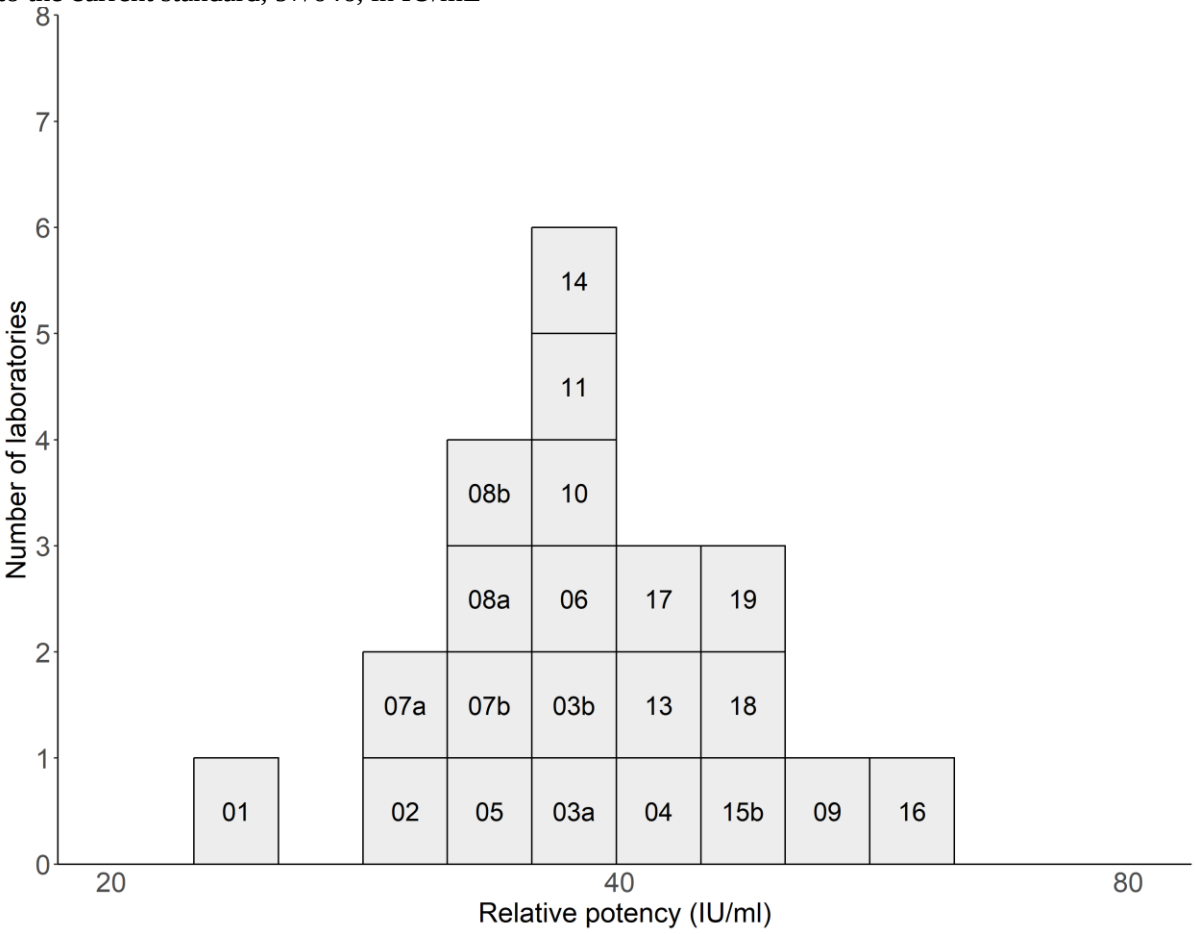


Table 8. Laboratory reported geometric mean estimates (IU/mL) for 97/646 and 23/260

Lab	97/646	23/260
01	128.1	47.2
05	79.8	28.0
08a	98.1	35.3
08b	94.8	34.1
10	101.8	41.4
13	90.9	42.0
14	104.4	40.9
16	102.2	49.6
18	154.2	54.5
GM	104.2	40.7
Robust GM	100.8	41.1
Median	101.8	41.4
GCV	21.36	22.87
N	9	9

Table 9. Potency estimates for immunoglobulin samples I-1 to I-4, expressed relative to the candidate IS, 23/260, in IU/mL

Lab	Potency vs 23/260				% of potency vs 97/646
	I-1	I-2	I-3	I-4	
01	53.8	31.5	67.5	49.7	159.3%
02	56.8	26.1	44.2	35.7	135.2%
03a	49.4	29.7	52.3	43.0	99.5%
03b	48.1	28.7	53.0	43.7	99.6%
04	38.5	21.8	43.2	29.4	95.2%
05	47.4	26.1	47.6	35.3	110.7%
06	43.4	24.3	51.8	38.6	103.4%
07a	48.1	27.6	53.3	36.5	125.7%
07b	49.9	27.8	53.8	37.0	121.2%
08a	47.4	26.2	44.6	33.0	111.8%
08b	45.1	26.4	45.0	32.6	110.7%
09	37.1	27.1	39.4	32.2	69.7%
10	38.4	21.3	40.6	32.1	99.6%
11	37.3	20.7	40.9	30.8	105.2%
13	35.6	NP	NP	NP	87.8%
14	40.2	24.1	47.4	36.2	102.0%
15b	39.9	34.0	47.0	35.3	84.1%
16	40.1	27.2	45.0	36.1	69.3%
17	43.2	24.2	55.6	35.7	97.3%
18	28.1	17.7	NP	20.4	78.2%
19	40.3	29.8	40.1	31.6	81.7%
GM	42.9	25.6	48.0	34.9	
Robust GM	43.0	26.1	47.2	34.9	
Median	43.2	26.3	47.0	35.5	
GCV	17.51	16.72	14.74	19.52	
N	21	20	19	20	

GM= Geometric mean, GCV= Geometric coefficient of variation, N= number of valid estimates used in calculations, NP= test sample non-parallel to reference sample

Table 10. Laboratory reported geometric mean estimates of immunoglobulin samples, I-1 to I-4, in IU/mL, compared with potency estimates relative to the candidate IS, 23/260, in IU/mL

Lab	Laboratory reported estimates				Potency vs 23/260				Potency as % of reported			
	I-1	I-2	I-3	I-4	I-1	I-2	I-3	I-4	I-1	I-2	I-3	I-4
01	55.0	29.5	60.2	39.7	53.8	31.5	67.5	49.7	97.9%	107.0%	112.1%	125.3%
05	34.6	20.1	35.3	26.0	47.4	26.1	47.6	35.3	136.9%	129.8%	135.1%	135.9%
08a	42.1	23.8	39.9	30.2	47.4	26.2	44.6	33.0	112.6%	110.0%	111.9%	109.3%
08b	40.6	23.1	39.4	28.6	45.1	26.4	45.0	32.6	111.0%	114.4%	114.2%	114.2%
10	41.8	22.4	43.0	34.2	38.4	21.3	40.6	32.1	92.0%	95.2%	94.4%	93.8%
13	42.5	29.9	50.2	37.4	35.6	NP	NP	NP	83.7%	n/a	n/a	n/a
14	42.4	25.9	51.1	38.9	40.2	24.1	47.4	36.2	94.7%	93.1%	92.8%	93.0%
16	49.2	45.2	49.2	26.2	40.1	27.2	45.0	36.1	81.4%	60.2%	91.4%	138.0%
18	57.6	28.2	69.2	47.1	28.1	17.7	NP	20.4	48.7%	62.7%	n/a	43.3%
GM	44.6	26.8	47.6	33.6	41.1	24.7	47.7	33.6				
Robust GM	43.2	25.9	47.6	33.4	41.9	25.4	45.9	34.2				
Median	42.4	25.9	49.2	34.2	40.2	26.1	45.0	34.1				
GCV	17.31	26.80	24.02	22.94	21.16	19.11	17.59	27.74				
N	9	9	9	9	9	8	7	8				

Table 11. Geometric mean estimates (IU/mL) for immunoglobulin, plasma and serum samples calculated relative to the current IS, 97/646. GM=geometric mean, GCV=geometric coefficient of variation, MAD=Median absolute deviation. Samples are listed by increasing median HepA concentration within each sample type. *Lab 13 excluded from calculations

Sample	Lab								GM*	Median	GCV*	10^MAD
	01	04	05	10	13	14	16	18				
I-2	19.8	22.9	23.6	21.4	NP	23.6	39.3	22.6	24.2	22.9	25.06	1.03
I-4	31.2	30.9	31.9	32.2	NP	35.5	52.1	26.1	33.5	31.9	23.97	1.03
I-1	33.8	40.5	42.8	38.6	40.5	39.4	57.9	35.9	40.7	39.9	18.97	1.05
I-3	42.4	45.4	43.1	40.8	NP	46.5	64.9	NP	46.6	44.2	18.50	1.05
P-11	0.1	0.1	0.2	0.1	1.9	0.1	0.1	0.2	0.1	0.1	46.63	1.33
P-13	0.2	0.1	0.3	0.2	1.7	0.3	0.2	0.2	0.2	0.2	40.57	1.37
P-5	0.3	0.2	0.3	0.2	2.4	0.4	0.3	0.3	0.3	0.3	25.36	1.19
P-14	0.4	0.2	0.4	0.1	3.0	0.5	0.1	0.3	0.3	0.4	86.51	1.69
P-9	0.4	0.6	0.5	0.5	2.8	0.4	0.5	0.5	0.5	0.5	15.76	1.09
P-10	0.5	0.6	0.7	0.3	2.9	0.7	0.9	0.8	0.6	0.7	48.10	1.21
P-3	0.5	0.7	0.6	0.6	2.7	1.2	0.7	0.6	0.7	0.6	36.11	1.11
P-7	1.4	2.2	1.9	2.0	5.0	1.8	1.7	2.3	1.9	2.0	19.63	1.13
P-6	2.1	0.7	1.6	0.3	4.9	1.4	2.8	1.6	1.2	1.6	107.09	1.53
P-8	2.2	2.5	3.9	3.4	6.3	4.2	3.6	3.5	3.3	3.6	26.61	1.13
P-12	3.9	n/a	3.9	3.3	5.3	3.7	5.2	3.5	3.9	3.9	17.26	1.13
P-2	16.7	1.2	18.3	35.1	19.8	19.9	25.1	23.7	14.7	19.8	213.80	1.19
S-4	0.1	0.1	0.1	0.05	2.6	0.1	0.05	0.1	0.1	0.1	31.74	1.29
S-6	0.1	0.1	0.1	0.04	3.9	0.1	0.1	0.1	0.1	0.1	59.02	1.25
S-5	0.2	0.2	0.2	0.5	2.7	0.5	0.5	0.3	0.3	0.4	59.33	1.45
S-9	0.4	n/a	0.5	0.3	2.1	0.5	0.5	0.3	0.4	0.5	19.36	1.08
S-2	0.3	0.5	0.5	0.5	4.0	0.6	0.7	0.6	0.5	0.5	28.10	1.12
S-3	4.2	1.3	4.2	3.4	6.7	4.7	4.5	4.6	3.6	4.4	59.55	1.06
S-7	7.2	n/a	7.3	0.1	9.3	8.6	6.2	4.3	3.5	7.2	393.42	1.19
S-8	31.5	n/a	29.8	57.8	39.0	37.0	30.7	30.3	35.1	31.5	29.31	1.06

Table 12. Geometric mean estimates for immunoglobulin, plasma and serum samples calculated relative to the current IS, 97/646, expressed as a % of the study median estimate; shaded cells are outside range 62.3-160.5%. Samples are listed by increasing median HepA concentration within each sample type.

Sample	Lab							
	01	**04**	**05**	**10**	**13**	**14**	**16**	**18**
I-2	86.5	100.0	103.0	93.6	n/a	103.4	171.8	98.8
I-4	97.9	96.8	100.0	101.1	n/a	111.3	163.5	81.8
I-1	84.6	101.4	107.3	96.6	101.5	98.6	144.9	89.8
I-3	95.8	102.6	97.4	92.3	n/a	105.2	147.0	n/a
GM	***91.0***	***100.2***	***101.9***	***95.9***	***101.5***	***104.5***	***156.4***	***89.9***
95% LCL	***81.1***	***96.3***	***95.4***	***89.8***	***n/a***	***96.5***	***137.1***	***71.1***
95%UCL	***102.2***	***104.3***	***108.8***	***102.3***	***n/a***	***113.1***	***178.4***	***113.6***
P-11	64.8	81.9	128.4	47.4	1649.7	89.4	111.8	137.4
P-13	69.1	49.4	137.4	73.0	720.6	106.6	104.4	95.8
P-5	79.7	66.2	102.8	64.8	745.6	112.6	103.7	97.3
P-14	116.5	48.1	114.3	30.1	824.6	138.2	35.3	87.5
P-9	79.1	126.4	108.3	98.4	575.4	91.8	93.1	101.6
P-10	80.3	86.3	101.1	39.3	434.4	99.0	130.7	116.7
P-3	70.6	106.2	93.9	94.2	422.8	192.3	114.5	92.9
P-7	69.2	111.3	98.2	101.9	254.7	92.5	87.7	120.2
P-6	133.0	41.7	98.5	21.2	310.4	90.8	175.3	101.5
P-8	62.4	68.8	109.2	94.9	175.8	116.4	101.2	98.8
P-12	100.0	n/a	100.6	84.9	135.4	94.0	133.2	88.6
P-2	84.3	5.9	92.3	176.9	99.6	100.4	126.3	119.4
GM	***81.8***	***58.6***	***106.3***	***66.6***	***395.1***	***107.5***	***103.8***	***103.8***
95% LCL	***70.5***	***33.2***	***98.4***	***45.5***	***234.2***	***93.3***	***81.2***	***95.0***
95%UCL	***95.0***	***103.4***	***114.9***	***97.4***	***666.6***	***123.9***	***132.7***	***113.5***
S-4	105.6	90.5	129.7	77.9	4350.1	94.7	77.4	164.5
S-6	106.1	81.6	135.9	33.7	3581.9	94.2	87.7	127.2
S-5	38.4	54.3	62.4	131.8	688.3	118.1	117.5	85.1

S-9	92.9	n/a	100.0	72.0	459.2	103.4	106.9	72.6
S-2	59.1	95.3	89.6	89.0	752.9	104.9	129.5	112.5
S-3	97.3	29.3	97.5	77.9	153.7	107.5	102.6	105.2
S-7	100.0	n/a	101.9	1.9	129.5	119.5	86.7	60.1
S-8	100.0	n/a	94.5	183.5	123.6	117.6	97.5	96.1
GM	***83.2***	***64.5***	***99.0***	***52.8***	***556.0***	***107.1***	***99.5***	***98.5***
95% LCL	***61.3***	***34.9***	***81.1***	***16.0***	***171.1***	***98.9***	***86.3***	***75.5***
95%UCL	***113.0***	***119.2***	***120.9***	***174.4***	***1806.8***	***115.9***	***114.6***	***128.5***

Figure 2. Geometric mean estimates calculated relative to the current IS, 97/646, expressed as a % of the study median estimate for (a, top) immunoglobulin samples, (b, middle) plasma samples, (c, bottom) serum samples

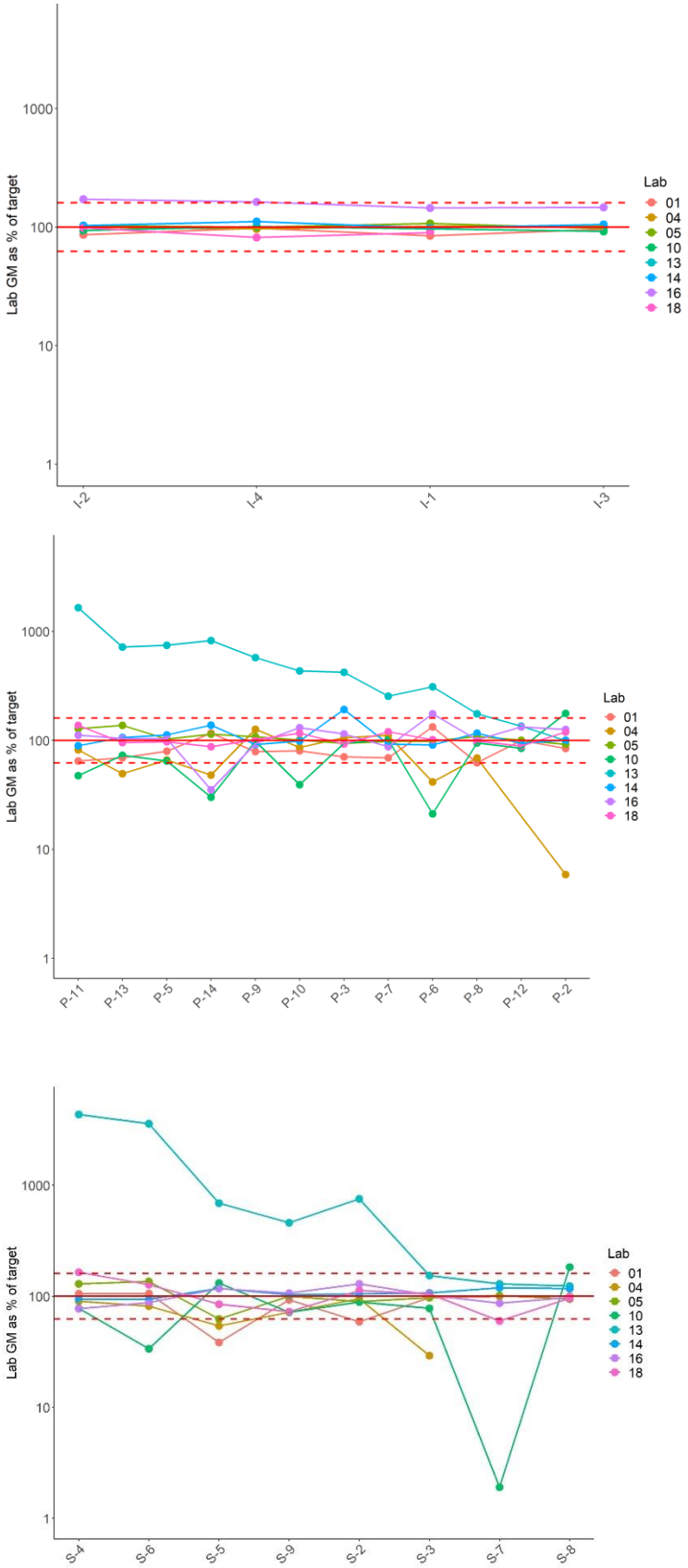


Table 13. Geometric mean estimates (IU/mL) for immunoglobulin, plasma and serum samples calculated relative to the candidate IS, 23/260. GM=geometric mean, GCV=geometric coefficient of variation, MAD=Median absolute deviation. Samples are listed by increasing median HepA concentration within each sample type; *Lab 13 excluded from calculations

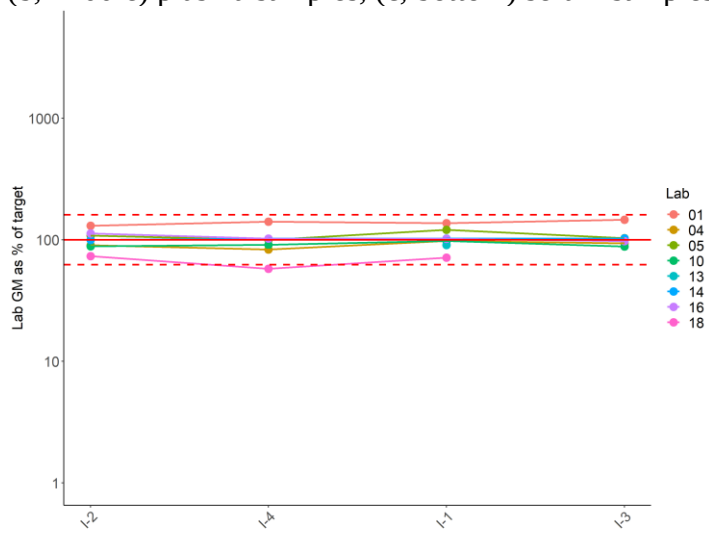
Sample	Lab								GM*	Median	GCV*	10^MAD
	01	04	05	10	13	14	16	18				
I-2	31.5	21.8	26.1	21.3	NP	24.1	27.2	17.7	23.9	24.1	20.80	1.13
I-4	49.7	29.4	35.3	32.1	NP	36.2	36.1	20.4	33.2	35.3	30.91	1.10
I-1	53.8	38.5	47.4	38.4	35.6	40.2	40.1	28.1	40.2	39.3	22.43	1.02
I-3	67.5	43.2	47.6	40.6	NP	47.4	45.0	NP	47.9	46.2	19.57	1.05
P-11	0.1	0.1	0.2	0.1	1.8	0.1	0.1	0.2	0.1	0.1	53.57	1.44
P-13	0.2	0.1	0.4	0.2	1.4	0.3	0.2	0.3	0.2	0.2	42.48	1.32
P-5	0.3	0.2	0.4	0.2	1.9	0.4	0.3	0.4	0.3	0.3	33.06	1.18
P-14	0.5	0.2	0.5	0.1	2.9	0.5	0.1	0.4	0.3	0.4	101.29	1.58
P-9	0.4	1.0	0.6	0.5	2.6	0.5	0.4	0.6	0.5	0.5	40.73	1.22
P-10	0.6	0.9	0.8	0.3	2.8	0.7	0.7	0.9	0.7	0.7	55.97	1.27
P-3	0.5	0.6	0.7	0.6	2.1	1.3	0.6	0.7	0.7	0.6	34.69	1.10
P-7	1.5	n/a	2.2	1.9	4.3	1.8	1.4	2.8	1.9	1.9	29.73	1.33
P-6	2.3	1.0	1.8	0.3	4.2	1.5	2.3	1.9	1.4	1.9	99.78	1.25
P-8	2.4	n/a	4.4	3.3	5.4	4.2	3.0	4.3	3.5	4.2	27.71	1.27
P-12	4.2	n/a	4.5	3.2	4.6	3.7	4.3	4.2	4.0	4.2	12.48	1.06
P-2	18.1	1.1	20.8	34.3	17.0	20.2	20.5	28.5	14.9	20.4	224.82	1.16
S-4	0.1	0.1	0.1	0.05	2.5	0.1	0.04	0.1	0.1	0.1	48.58	1.39
S-6	0.1	0.1	0.2	0.04	3.3	0.1	0.1	0.2	0.1	0.1	70.74	1.46
S-5	0.2	0.2	0.3	0.5	2.6	0.5	0.3	0.4	0.3	0.4	51.51	1.35
S-9	0.5	n/a	0.5	0.3	2.0	0.5	0.4	0.4	0.4	0.5	18.59	1.15
S-2	0.3	0.7	0.5	0.5	3.5	0.6	0.6	0.7	0.5	0.6	30.25	1.25
S-3	4.6	26.2	4.8	3.3	5.8	4.8	3.7	5.5	5.7	4.8	101.00	1.18
S-7	7.8	n/a	8.3	0.1	8.0	8.7	5.1	5.2	3.6	7.8	406.38	1.12
S-8	34.0	n/a	34.8	56.5	33.5	37.6	25.2	36.5	36.4	34.8	29.65	1.05

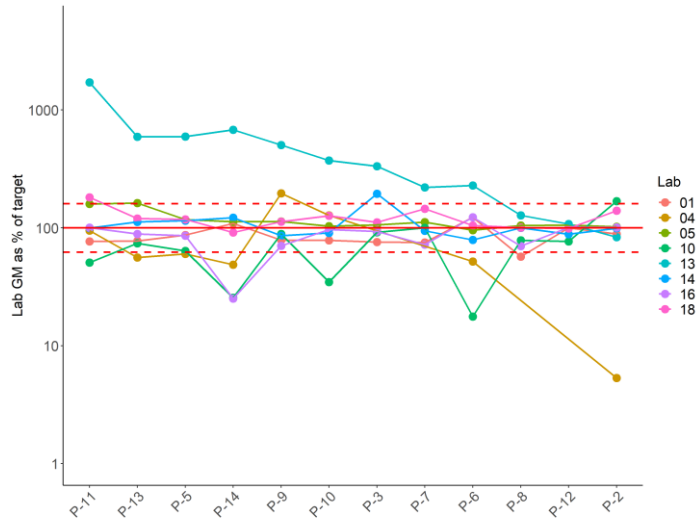
Table 14. Geometric mean estimates for immunoglobulin, plasma and serum samples calculated relative to the candidate IS, 23/260, expressed as a % of the study median estimate. Shaded cells are outside range 62.3-160.5%. Samples are listed by increasing median HepA concentration within each sample type.

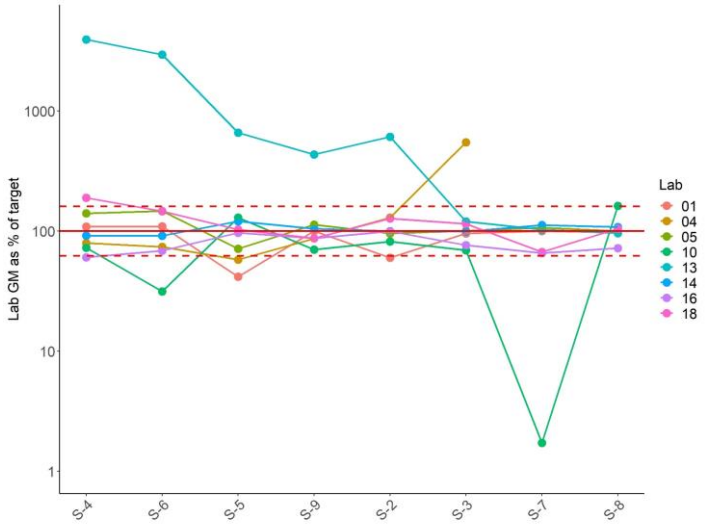
Sample	Lab							
	01	**04**	**05**	**10**	**13**	**14**	**16**	**18**
I-2	130.8	90.3	108.2	88.4	n/a	100.0	112.9	73.3
I-4	141.0	83.3	100.0	91.0	n/a	102.5	102.3	57.8
I-1	137.0	98.0	120.7	97.8	90.5	102.2	102.0	71.4
I-3	146.2	93.5	103.2	88.0	n/a	102.6	97.4	n/a
GM	***138.6***	***91.1***	***107.7***	***91.2***	***90.5***	***101.8***	***103.5***	***67.1***
95% LCL	***128.6***	***81.6***	***94.5***	***84.4***	***n/a***	***99.9***	***93.8***	***48.6***
95% UCL	***149.4***	***101.7***	***122.8***	***98.6***	***n/a***	***103.8***	***114.3***	***92.8***
P-11	76.7	94.9	159.8	50.8	1716.8	99.6	100.4	181.4
P-13	77.5	56.0	162.0	74.1	592.4	112.5	88.9	119.8
P-5	86.8	60.2	117.6	63.8	594.7	115.2	85.7	118.0
P-14	109.3	48.8	112.7	25.6	680.2	122.0	25.2	91.5
P-9	78.7	195.9	113.3	88.7	503.5	86.0	70.3	112.7
P-10	78.3	127.5	103.5	34.7	372.1	90.7	96.6	126.7
P-3	75.9	94.3	106.1	91.6	333.0	194.4	93.4	111.3
P-7	75.1	n/a	111.9	100.0	220.0	94.3	72.2	145.3
P-6	122.8	51.8	95.6	17.7	228.4	78.9	122.9	104.6
P-8	57.0	n/a	104.8	78.5	127.9	100.0	70.1	100.6
P-12	100.0	n/a	105.7	76.9	107.9	88.4	101.0	98.7
P-2	88.6	5.3	102.0	168.4	83.4	99.2	100.8	139.9
GM	***84.0***	***60.0***	***114.7***	***61.8***	***326.8***	***103.8***	***80.8***	***118.8***
95% LCL	***73.9***	***27.4***	***103.1***	***41.4***	***187.2***	***89.4***	***62.5***	***105.2***
95% UCL	***95.4***	***131.5***	***127.5***	***92.3***	***570.6***	***120.5***	***104.5***	***134.1***
S-4	108.9	79.6	140.6	72.7	3943.5	91.8	60.6	189.1
S-6	109.4	74.1	147.3	31.5	2941.7	91.4	68.6	146.2
S-5	41.8	57.9	71.3	129.7	657.9	120.7	96.9	103.2
S-9	100.0	n/a	113.1	70.1	434.4	104.6	87.3	87.1

S-2	60.0	129.4	95.7	81.8	608.9	100.2	99.8	127.3
S-3	95.7	547.4	100.7	69.3	120.3	99.3	76.5	115.2
S-7	100.0	n/a	107.1	1.7	103.2	112.4	65.8	67.0
S-8	97.7	n/a	100.0	162.3	96.2	108.0	72.3	104.7
GM	***85.3***	***119.3***	***107.0***	***49.0***	***470.7***	***103.1***	***77.3***	***112.5***
95% LCL	***63.8***	***39.0***	***88.4***	***14.7***	***139.9***	***95.2***	***66.3***	***86.3***
95% UCL	***113.8***	***364.9***	***129.5***	***162.7***	***1584.0***	***111.8***	***90.1***	***146.5***

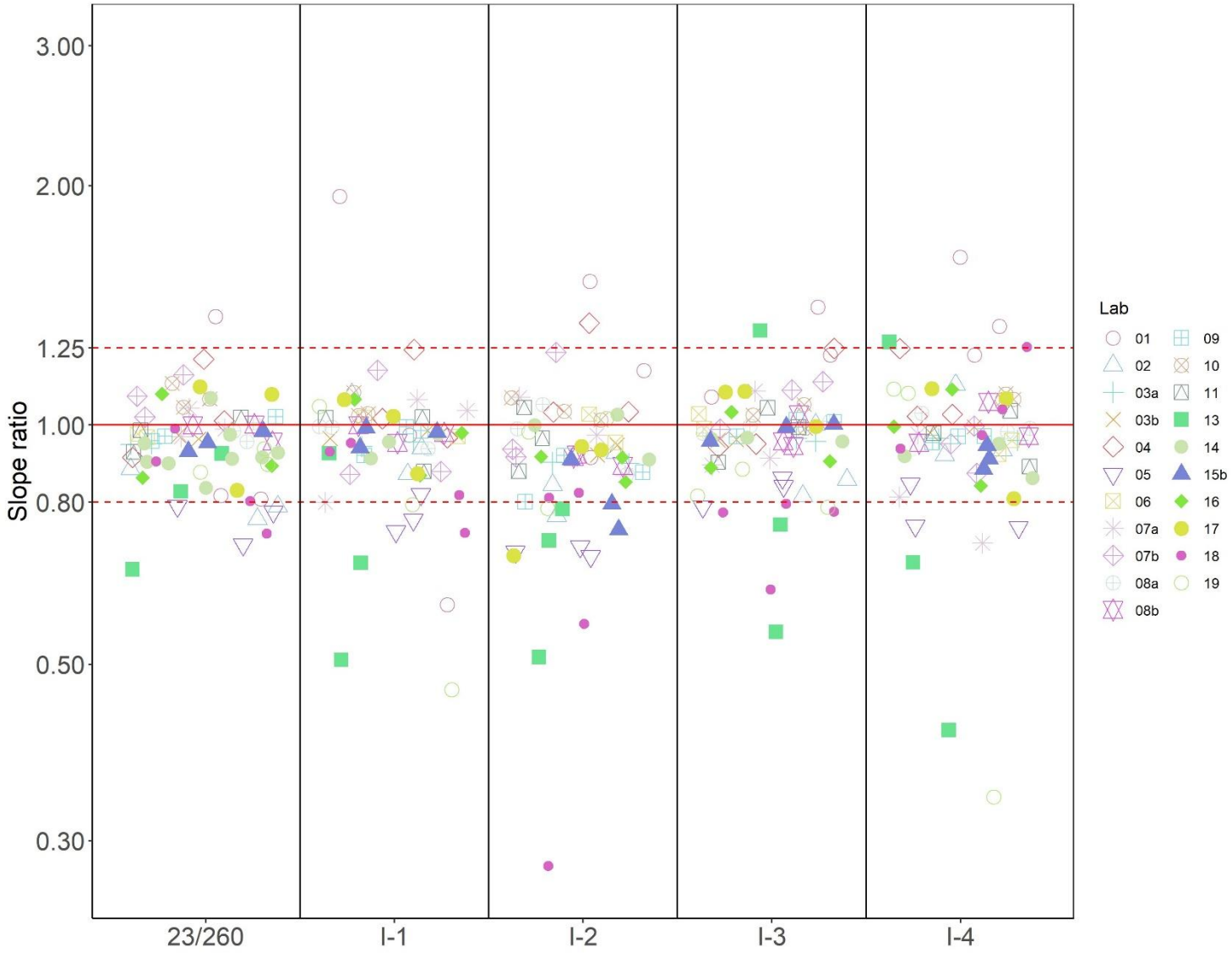
Figure 3. Geometric mean estimates calculated relative to the candidate replacement IS, 23/260, expressed as a % of the study median estimate for (a, top) immunoglobulin samples, (b, middle) plasma samples, (c, bottom) serum samples







Supplementary Figure 1. Slope ratios for candidate standard, 23/260, and immunoglobulin samples, I-1 to I-4, relative to the current standard, 97/646



Supplementary Table 1. Individual assay potency estimates calculated relative to the current standard, 97/646, in IU/mL

Lab	Assay	23/260	I-1	I-2	I-3	I-4
01	1	22.5	NP	23.2	41.0	NP
01	2	26.6	NP	18.7	43.7	31.2
01	3	NP	33.8	21.0	NP	NP
02	1	NP	40.7	19.4	31.7	25.7
02	2	28.9	43.3	19.1	33.2	28.4
02	3	NP	42.1	19.6	33.2	25.3
03a	1	39.5	50.0	29.6	52.6	43.1
03a	2	38.9	49.4	30.0	52.5	43.5
03b	1	39.5	48.6	29.0	53.4	43.9
03b	2	38.9	48.1	28.7	53.1	43.8
04	1	42.0	40.2	24.1	45.2	31.1
04	2	39.3	40.6	23.0	45.6	29.7
04	3	41.8	40.7	22.8	45.3	31.8
05	1	35.0	41.0	21.7	39.5	29.8
05	2	NP	44.8	25.6	45.4	34.5
05	3	35.5	NP	23.5	44.5	31.5
06	2	39.0	45.8	23.1	52.3	36.8
06	3	36.5	38.4	23.9	48.0	37.9
07a	1	30.1	38.2	22.1	40.8	NP
07a	2	30.8	NP	21.6	40.6	26.5
07a	3	32.3	38.3	22.2	45.9	31.9
07b	1	32.4	39.2	23.1	42.1	30.4
07b	2	31.9	41.0	22.5	44.0	27.9
07b	3	32.3	43.4	23.2	47.4	33.6
08a	1	34.3	41.0	21.6	37.9	28.7
08a	2	33.7	42.8	26.4	40.7	31.8
08a	3	36.7	43.6	22.4	41.2	28.1
08b	1	34.7	38.1	22.9	40.7	29.6
08b	2	33.4	43.0	25.2	38.3	29.3
08b	3	37.8	41.3	23.5	43.2	29.5
09	1	56.2	52.6	32.6	52.6	41.7
09	2	57.9	56.2	35.3	62.1	48.4
09	3	53.8	51.0	51.0	55.1	48.8
10	1	40.5	39.2	22.7	47.2	31.6
10	2	37.7	38.3	21.5	38.7	33.6
10	3	39.3	38.3	20.1	37.1	31.6
11	1	35.3	34.5	18.4	37.1	26.9
11	2	40.6	37.4	22.0	43.5	33.6
11	3	35.6	34.5	18.9	36.5	27.8
13	1	49.4	NP	37.5	NP	NP
13	2	39.9	40.5	19.9	NP	NP
13	3	NP	NP	23.4	NP	NP
14	1	36.8	36.9	23.2	44.8	33.8
14	2	39.4	-	-	-	-
14	3	40.0	-	-	-	-

14	4	41.9	41.0	24.4	48.5	39.5
14	5	39.1	-	-	-	-
14	6	36.9	-	-	-	-
14	7	37.5	40.4	23.4	46.2	33.4
14	8	37.3	-	-	-	-
14	9	35.6	-	-	-	-
15b	1	45.5	43.5	25.2	53.6	41.9
15b	2	45.9	48.1	40.5	57.1	40.6
15b	3	47.7	50.9	26.9	57.0	43.6
16	1	43.5	47.1	28.9	47.4	32.4
16	2	50.2	54.4	41.8	62.5	61.6
16	3	81.8	75.6	50.3	92.4	70.9
17	1	34.1	41.8	22.3	64.1	31.3
17	2	39.7	37.3	28.3	55.7	37.9
17	3	47.6	56.2	21.9	52.2	41.7
18	1	64.8	54.7	96.5	NP	NP
18	2	38.6	32.9	28.1	NP	26.0
18	3	49.6	NP	25.1	NP	36.4
18	4	NP	25.7	20.3	NP	18.7
19	1	54.2	NP	40.4	53.4	NP
19	2	45.5	49.3	27.0	45.2	34.1
19	3	44.1	NP	32.9	NP	43.8

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

Participant	Organisation	Country
Dorothee Fried and Andreas Sander	Abbott GmbH	Germany
Thomas Robin	Beckman Coulter	France
Sang-hyeon Son, Hee Won Woo, Yeonjae Kim	Biologics Research Division, National Institute of Food and Drug Safety Evaluation (NIFDS)	South Korea
Nathalie Auberger and Stéphane Berrez	BioMerieux	France
Tomoko Kiyohara, Mami Matsuda, Kuniko Tanno, Tiancheng Li and Ryosuke Suzuki	Department of Virology II, National Institute of Infectious Diseases (NIID), Japan Institute for Health Security	Japan
Angela Cuzzocrea, Selene Tiberti, Roberta Fonte and Marino Marchisio	DiaPro Diagnostic Bioprobes	Italy
Ginevra Di Matteo, Laura Spina, Valerio Minero and Francesco Capuano	Diasorin Italia S.p.A	Italy
Raissa Cabore, Emmanuel Di Paolo and Dominique Labbe	GlaxoSmithKline Biologicals SA	Belgium
Yves Zounli, Juan De Dios Reta Gonzalez, Derek Davis, Robin Flynn and Michael Gray	Grifols Therapeutics	USA
Daniela Huzly	Institute of Virology, University Medical Center Freiburg	Germany
Chitra Tejpal and Emma Bentley	Medicines and Healthcare products Regulatory Agency (MHRA)	UK
Qian He, Ying Wang, Xing Wu, Xiao Ma	National Institutes for Food and Drug Control (NIFDC)	China
Andreas Forsthuber and Felix Pliszka Lilge	Octapharma Produktionsges. mbH	Austria
Clara Arrivé, Léonard Aykan and Aurélie Thouvenin	Octapharma S.A.S	France
Katharina Schlosser and Lilija Miller	Paul-Ehrlich-Institut	Germany
Charles Noeson	QuidelOrtho	USA
Dieter Roessler and Vanessa Balassa	Roche Diagnostics GmbH	Germany

Sai Patibandla and Maximilien Ngbokoli	Siemens Healthineers	USA
Cindy Cambier, Caroline Sebret, Adeline Wannez and Sebastien Richard	Takeda - Baxalta Belgium Mfg SA	Belgium

Appendix 2. Study protocol provided to study participants

Medicines & Healthcare products Regulatory Agency

STUDY PROTOCOL

Collaborative Study CS723

Development of the 3rd WHO International Standard for anti-Hepatitis A Immunoglobulin

Study Background and Aims

This multi-center International collaborative study will evaluate a candidate preparation to serve as the 3rd WHO International Standard (IS) for anti-Hepatitis A Immunoglobulin. This will replace the current 2nd WHO International Standard for anti-Hepatitis A immunoglobulin (NIBSC product code: 97/646) which was established by WHO in 1998 [1]. The study is organized by the MHRA on behalf of the World Health Organization (WHO).

International Standards are recognized as the highest order of reference material for biological substances and are assigned a potency in International Units (IU). They are intended to be used to calibrate assays to report the biological activity of samples or products in terms of IU. This allows for comparability between assays/laboratories and enables parameters, such as analytical sensitivity of tests and the measurement of antibody levels in immunoglobulin preparations or clinical measures of immunity, to be better defined. Replacement IS are intended to maintain continuity in the calibration and reporting of results in IU. There is also a requirement to ensure the candidate replacement remains fit-for-purpose, accounting for advancements in technology and the clinical landscape since the current WHO IS was established. This study will evaluate these factors following published WHO guidelines [2] and be submitted for formal establishment by WHO.

The aims of this study are to:

- Evaluate the candidate replacement's potency/readout, in parallel to the 2nd WHO International Standard, in a range of typical assays performed in different laboratories.
- Characterise the candidate's reactivity/specificity in different assay systems.
- Assess commutability, i.e. establish the extent to which the candidate is suitable to serve as a standard for a variety of different samples.
- Propose a unitage for the candidate 3rd WHO International Standard that will ensure continuity in the use of the International Unit.

Study Samples

All study samples are provided coded and blinded to participants (Table 1.). Each participant is provided with 4 study sample panels to allow for 3 independent tests per method, plus one spare which can be used for a preliminary assay. Laboratories with more than one method will receive additional sample sets.

The samples comprise plasma, sera or purified immunoglobulin of human origin which have been collected from healthy donors. All donations have been screened and tested negative for blood borne pathogen markers HBsAg, anti-HIV Ab and HCV RNA. Plasma/sera samples have been clarified by centrifugation and filtration.

Table 1. CS723 Study Sample Panel.

All samples should be stored at -20°C or below.

*Further details of the testing requirements are provided in Section 2 of the Study Protocol

Sample Code	Formulation	Volume (mL)	Vial	Testing Protocol*
HA-01	Freeze-dried	0.5	DIN Ampoule	Dilutions beyond endpoint
HA-02				
HA-03	Liquid frozen	0.5	Screw Cap Vial	
HA-04				
HA-05				
HA-06				
HA-07	Liquid frozen	0.5	Screw Cap Vial	As per local, routine sample testing, SOP
HA-08				
HA-09				
HA-10				
HA-11				
HA-12				
HA-13				
HA-14				
HA-15				
HA-16				
HA-17				
HA-18				
HA-19				
HA-20				
HA-21				
HA-22				
HA-23				
HA-24				
HA-25				
HA-26				
HA-27				
HA-28				
HA-29				
HA-30				

CAUTION: As with all materials of biological origin, the material should be regarded as potentially hazardous to health.

Study Protocol

1. Sample Preparation

As per the Instructions for Use (IFU), prior to assay the freeze-dried samples HA-01 and HA-02 require reconstitution in 0.5mL of sterile distilled H_2O. Complete dissolution may take 30-60 minutes* and require gentle mixing. Reconstitution should be performed at room temperature.

**If complete dissolution has not occurred after 30-60 minutes, it is recommended to increase the reconstitution volume to 1.0mL of sterile distilled H_2O. Please be aware this will result in a 2-fold dilution of the sample. Report this to the study coordinator, as well as recording within the results reporting sheet.*

All other samples (HA-03 to HA-30) are provided liquid frozen and should be thawed at room temperature prior to assay.

2. Sample Testing

All participants are requested to:

- Perform 3 independent tests on different days or with different operators.
- Use a fresh set of samples for each independent test, ideally testing all samples within the same assay run/plate. If not possible, please prioritise samples HA-01 to HA-06 for concurrent testing and include samples HA-01 and HA-02 within additional assay runs/plates. Please indicate in the results reporting sheet if samples have not been tested concurrently.
- Test samples in duplicate.
- Where required, prepare dilutions of the samples in the buffer or sample matrix stated in your local SOP.
- Include all routine assay controls/calibrants/references when performing testing and report any anomalies in test performance.

2.2 Quantitative Methods

For the purpose of this study, this includes all test methods which can provide quantitative results for analysis of sample potency (i.e. results reported in IU/mL).

Samples HA-01 to HA-06:

- Prepare serial dilutions of samples to include at least 4 positive points, with at least one point beyond the endpoint dilution.
- It is recommended to set initial dilutions based on an assumed potency of 100 IU/mL
- If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.

Samples HA-07 to HA-30:

- Test samples according to routine sample testing procedure, to provide a quantitative sample result.
- Where samples record a result above the assay cut-off, if the testing procedure allows, please repeat the test after diluting the sample to report an absolute result. Record in the results reporting sheet where samples have been diluted.

2.3 Qualitative Methods

This includes test methods which cannot be used to quantify the potency of samples (i.e. results reported as pos/neg).

Samples HA-01 to HA-06:

- Prepare serial dilutions of samples to include at least 2 positive points, with at least one point beyond the endpoint dilution.
- It is recommended to test samples using 2-fold dilutions.
- It is recommended to set initial dilutions based on the potency of the current WHO IS (97/646; 98 IU/mL)
- If needed, dilutions can be adjusted for subsequent assays and recorded clearly in the results reporting sheet.

Samples HA-07 to HA-30:

- Test samples according to routine sample testing procedure, reporting results as positive/negative.

Results Reporting

An Excel reporting sheet is provided so that all essential information can be recorded, including details of test method and the raw data obtained from each assay. The use of the reporting sheet facilitates the consistent analysis and interpretation of results.

- Under the 'Result Summary' record the qualitative (+/-) and where applicable quantitative result for all samples tested, as per analysis in your laboratory. It is important to know whether the samples are considered positive and provide any additional comments on the sample result which will support data analysis.
- Under the 'Raw data' record the raw assay readout (e.g. absorbance OD, RLU, etc.) and dilutions tested for samples HA-01 to HA-06. For quantitative methods, record where samples HA-07 to HA-30 have been diluted for testing. Include the assay cut-off value indicating sero-reactivity for each assay.
- Where multiple methods have been used, complete one reporting sheet per method.
- Record in the Excel reporting sheet any deviations from the study protocol and complete all fields requesting additional method details.

Deadline for return of results is 12th September 2025

All completed results spreadsheets should be returned electronically to:

Project Leader: Emma Bentley Emma.Bentley@mhra.gov.uk

Deputy: Chitra Tejpal Chitra.Tejpal@mhra.gov.uk

Data Analysis

Analysis of the study data will assess the potencies of each of the study samples HA-01 to HA-06 relative to each other, and the interpretation of the other study samples HA-07 to HA-30 will be used to evaluate the performance of the samples within the different assay methods. The anonymity of each laboratory is maintained by reporting datasets with a blind code, details of the assay performed will be included.

A draft study report will be sent to participants for comment. The report will include data analysis, proposed conclusions and recommendations on the use and unitage of the candidate 3rd WHO International Standard for anti-Hepatitis A Immunoglobulin. Participants' comments will be included in the report prior to submission to WHO in December 2025. Study participants will be notified of the outcome of the study after the WHO ECBS meeting due to be held in March 2026.

References

[1] Ferguson M, Sands D, Lelie N. Hepatitis A immunoglobulin: an international collaborative study to establish the second international standard. Biologicals. 2000 Dec;28(4):233-40. doi: 10.1006/biol.2000.0262. PMID: 11237359

[2] WHO, Recommendations for the preparation, characterization and establishment of international and other biological reference standards. WHO Technical Report Series, No. 932., in Expert Committee on Biological Standardization. 2006.

As outlined in the study questionnaire, participation in the collaborative study is conducted under the following WHO Collaborative Study Terms and Conditions:

By joining the WHO International collaborative study to establish the 3rd WHO International Standard for anti-Hepatitis A immunoglobulin

The participant agrees that:

- The study samples have been prepared from Materials provided by donors and therefore must be treated as proprietary;
- The Materials provided must not be shared with anyone outside of the study;
- The Materials must not be used for application in human subjects or animals in the human food chain in any manner or form;
- There must be no attempt to reverse engineer, ascertain the chemical structure of, modify, or make derivatives of, any of the Materials;
- Participants accept responsibility for safe handling and disposal of the materials provided in according to the local regulations in their organization/country.
- Data obtained through testing of the Materials must not be published or cited before the formal establishment of the standard by World Health Organization, without the express permission of the MHRA study organiser.

MHRA, as the Collaborative Study coordinator, notes that:

- It is normal practice to acknowledge all participants as contributors of data rather than co-authors in publications;
- Data published from participating labs will be anonymised;
- Participation of this study is at your discretion and does not include remuneration costs;
- Prior to the establishment of the standard MHRA reserves the right to disclose specific information about the use of the Material(s), without acknowledgement of the study participants;
- Participants will receive a copy of the report of the study with proposed conclusions and recommendations for comment before it is further distributed.

Appendix 3. Draft Instructions for Use

Medicines & Healthcare products Regulatory Agency

Standard Type

WHO International Standard

3rd WHO International Standard for Anti-hepatitis A Immunoglobulin
NIBSC code: 23/260
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

§

1. INTENDED USE

The 3rd WHO International Standard for Hepatitis A Immunoglobulin is intended for the calibration of immunoassays that measure anti-hepatitis A antibody levels in human serum or plasma.

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

The standard has an assigned value of 20 IU per ampoule.

4. CONTENTS

Country of origin of biological material: The Netherlands.
Each ampoule contains the freeze-dried residue of a 0.5 mL fill of an 8% human immunoglobulin preparation.

5. STORAGE

The recommended storage temperature is -20
Please note because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

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

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
After adding water to reconstitute the contents of the ampoule we recommend that the ampoule is left for a few minutes with occasional GENTLE mixing (e.g. swirling by hand) to ensure that the contents are fully reconstituted

8. STABILITY

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

NIBSC Confidence in Biological Medicines

NIBSC follows the policy of WHO with respect to its reference materials. Stability after reconstitution has not been assessed and should be assessed by the end user.

9. REFERENCES

A comprehensive report of the production and characterisation of this standard has been published on the WHO website and is available here: CITATION TO BE ADDED

10. ACKNOWLEDGEMENTS

We thank the donors of the original source material used to produce the standard and all of the participants in the collaborative study

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: White freeze-dried cake		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	N/A		
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	

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

World Health Organization

Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

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

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.05g
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, characterization and establishment of international and other biological reference standards https://www.who.int/publications/m/item/annex2-trs932(revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.