

**EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
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**INTERNATIONAL COLLABORATIVE STUDY TO CALIBRATE
PROPOSED 4th WHO INTERNATIONAL STANDARD FOR
FERRITIN (HUMAN, RECOMBINANT)**

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NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments **MUST** be received by **17 September 2021** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

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Summary

The candidate 4th International Standard for Ferritin (Human, Recombinant) (19/118), was evaluated and calibrated against the 3rd International Standard for Ferritin (Human, Recombinant) (94/572), a second candidate preparation (19/162) and three serum commutability samples in an international collaborative study involving twelve laboratories in nine countries. Eleven of the twelve participating laboratories performed ferritin quantitation using automated assay platforms and one laboratory used a manual ELISA kit.

The overall geometric mean potency (from all methods) of the candidate 4th International Standard, 19/118, was 10.5 µg/ampoule, with inter-laboratory variability, expressed as % GCV, of 4.7%. Accelerated stability studies have predicted both 19/118 and 19/162 to be very stable for long term storage at -20°C.

It is recommended that WHO establish 19/118 as the 4th International Standard for Ferritin (Human, Recombinant) with an assigned potency of 10.5 µg/ampoule and expanded uncertainty limits 10.2-10.8 µg/ampoule (95% confidence; k=2.23).

Introduction

Ferritin is the main storage protein for iron in tissues and is engaged in its uptake, accumulation and release in cells. The ferritin molecule is a heterogenous (made up varying proportions of H and L subunits) intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000-4500 iron atoms. Circulating ferritin is normally predominantly in the L subunit form and is not iron-bearing.

The level of serum ferritin directly reflects the level of stored iron and is normally quantified using an antibody test, that detects the ferritin protein, to diagnose iron-related disorders and in surveys of iron status. See Worwood [1, 2] and WHO [3] for reviews of serum ferritin.

To help standardize results from different assay methodologies, WHO International Standards (IS) have been prepared using ferritin derived from human liver and spleen tissue[4, 5]. Due to difficulties in acquiring suitable tissue the current 3rd IS was prepared using a recombinant L chain which was shown to be immunologically similar to the 2nd IS derived from spleen[6]. The 3rd IS, in use since 1997, has been distributed to more than 200 companies in 38 countries and stocks are almost depleted. In this study we have produced candidate preparations of recombinant ferritin L chain (FLC) expressed in *E. coli* and CHO cells and subjected the materials to an international collaborative study. The results of the collaborative study to replace the 3rd IS for ferritin are presented in this report.

3 Materials and Methods

3.1 Materials for the collaborative study

The codon optimized DNA for human FLC was cloned into pET-21a for *E. coli* BL21 (DE3) expression or pcDNA™ 3.4 for ExpiCHO-S™ expression (Figure 1) and the sequences verified by Sanger sequencing. FLC was expressed in each cell type following the manufacturer's instructions {[pET-21a\(+\) DNA - Novagen | 69740 \(merckmillipore.com\)](#), [MAN0014337_expicho_expression_system_UG.pdf \(thermofisher.com\)](#)} and the cell pellets harvested by centrifugation. *E. coli* cell pellets were lysed, on ice, with 1% Lysozyme and 0.1% Benzonase nuclease in PBS, sonicated and the supernatant containing the crude FLC extract isolated by centrifugation. ExpiCHO-S™ cell pellets were lysed in ice cold deionized water containing 0.1% Benzonase nuclease and the supernatant containing the crude FLC extract again isolated by centrifugation. Both crude FLC extracts were further purified as described by Levi *et al* [7, 8], dialysed into PBS, aliquoted and stored at -80°C prior to lyophilization. The purified FLC amino acid sequence from both expression systems was confirmed by LC-MS/MS using Swiss-Prot for data analysis.

Three commutability (clinical) serum samples containing low, normal and high levels of ferritin were purchased from the WEQAS and stored at -80°C prior to lyophilization.

Participants were allocated three ampoules of each of the following coded materials:

- **Sample S:** WHO 3rd International Standard for Ferritin, human recombinant (lyophilized; 94/572). Contents: **6.3 µg/ampoule.**
- **Sample A*:** Lyophilized recombinant ferritin in serum (19/118). Contents: **approximately 10 µg/ampoule.**
- **Sample B**:** Lyophilized recombinant ferritin in serum (19/162). Contents: **approximately 7 µg/ampoule.**
- **Sample C***:** Lyophilized high serum ferritin. Contents: **approximately 0.9 µg/ampoule.**
- **Sample D***:** Lyophilized normal serum ferritin. Contents: **approximately 0.1 µg/ampoule.**
- **Sample E***:** Lyophilized low serum ferritin. Contents: **approximately 0.01 µg/ampoule.**

***Sample A** Consisted of FLC expressed in *E. coli* BL21 (DE3) transformed with plasmid pET-21a containing the full FLC sequence.

Purified FLC protein was diluted to ~10µg/ml in human serum containing 40mM HEPES and 1% Trehalose, dispensed into ampoules (~1ml/ampoule), lyophilized (17/05/2019) and coded 19/118. The mean weight of the dispensed solution in 220 ampoules was 1.0089 g. The imprecision of the filling (CV) was 0.171%, the mean oxygen head space was 0.15% and the mean residual moisture was 0.38%. 5,700 ampoules are available for distribution.

****Sample B** Consisted of FLC expressed in ExpiCHO-S™ cells transfected with Plasmid pcDNA™ 3.4 containing the full FLC sequence.

Purified FLC protein was diluted to ~7µg/ml in human serum containing 40mM HEPES and 1% Trehalose, dispensed into ampoules (~1ml/ampoule), lyophilized (09/08/2019) and coded 19/162. The mean weight of the dispensed solution in 143 ampoules was 1.0094 g. The imprecision of the filling (CV) was 0.160%, the mean oxygen head space was 0.17% and the mean residual moisture was 0.42%. 3,000 ampoules are available for distribution.

*****Samples C, D and E** Each sample consisted of ~100ml human serum from patients with high, normal and low levels of ferritin respectively, and reformulated with the addition of 40mM HEPES and 1% Trehalose, prior to dispensing into ampoules (~1ml/ampoule) and lyophilization (24/01/2020). The mean weight of the dispensed solutions in 9 ampoules was 1.0285 g. The imprecision of the filling (CV) was 0.130%, the mean oxygen head space was 0.13% and the mean residual moisture was 0.15%. Note, these ampoules are only for distribution in the collaborative study.

For all samples the sera were tested and found negative for anti-HIV, anti-HCV, HCV RNA and HBsAg.

All the bulk materials were processed into final containers at the National Institute for Biological Standards and Control (NIBSC), Potters Bar, Herts EN6 3QG, UK. NIBSC is the custodian of the materials and stores the materials at the above address.

3.2 Participants

A total of fifteen laboratories in ten countries across the globe agreed to participate in the study, twelve laboratories from nine countries returned results (detailed in Appendix 2), each of which has been assigned a code number. This coding does not reflect the order of listing.

3.3 Ferritin quantitation methods

A WHO International Standard should be suitable for use in as many different assay methods as possible. Therefore, all participating laboratories were requested to perform their usual method of analysis (Table 1). Where a laboratory performed more than one assay method, or an additional variation of the same assay method, each was treated as if performed by different laboratories.

3.4 Study design

Each participant was provided with a protocol (Appendix 4) and three ampoules of each study sample detailed in section 3.1. For each assay method, the layouts resulted in six assay estimates for each study sample relative to their in-house calibrant. For all assay methods, participants were requested to perform two assays on each of three days and regardless of the assay method used, participants were requested to prepare two independent sets of fresh dilutions of each preparation for each assay run.

Raw assay data were requested together with a summary of the participants' own estimates of potency for the six ferritin samples.

3.5 Statistical analysis

The potencies of coded samples A-E were calculated relative to the 3rd International Standard 94/572. Individual assay potency estimates were calculated using parallel line analysis with a log transformation of assay response. Calculations were performed using the R software program[9]. Non-linearity and non-parallelism of dose-response relationships were considered in the assessment of assay validity. Linearity was assessed by calculating the coefficient of determination R^2 and parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference sample. Instances where the R^2 value was <0.99 , or where the ratio of fitted slopes was outside the range 0.90-1.11, were considered invalid 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 (within laboratories) and between laboratories has been expressed using the geometric coefficient of variation ($GCV = \{10^s - 1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates).

Individual assay estimates of relative potencies were log transformed and a variance components analysis was performed in order to determine the intra-lab and inter-lab components of variation. These were used to determine standard uncertainty estimates for samples A and B. The expanded uncertainty was obtained by multiplying the standard uncertainty by the coverage factor $k=2.23$, taken to correspond to a 95% level of confidence.

3.6 Stability

To predict stability on extended storage, ampoules of samples A (19/118) and B (19/162) were stored at elevated temperatures for longer than 1 year before analysis. The relative potencies of the accelerated thermal degradation samples were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay[10], and hence predict the degradation rates when stored at a range of temperatures.

4 Results

4.1 Assay data

The twelve participants contributed data from a total of 75 assays, deviations from the study protocol and other anomalies were as follows:

- (i) Laboratory 5 provided two data sets from separate operators.
- (ii) Laboratory 9 reported results from one assay on each of three days due to staff shortage.

4.2 Assay validity

The majority of assays gave valid potency estimates when assessed using the validity criteria described in section 3.5, which were intended for use in the analysis of data from this study only and should not be interpreted as suitable for routine use in the assessment of assay validity within all collaborating laboratories. Around 10% of cases were excluded due to non-linearity or non-parallelism. Where the number of valid assays was less than three ($N < 3$) a GCV was not calculated.

4.3 Laboratory reported potencies and potencies relative to the WHO 3rd International Standard for Ferritin (Human recombinant), 94/572

Potency estimates from individual assays for each laboratory, calculated by NIBSC and reported by the laboratories are respectively presented in Appendix 1, Tables 1 and 2. Details of the individual laboratory geometric mean (GM) potencies of samples S, A- E based on laboratory reported estimates are shown in Table 2. Individual laboratory GM potencies of samples A- E relative to sample S, the 3rd IS for Ferritin (Human recombinant) based on NIBSC calculated estimates are shown in Table 3. For some manufacturers, the IS used to calibrate an assay test kit varies dependent on the region where the assay test kit is marketed. Seven of the twelve laboratories use the 3rd IS for Ferritin (Human, Recombinant) to calibrate their test kits directly or indirectly, with the remaining five laboratories claiming traceability to either the 1st or 2nd IS for Ferritin (see Table 1 for details). The GM laboratory reported results and GM NIBSC calculated results relative to sample S are presented as histograms in Figures 3i-v and 4i-vi. All participating laboratories identified commutability (clinical) samples C, D and E as containing high, normal, and low levels of ferritin respectively.

4.4 Intra-laboratory variability

For laboratory reported results, GCV's ranged from 0.4-7.2% (median 2.8%) for samples S, A & B; 0.5-10.1% (median 2.6%) for commutability samples C & D and 2.0-21.7% (median 6.6%) for commutability sample E (Table 2). Using the NIBSC calculations relative to sample S (Table 3), GCV's for samples A and B were $\leq 5\%$ (representing good repeatability); ranged from 1.1- 13.1% (median 3.9%) for commutability samples C & D and 1.2- 42.8% (median 11.3%) for commutability sample E. Sample E is below the limit of quantitation (LOQ) for

the dilution series used for samples S, A and B, thereby contributing to the higher intra-laboratory variability for this sample. For three laboratories (08, 09 & 10) no %GCV's were available for one or more samples due to insufficient valid assays.

4.5 Inter-laboratory variability

Inter-laboratory variability is much improved when potencies for samples A and B are determined relative to the 3rd IS for Ferritin (Human recombinant) as shown in Table 3 and histograms in Figure 3ii-v. Inter-laboratory variability is greater for clinical (heterogeneous) samples C-E which is reduced to around 20% for samples C & D and 29% for sample E when excluding laboratories 09 and 10 due to insufficient valid results. Again, sample E is below the LOQ for the dilution series used for samples S, A and B, thereby contributing to the higher intra- and inter-laboratory variability for this sample. Laboratories 09 and 10 have been excluded from the final inter-laboratory GM and GCV calculations because of insufficient valid results. For comparison, Tables 2-4 present the data including and excluding Laboratories 09 and 10.

Table 5 summarises inter-laboratory variability and shows inter-laboratory variability is independent of the standard used for quantifying samples C & D. Sample E has been excluded because it is below the LOQ.

4.6 Stability

Estimates of the potency for ampoules of samples A (19/118) and B (19/162) stored at elevated temperatures for a period of 2.0 and 1.7 years respectively are summarised in Appendix 3. The stability analysis was carried out at NIBSC using a sandwich chemiluminescence immunoassay kit on an automated immunoassay platform.

The analysis has shown a predicted loss of potency per year of 0.01% for 19/118 and 0.02% for 19/162 when stored at -20°C. We can be confident that 19/118 and 19/162 will be stable for decades when stored at -20°C and are suitable for shipment at ambient temperature.

5 Conclusions and Discussion

Eleven of the twelve participating laboratories performed ferritin quantitation using automated assay platforms and one laboratory used a manual ELISA kit.

5.1 Potencies of samples A and B relative to the 3rd IS

There was better overall agreement between all laboratories and between assay methods for the potency of sample A (19/118), the candidate 4th International Standard, than for sample B (19/162).

Summary statistics of concentration estimates for samples A and B relative to sample S are shown in Table 6. The overall geometric mean potency for sample A is 10.5µg/ ampoule with expanded uncertainty limits 10.2-10.8 µg/ampoule.

5.2 Potencies for samples B relative to the candidate 4th International Standard for Ferritin (Human recombinant), 19/118

Table 4 shows the estimates for samples B- E relative to sample A. The relative value of sample B can be converted to µg following establishment of the new International Standard. We suggest that sample B can be distributed and used as an internal assay/run control.

6 Comments from Participants

The draft report was distributed between participants for comment and all participants either agreed with the draft proposals or did not express an opinion.

7 Proposal

Together with the agreement of the participants, scientific advisor and co-ordinator, it is recommended that sample A, 19/118, is established by WHO as the **4th International Standard for Ferritin (Human, Recombinant)** with an assigned potency of **10.5 µg/ampoule** and expanded uncertainty limits **10.2-10.8 µg/ampoule** (95% confidence; k=2.23).

8 Instructions for Use

The draft Instructions for Use to accompany 19/118 are provided in Appendix 5.

9 Acknowledgements

We thank the staff of the Standards Processing Division and Standardisation Science, NIBSC, for ampouling samples A-E and Min Fang (NIBSC) for MS analysis of the candidate samples. We also thank all the participants of the collaborative study.

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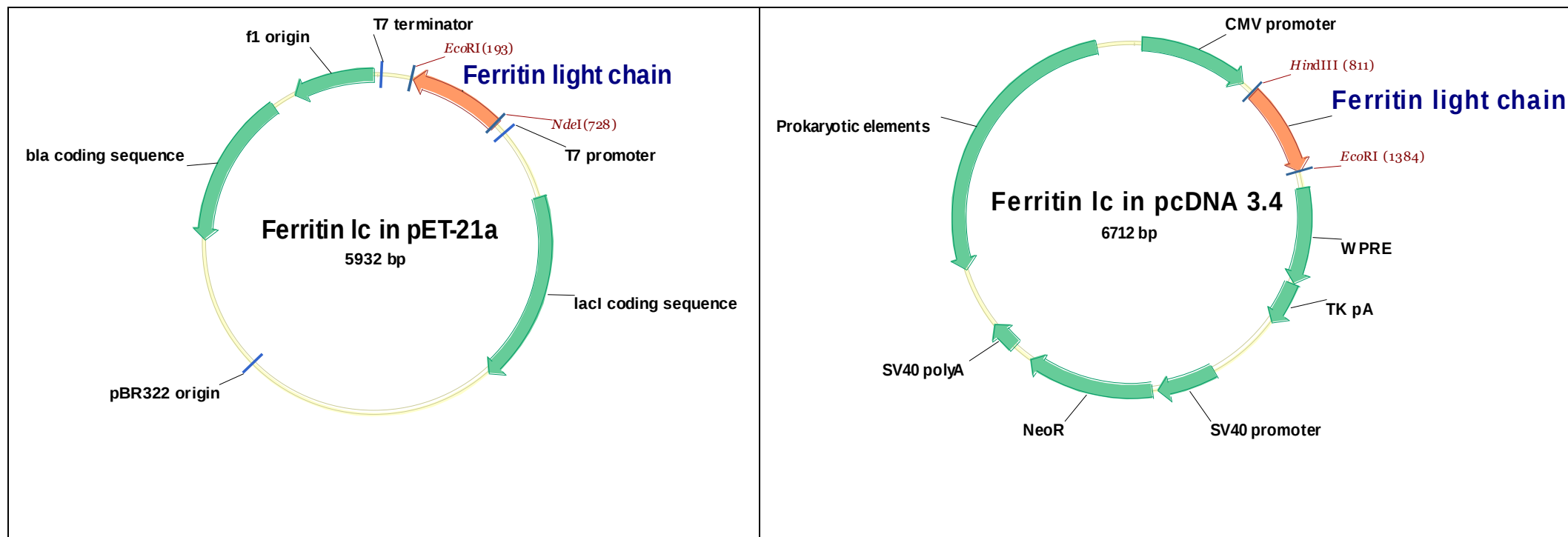
Figure 1. pET-21a and pcDNA™ 3.4 plasmids containing the FLC DNA sequence

Figure 2. Human FLC amino acid sequence

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1 mssqirqnys tdveaavns1 vnlylqasyt ylslgfyfdr ddvalegvsh ffrelaeekr
61 egyerllkmq nqrgralfq dikkpaedew gktpdamkaa malekklnqa lldlhalgsa
121 rtdphlcdf1 ethfldeevk likkmgdhlt nlhrlggpea glgeylferl tlkhd

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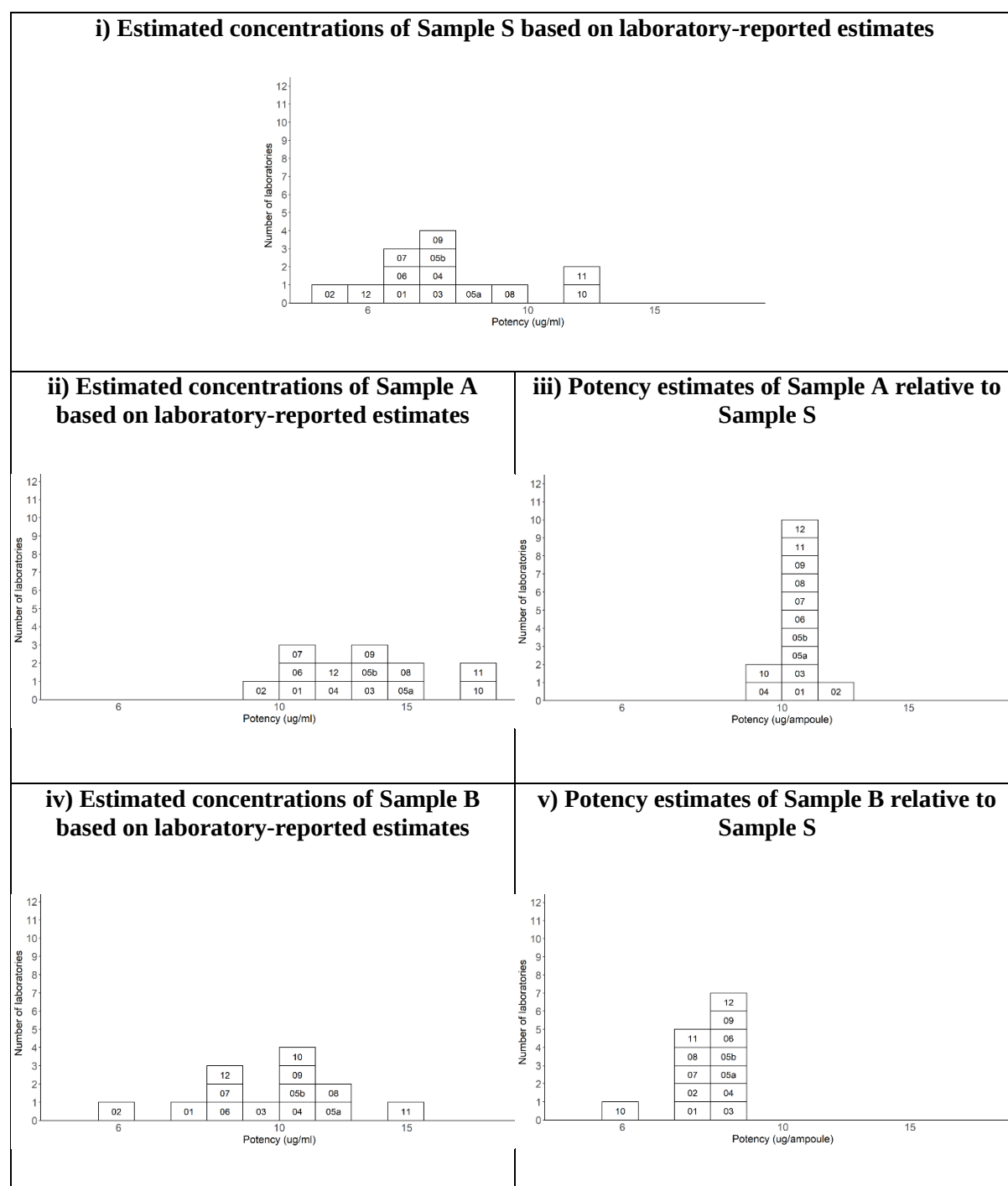
Figure 3. Histograms of GM laboratory reported results and GM NIBSC calculated results for samples S, A and B

Figure 4. Histograms of GM laboratory reported results and GM NIBSC calculated results for samples C-E

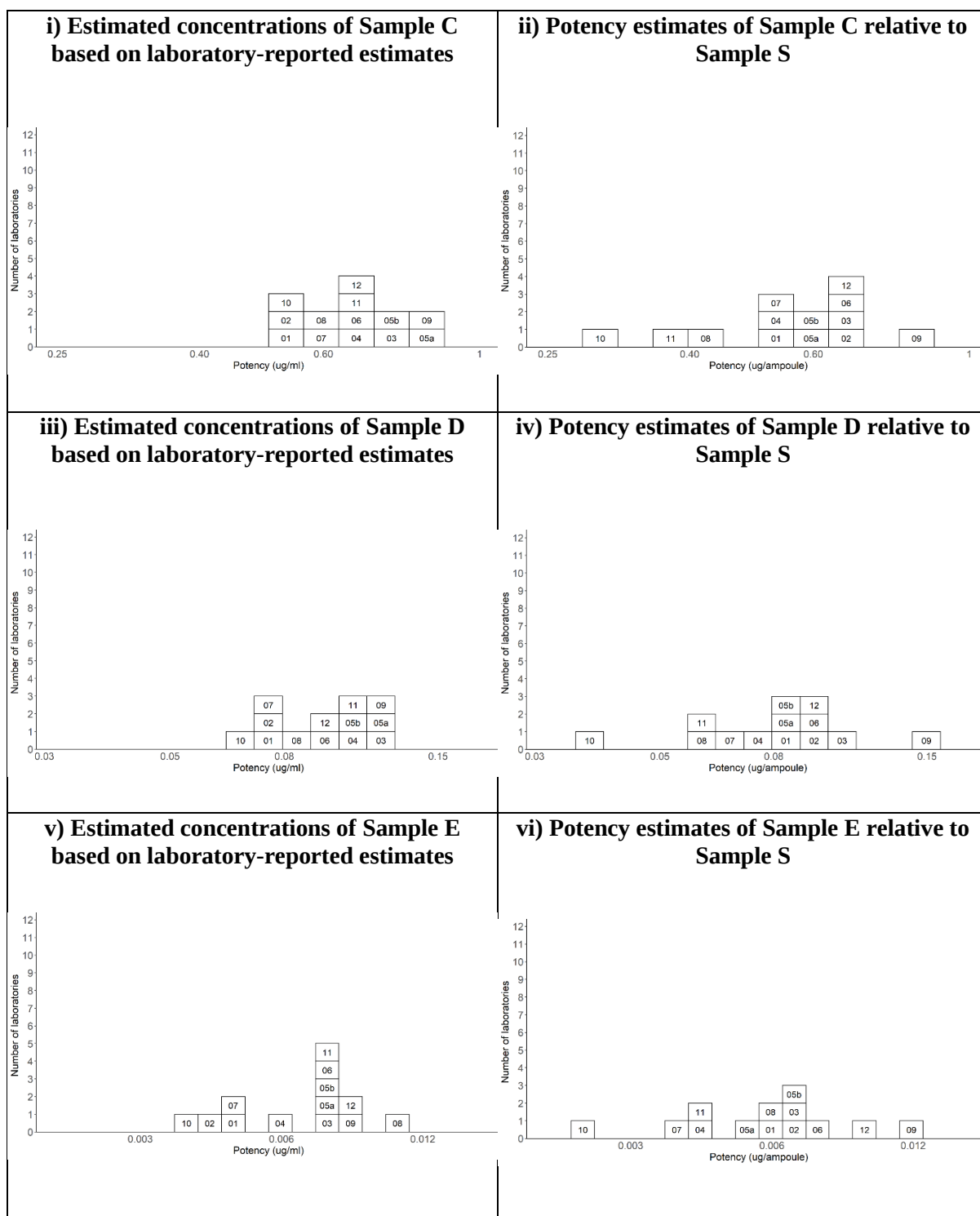


Table 1: Instruments and assay test kits used in the collaborative study and WHO IS used to calibrate the corresponding in-house standard

Instruments/assay platform used in the collaborative study:	In-house standard calibrated using:
Abbott Architect iSR2000	1 st IS (80/602)
Beckman Coulter Access Immunoassay	3 rd IS (94/572)
Beckman Coulter Olympus AU400 using Medicon Ferritin test kit	3 rd IS (94/572)
DBC Sandwich ELISA using the Tecan Infinite F50 ELISA absorbance microplate reader	3 rd IS (94/572)
DiaSorin Liaison XL sandwich chemiluminescence immunoassay	2 nd IS (80/578)
Human Huma CLIA150 immunoassay	2 nd IS (80/578) and evaluated against 3 rd IS (94/572) with acceptable recovery
Roche Cobas e601 using Elecsys® Ferritin test kit	1 st IS (80/602) with established traceability to 3 rd IS (94/572)
Roche Cobas e801 (CobasPro) using Elecsys® Ferritin test kit	1 st IS (80/602) with established traceability to 3 rd IS (94/572)
Siemens Advia Centaur XPT using Advia Centaur Ferritin test kit	2 nd IS (80/578)
Siemens BNII System N latex Ferritin	3 rd IS (94/572)
Thermo Fisher Scientific Indiko	3 rd IS (94/572)
Tosoh AIA-2000	1 st IS (80/602)

Table 2: Geometric mean estimated concentration (µg/ml) based on laboratory-reported estimates

Lab Code	Sample S			Sample A			Sample B			Sample C			Sample D			Sample E		
	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N
01	6.718	7.230	6	10.921	3.538	6	7.245	3.904	6	0.515	1.888	6	0.079	1.595	6	0.005	5.606	6
02	5.532	2.223	6	9.980	1.340	6	6.176	1.309	6	0.551	1.132	6	0.074	1.951	6	0.004	2.054	6
03	7.177	1.315	6	12.620	1.705	6	9.318	3.026	6	0.764	1.960	6	0.117	1.932	6	0.008	2.249	6
04	7.584	3.358	6	11.852	4.472	6	10.810	5.712	6	0.673	2.465	6	0.100	2.702	6	0.006	6.606	6
05a	8.719	2.691	6	14.309	2.602	6	11.753	3.202	6	0.863	3.092	6	0.113	4.144	6	0.008	2.315	6
05b	7.935	5.637	6	13.298	4.047	6	10.753	4.132	6	0.747	3.033	6	0.105	3.557	6	0.008	3.219	6
06	6.320	2.091	6	10.990	1.249	6	8.040	1.770	6	0.675	1.445	6	0.098	0.471	6	0.007	11.614	6
07	6.792	5.234	6	10.981	2.319	6	8.011	1.393	6	0.593	10.089	6	0.075	3.604	6	0.005	5.690	6
08	9.041	1.981	6	15.038	2.253	6	11.375	2.824	6	0.610	2.240	6	0.087	2.201	6	0.010	14.231	6
09	7.575	1.020	3	12.737	0.387	3	10.011	2.802	3	0.840	5.616	3	0.122	3.828	3	0.008	7.037	3
10	11.858	2.788	6	19.430	7.191	6	11.148	4.070	6	0.558	3.640	6	0.066	9.428	6	0.004	14.604	6
11	11.485	1.778	6	18.940	3.654	6	14.178	2.349	6	0.707	2.331	6	0.109	1.772	6	0.008	7.139	6
12	6.192	3.820	6	11.354	3.315	6	8.487	2.531	6	0.673	4.696	6	0.092	4.807	6	0.008	21.711	6
All	7.722	25.611	13	12.989	23.123	13	9.564	25.676	13	0.666	17.472	13	0.094	21.739	13	0.007	34.315	13
All*	7.439	22.874	11	12.544	20.356	11	9.393	27.694	11	0.663	16.224	11	0.094	17.890	11	0.007	30.878	11

*Excluding Lab 09 and Lab 10

GM: Geometric mean

GCV: Geometric coefficient of variation (%)

N: Number of valid estimates

n/a: not calculated as N < 3

Table 3: Geometric mean potency estimates (µg/ampoule) relative to Sample S calculated at NIBSC

Lab Code	Sample A			Sample B			Sample C			Sample D			Sample E		
	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N
01	10.226	2.760	6	7.083	4.971	6	0.507	5.823	6	0.081	8.048	6	0.006	12.126	6
02	11.235	1.366	6	7.107	1.513	6	0.640	1.763	6	0.092	3.375	6	0.007	6.374	6
03	11.092	2.758	6	8.189	2.642	6	0.671	1.149	6	0.101	1.455	6	0.006	1.197	6
04	9.632	4.640	5	8.863	5.101	6	0.534	3.621	6	0.078	8.699	6	0.004	13.868	6
05a	10.354	1.838	6	8.522	3.597	6	0.604	3.864	6	0.083	3.098	6	0.005	2.655	6
05b	10.554	2.211	6	8.526	2.566	6	0.597	3.859	5	0.084	7.284	6	0.007	3.910	6
06	10.900	1.595	6	7.967	1.487	6	0.672	1.908	6	0.097	1.710	6	0.008	9.469	6
07	10.199	3.043	6	7.434	4.750	6	0.525	13.048	5	0.068	5.473	6	0.004	17.126	6
08	10.469	4.250	5	7.886	1.938	5	0.412	n/a	2	0.060	3.210	5	0.006	21.898	5
09	10.545	n/a	2	8.157	n/a	2	0.846	5.970	3	0.144	6.269	3	0.012	11.935	3
10	9.356	n/a	1	6.003	n/a	2	0.309	9.046	3	0.036	13.064	4	0.002	42.825	4
11	10.233	2.322	6	7.749	1.443	6	0.373	3.144	6	0.060	2.587	6	0.004	7.927	6
12	11.022	3.454	4	8.376	1.316	4	0.673	5.684	3	0.095	4.573	5	0.009	11.316	5
All	10.434	5.470	13	7.798	11.014	13	0.548	31.922	13	0.079	39.473	13	0.006	52.324	13
All*	10.528	4.706	11	7.953	7.790	11	0.555	22.206	11	0.081	20.283	11	0.006	28.982	11

*Excluding Lab 09 and Lab 10

GM: Geometric mean

GCV: Geometric coefficient of variation (%)

N: Number of valid estimates

n/a: not calculated as N < 3

Table 4: Geometric mean potency estimates relative to Sample A calculated at NIBSC

Lab Code	Sample B			Sample C			Sample D			Sample E		
	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N	GM	% GCV	N
01	0.6954	4.788	6	0.0496	4.970	6	0.0081	7.204	6	0.0006	10.877	6
02	0.6335	1.530	6	0.0563	1.804	6	0.0080	5.776	6	0.0006	10.741	6
03	0.7386	4.871	6	0.0607	1.946	6	0.0091	2.203	6	0.0006	1.832	6
04	0.9118	5.401	5	0.0561	4.784	5	0.0084	5.179	5	0.0005	12.503	5
05a	0.8227	2.585	6	0.0585	2.969	6	0.0080	2.410	6	0.0005	3.218	6
05b	0.8080	1.031	6	0.0565	3.295	5	0.0079	6.614	6	0.0006	3.203	6
06	0.7306	2.291	6	0.0618	2.077	6	0.0089	1.461	6	0.0007	8.825	6
07	0.7321	1.910	6	0.0528	11.916	5	0.0070	2.860	6	0.0004	11.061	6
08	0.7558	3.363	6	0.0376	n/a	2	0.0058	3.485	6	0.0007	10.764	6
09	0.7267	n/a	1	0.0768	n/a	2	0.0136	n/a	2	0.0012	n/a	2
10	0.6471	n/a	1	NP	n/a	n/a	0.0035	n/a	1	0.0003	n/a	1
11	0.7575	3.363	6	0.0375	n/a	2	0.0062	2.301	6	0.0005	7.170	6
12	0.7656	4.679	3	0.0601	6.264	3	0.0085	6.649	5	0.0008	19.135	5
All	0.7449	10.081	13	0.0543	22.432	12	0.0076	35.947	13	0.0006	39.319	13
All*	0.7562	9.823	11	0.0527	19.607	11	0.0078	15.349	11	0.0006	19.498	11

*Excluding Lab 09 and Lab 10

GM: Geometric mean

GCV: Geometric coefficient of variation (%)

N: Number of valid estimates

n/a: not calculated as $N < 3$

NP: Non-parallel

Table 5: Summary of Inter-laboratory %GCV*

Standard	Test Sample			
	Sample A	Sample B	Sample C	Sample D
S	5%	8%	22%	20%
IH	20%	28%	16%	18%
A	-	10%	20%	15%
B	10%	-	22%	21%

*Excluding Lab 09 and Lab 10

IH: In-house

Table 6: Summary statistics of concentration estimates (µg/ampoule) relative to sample S

Statistic	Sample A	Sample B
Overall GM	10.5	8.0
Intra-lab variance component (as GCV)	2.8%	3.2%
Inter-lab variance component (as GCV)	4.7%	7.8%
Expanded uncertainty (95% confidence, k=2.23)	10.2 - 10.8	7.6 – 8.4

Appendix 1

Table 1: Individual assay potency estimates relative to Sample S (µg/ampoule) calculated at NIBSC

Lab	Day	Assay	Sample A	Sample B	Sample C	Sample D	Sample E
01	1	1	10.004	6.477	0.460	0.070	0.005
01	1	2	10.103	7.254	0.493	0.082	0.006
01	2	1	10.626	7.167	0.511	0.083	0.005
01	2	2	10.381	6.956	0.515	0.082	0.006
01	3	1	10.376	7.409	0.543	0.086	0.006
01	3	2	9.882	7.275	0.522	0.086	0.006
02	1	1	11.066	7.076	0.638	0.090	0.007
02	1	2	11.127	7.049	0.634	0.093	0.007
02	2	1	11.475	7.055	0.635	0.089	0.006
02	2	2	11.359	7.283	0.658	0.097	0.007
02	3	1	11.176	6.994	0.626	0.091	0.006
02	3	2	11.210	7.118	0.648	0.093	0.007
03	1	1	10.927	8.272	0.664	0.099	0.006
03	1	2	11.062	8.423	0.662	0.101	0.006
03	2	1	11.185	8.320	0.672	0.103	0.006
03	2	2	11.666	7.831	0.683	0.103	0.006
03	3	1	10.833	8.068	0.670	0.102	0.006
03	3	2	10.901	8.236	0.675	0.101	0.006
04	1	1	9.504	8.987	0.550	0.079	0.004
04	1	2	9.018	8.575	0.503	0.067	0.004
04	2	1	NL	9.450	0.535	0.078	0.004
04	2	2	10.199	9.308	0.538	0.080	0.005
04	3	1	9.837	8.377	0.523	0.077	0.004
04	3	2	9.643	8.538	0.555	0.087	0.005

05a	1	1	10.480	8.402	0.596	0.083	0.005
05a	1	2	10.485	8.655	0.599	0.088	0.005
05a	2	1	10.343	8.851	0.610	0.083	0.005
05a	2	2	10.497	8.810	0.647	0.083	0.005
05a	3	1	10.321	8.368	0.594	0.082	0.005
05a	3	2	10.008	8.073	0.579	0.081	0.005
05b	1	1	10.961	8.808	0.599	0.084	0.007
05b	1	2	10.535	8.504	NL	0.088	0.007
05b	2	1	10.686	8.752	0.636	0.091	0.007
05b	2	2	10.390	8.313	0.596	0.086	0.006
05b	3	1	10.410	8.505	0.580	0.077	0.006
05b	3	2	10.354	8.285	0.579	0.077	0.006
06	1	1	10.871	8.016	0.676	0.097	0.008
06	1	2	11.098	8.091	0.691	0.099	0.007
06	2	1	11.052	7.954	0.660	0.098	0.008
06	2	2	10.958	7.785	0.678	0.097	0.007
06	3	1	10.795	7.886	0.656	0.094	0.008
06	3	2	10.635	8.072	0.672	0.097	0.008
07	1	1	10.418	7.621	0.616	0.073	0.005
07	1	2	10.297	7.438	0.581	0.070	0.004
07	2	1	9.639	6.837	NL	0.063	0.003
07	2	2	10.116	7.326	0.468	0.066	0.004
07	3	1	10.295	7.629	0.481	0.068	0.005
07	3	2	10.453	7.795	0.495	0.066	0.004
08	1	1	10.282	7.721	NP	0.059	0.007
08	1	2	10.882	7.827	0.408	0.060	0.008
08	2	1	9.837	7.802	NP	0.058	0.005
08	2	2	10.835	7.993	0.416	0.063	0.006
08	3	1	10.543	8.093	NP	0.059	0.005
08	3	2	Std NL	Std NL	Std NL	Std NL	Std NL
09	1	1	10.637	7.600	0.811	0.137	0.011

09	1	2	n/t	n/t	n/t	n/t	n/t
09	2	1	NL	8.755	0.904	0.154	0.014
09	2	2	n/t	n/t	n/t	n/t	n/t
09	3	1	10.453	NL	0.826	0.141	0.012
09	3	2	n/t	n/t	n/t	n/t	n/t
10	1	1	NL/NP	NL/NP	NL/NP	0.034	0.004
10	1	2	NL	NL/NP	0.334	0.042	0.001
10	2	1	9.356	6.050	0.313	0.031	0.002
10	2	2	Std NL	Std NL	Std NL	Std NL	Std NL
10	3	1	NL/NP	5.955	0.282	0.037	0.002
10	3	2	Std NL	Std NL	Std NL	Std NL	Std NL
11	1	1	9.868	7.729	0.368	0.062	0.005
11	1	2	10.392	7.759	0.356	0.057	0.004
11	2	1	10.565	7.550	0.365	0.061	0.004
11	2	2	10.197	7.796	0.382	0.061	0.004
11	3	1	10.228	7.781	0.384	0.060	0.004
11	3	2	10.162	7.882	0.382	0.060	0.004
12	1	1	Std NL	Std NL	Std NL	Std NL	Std NL
12	1	2	NL/NP	8.481	NP	0.092	0.008
12	2	1	10.719	8.223	NL	0.091	0.009
12	2	2	10.718	8.406	0.694	0.095	0.010
12	3	1	11.188	NL/NP	0.632	0.096	0.009
12	3	2	11.483	8.396	0.696	0.102	0.009

NL: Non-linear

NP: Non-parallel

Std NL: Standard non-linear

n/t: not tested

Table 2: Individual laboratory reported concentration estimates (µg/ml)

Lab	Day	Assay	Sample S	Sample A	Sample B	Sample C	Sample D	Sample E
01	1	1	7.118	11.363	7.340	0.519	0.079	0.005
01	1	2	6.632	11.091	6.792	0.506	0.079	0.005
01	2	1	6.451	11.273	7.383	0.513	0.080	0.005
01	2	2	7.510	10.771	7.046	0.507	0.077	0.005
01	3	1	6.287	10.670	7.493	0.533	0.080	0.005
01	3	2	6.397	10.389	7.443	0.516	0.081	0.005
02	1	1	5.654	10.023	6.273	0.557	0.072	0.004
02	1	2	5.631	10.053	6.220	0.555	0.075	0.004
02	2	1	5.521	10.139	6.124	0.547	0.072	0.004
02	2	2	5.314	9.748	6.092	0.545	0.075	0.004
02	3	1	5.552	9.972	6.100	0.543	0.074	0.004
02	3	2	5.530	9.948	6.249	0.556	0.075	0.004
03	1	1	7.190	12.457	9.428	0.756	0.114	0.008
03	1	2	7.133	12.510	9.520	0.743	0.116	0.007
03	2	1	7.137	12.651	9.411	0.758	0.118	0.008
03	2	2	7.045	13.041	8.779	0.764	0.116	0.008
03	3	1	7.311	12.557	9.347	0.780	0.120	0.008
03	3	2	7.247	12.512	9.444	0.781	0.119	0.008
04	1	1	7.393	11.263	10.758	0.669	0.100	0.006
04	1	2	8.095	11.692	11.293	0.704	0.100	0.007
04	2	1	7.541	12.661	11.399	0.666	0.097	0.006
04	2	2	7.540	12.259	11.272	0.670	0.101	0.006
04	3	1	7.524	11.861	10.113	0.660	0.099	0.006
04	3	2	7.431	11.434	10.110	0.670	0.105	0.007
05a	1	1	8.553	14.214	11.352	0.832	0.112	0.008
05a	1	2	8.881	14.741	12.123	0.874	0.122	0.008
05a	2	1	8.595	14.091	12.044	0.852	0.112	0.008
05a	2	2	8.443	14.026	11.753	0.897	0.109	0.008

05a	3	1	9.062	14.817	12.005	0.885	0.115	0.008
05a	3	2	8.798	13.987	11.271	0.839	0.111	0.008
05b	1	1	7.559	13.160	10.590	0.714	0.100	0.007
05b	1	2	7.862	13.159	10.631	0.747	0.109	0.008
05b	2	1	7.433	12.622	10.350	0.741	0.108	0.007
05b	2	2	7.901	13.036	10.429	0.740	0.108	0.007
05b	3	1	8.521	14.079	11.519	0.780	0.104	0.008
05b	3	2	8.398	13.782	11.043	0.764	0.101	0.008
06	1	1	6.169	10.951	8.085	0.682	0.098	0.007
06	1	2	6.198	10.923	7.975	0.676	0.097	0.006
06	2	1	6.309	11.075	7.977	0.657	0.098	0.008
06	2	2	6.331	11.022	7.830	0.676	0.097	0.006
06	3	1	6.528	11.184	8.179	0.681	0.097	0.008
06	3	2	6.390	10.788	8.200	0.681	0.098	0.008
07	1	1	6.527	10.913	7.909	0.662	0.076	0.005
07	1	2	6.739	10.868	7.928	0.670	0.078	0.004
07	2	1	7.467	11.379	8.101	0.587	0.078	0.004
07	2	2	6.907	11.171	8.042	0.539	0.073	0.005
07	3	1	6.546	10.660	7.915	0.543	0.072	0.005
07	3	2	6.608	10.909	8.173	0.567	0.073	0.004
08	1	1	9.099	14.826	11.132	0.602	0.086	0.011
08	1	2	8.877	15.280	11.017	0.589	0.085	0.012
08	2	1	9.319	14.483	11.497	0.616	0.088	0.009
08	2	2	8.843	15.061	11.169	0.607	0.091	0.010
08	3	1	9.132	15.246	11.665	0.624	0.087	0.008
08	3	2	8.984	15.349	11.793	0.622	0.086	0.011
09	1	1	7.570	12.784	9.796	0.820	0.119	0.008
09	1	2	n/t	n/t	n/t	n/t	n/t	n/t
09	2	1	7.655	12.686	10.327	0.894	0.127	0.009
09	2	2	n/t	n/t	n/t	n/t	n/t	n/t
09	3	1	7.501	12.742	9.917	0.808	0.119	0.008

09	3	2	n/t	n/t	n/t	n/t	n/t	n/t
10	1	1	11.694	19.328	10.926	0.567	0.073	0.004
10	1	2	12.264	22.250	12.036	0.595	0.066	0.004
10	2	1	11.822	19.223	11.199	0.544	0.064	0.004
10	2	2	11.801	18.769	10.755	0.554	0.056	0.003
10	3	1	12.197	19.002	10.955	0.539	0.071	0.005
10	3	2	11.390	18.252	11.059	0.552	0.065	0.004
11	1	1	11.410	18.080	14.070	0.690	0.109	0.008
11	1	2	11.860	19.860	14.630	0.690	0.109	0.007
11	2	1	11.490	19.640	13.780	0.700	0.113	0.008
11	2	2	11.450	18.790	14.260	0.730	0.108	0.008
11	3	1	11.240	18.470	13.890	0.710	0.108	0.007
11	3	2	11.470	18.860	14.460	0.720	0.108	0.008
12	1	1	6.213	11.643	8.480	0.676	0.089	0.007
12	1	2	6.167	11.743	8.387	0.663	0.086	0.006
12	2	1	6.563	10.959	8.578	0.719	0.096	0.011
12	2	2	5.857	10.877	8.481	0.692	0.094	0.010
12	3	1	6.287	11.598	8.826	0.626	0.094	0.008
12	3	2	6.088	11.334	8.182	0.668	0.096	0.008

Appendix 2

Participants of the collaborative study (in alphabetical order of country):

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Roland Knauer	Human Gesellschaft für Biochemica & Diagnostica mbH, Germany
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JiYoon Kim, Elaine O'Doherty	Abbot Ireland Diagnostic Division, Ireland
Hisao Tsukamoto	Tosoh Corporation, Japan
Goran Brattsand	Clinical Chemistry, NUS, Sweden
Paul Pettersson-Pablo	Clinical Chemistry, Örebro University Hospital, Sweden
Graham Roberts, Bernard Fox	NIBSC, UK
Ryan Masica	Beckman Coulter, USA

Appendix 3

Stability prediction of Sample A (19/118)

Measured activities (relative to -70°C) (relative potency estimates from 2 ampoules)				
Temp (°C)	Potency	Time (years)	95% LCL	95% UCL
-20	0.9953	2.0	0.9772	1.0138
-20	1.0123	2.0	0.9860	1.0393
4	0.9716	2.0	0.9539	0.9896
4	1.0279	2.0	1.0012	1.0552
20	0.9199	2.0	0.9031	0.9369
20	1.0032	2.0	0.9772	1.0300
37	0.8511	2.0	0.8290	0.8738
37	0.8533	2.0	0.8377	0.8691
45	0.7116	2.0	0.6986	0.7248
45	0.7274	2.0	0.7084	0.7468
56	0.3726	2.0	0.3626	0.3829
56	0.4367	2.0	0.4286	0.4450
Predicted degradation rates				
Temp (°C)	K	S.E. (K)	% Loss per Year	95% UCL % Loss
-70	0	0	0	0
-20	0.00011	0.00002	0.011	0.021
+4	0.00250	0.00023	0.250	0.364
+20	0.01493	0.00089	1.482	1.919
+37	0.08126	0.00235	7.805	8.882

Stability prediction of Sample B (19/162)

Measured activities (relative to -70°C) (relative potency estimates from 2 ampoules)				
Temp (°C)	Potency	Time (years)	95% LCL	95% UCL
-20	0.9872	1.75	0.9540	1.0215
-20	0.9777	1.75	0.9529	1.0031
4	0.9760	1.75	0.9432	1.0100
4	0.9753	1.75	0.9505	1.0006
20	0.9523	1.75	0.9203	0.9854
20	0.9505	1.75	0.9264	0.9753
37	0.8584	1.75	0.8295	0.8882
37	0.8435	1.75	0.8221	0.8655
45	0.7145	1.75	0.6903	0.7394
45	0.7434	1.75	0.7245	0.7628
56	0.4743	1.75	0.4580	0.4911
56	0.4663	1.75	0.4542	0.4786
Predicted degradation rates				
Temp (°C)	K	S.E. (K)	% Loss per Year	95% UCL % Loss
-70	0	0	0	0
-20	0.00024	0.00005	0.024	0.049
+4	0.00403	0.00052	0.402	0.661
+20	0.02025	0.00168	2.005	2.824
+37	0.09374	0.00376	8.948	10.644

Appendix 4

COLLABORATIVE STUDY PROTOCOL TO CALIBRATE PROPOSED 4th INTERNATIONAL STANDARD FOR FERRITIN, HUMAN RECOMBINANT

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Statistician

Peter Rigsby (NIBSC)

1. AIM

To calibrate the candidate preparations for the 4th International Standard for Ferritin, human recombinant.

2. MATERIALS PROVIDED

- **Sample S:** 3 ampoules of the WHO 3rd International Standard for Ferritin, human recombinant (lyophilized; 94/572), labelled as S1, S2 and S3. Contents: **6.3 µg/ampoule**.
- **Sample A:** 3 ampoules of lyophilized recombinant ferritin in serum labelled as A1, A2 and A3. Contents: **approximately 10 µg/ampoule**.
- **Sample B:** 3 ampoules of lyophilized recombinant ferritin in serum, labelled as B1, B2 and B3. Contents: **approximately 7 µg/ampoule**.
- **Sample C:** 3 ampoules of lyophilized high serum ferritin, labelled as C1, C2 and C3. Contents: **approximately 0.9 µg/ampoule**.
- **Sample D:** 3 ampoules of lyophilized normal serum ferritin, labelled as D1, D2 and D3. Contents: **approximately 0.1 µg/ampoule**.
- **Sample E:** 3 ampoules of lyophilized low serum ferritin, labelled as E1, E2 and E3. Contents: **approximately 0.01 µg/ampoule**.

The concentrations for samples A-E above are approximations and intended only as a guide for making dilution series (Examples of the dose-response curves obtained at NIBSC for samples S, A, B & C are shown in the Appendix).

Store all ampoules at –20°C until reconstitution and use. Do not reconstitute until the day of use.

Reconstitute the contents of each ampoule with 1.0 ml distilled/deionized H₂O according to the 'Instructions for Use' sheets supplied with the materials. Vortex gently and inspect contents to ensure complete dissolution. Transfer reconstituted contents to a labelled capped tube. The tubes may be stored at 4°C after use for repeat assays, if necessary.

We have a limited number of additional ampoules that can be provided on request in the case of breakages or errors.

3. ASSAY DESIGN

DAY 1:

Reconstitute ampoules S1, A1, B1, C1, D1, E1.

Using your assay platform, assay 2 independent dilution series including a minimum of 4 dilutions (e.g., neat, 1 in 2, 1 in 4, 1 in 8 etc) in your usual assay diluent of the preparations S1, A1, B1 and C1 alongside your usual standard (calibrant) dilutions in the same assay run. Perform a duplicate single point measurement of preparations D1 and E1 with a pre-dilution appropriate for your assay. **Each replicate dilution series should be prepared separately from the neat, reconstituted ampoule contents, including any pre-dilution required by your assay; do not assay duplicates of a single dilution series.** Please indicate clearly on the results sheets whether the dilutions tested relate to a pre-dilution or the neat, reconstituted ampoule contents.

Perform a second assay to include all preparations as above, **with 2 fresh independent dilution series prepared separately from the ampoule contents of each preparation, including any pre-dilution required by your assay**, adjusting the dilutions if necessary to ensure responses fall within the measurable range of your assay.

Therefore, a total of 4 independent dilution series from each reconstituted preparation should have been tested in 2 assays (2 dilution series per assay) for preparations S1, A1, B1 and C1. You should also have obtained 4 single point measurements for each of preparations D1 and E1.

DAY 2:

Reconstitute ampoules S2, A2, B2, C2, D2, E2.

Perform 2 separate assays as for Day 1.

DAY 3:

Reconstitute ampoules S3, A3, B3, C3, D3, E3.

Perform 2 separate assays as for Day 1.

4. **RECORDING RESULTS**

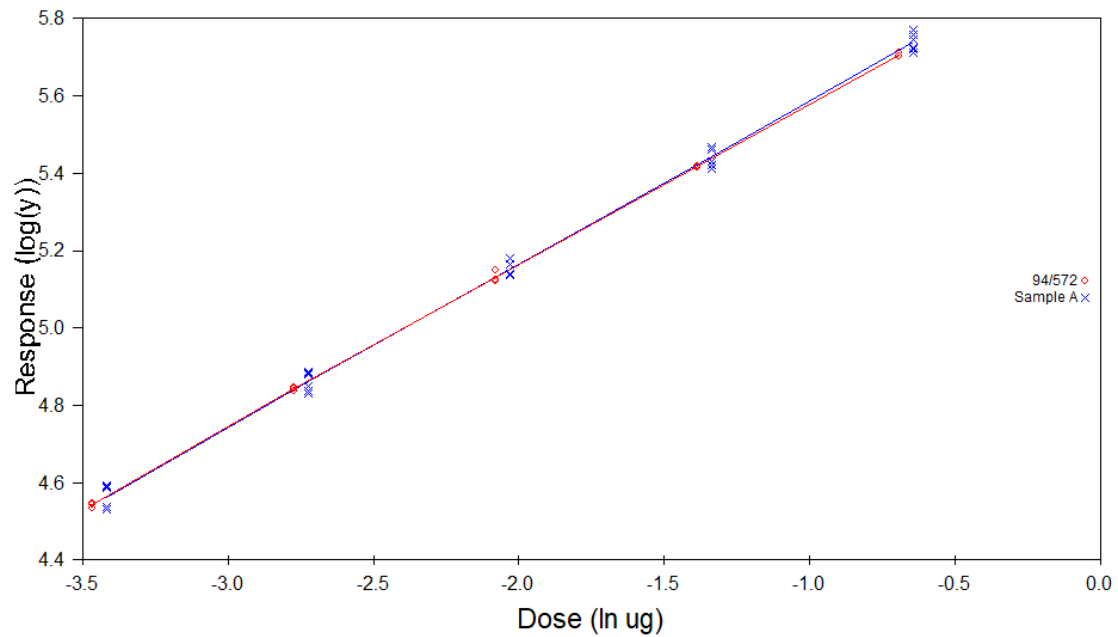
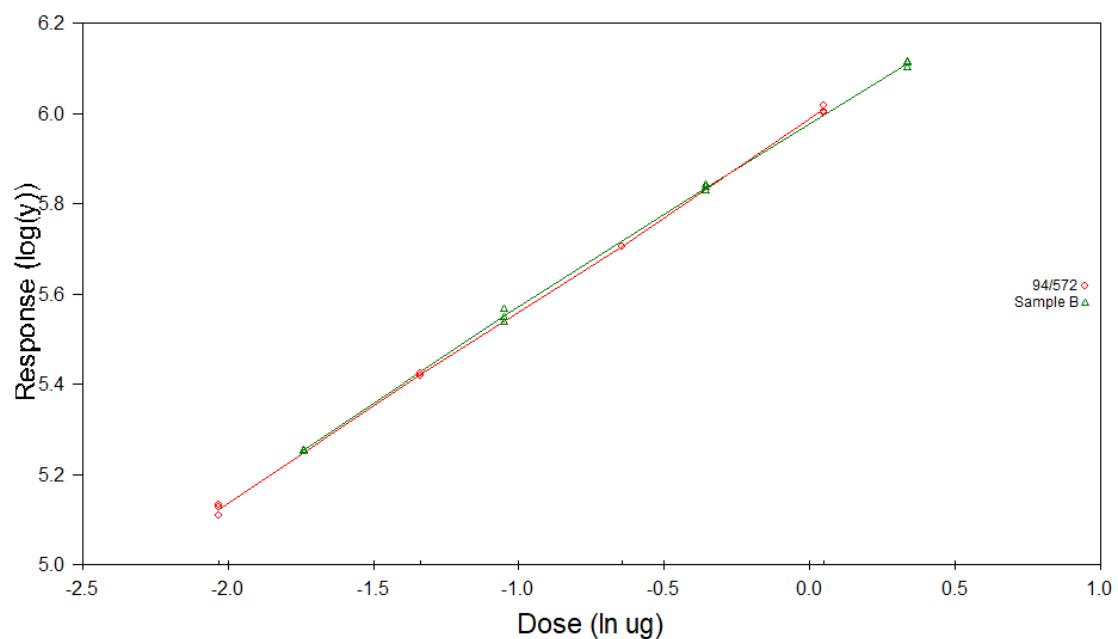
Please enter the raw data, i.e., dilution factor and response (e.g., absorbance, relative light units or concentration), on the Excel results sheets provided, along with your own estimates of the concentration of S, A, B, C, D and E relative to your in-house standard (calibrant).

Please return electronic copies of the results to bernard.fox@nibsc.org

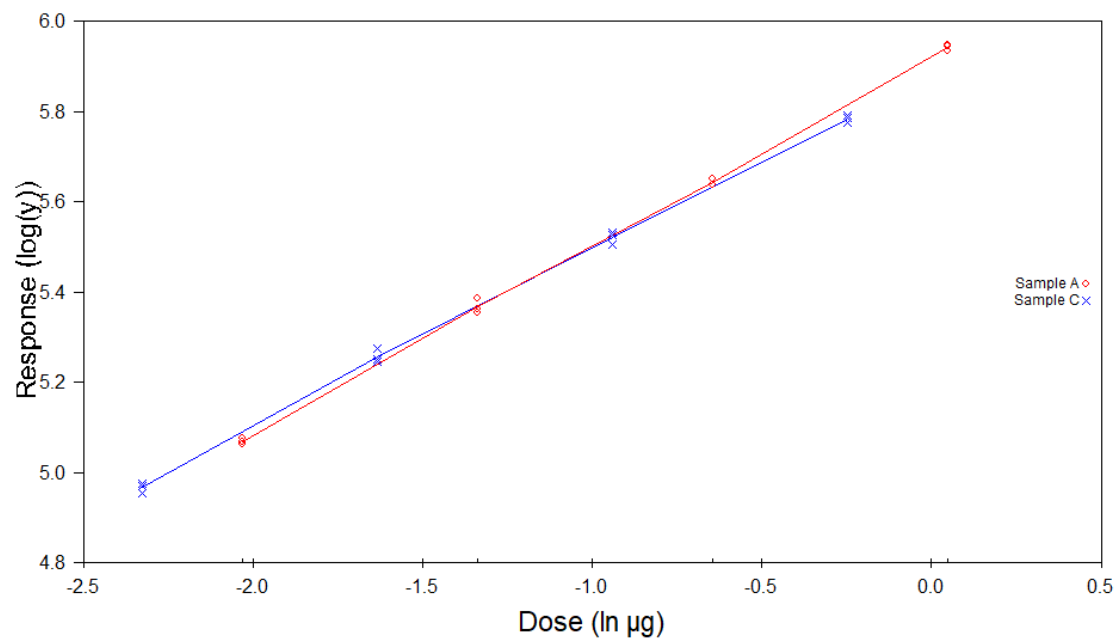
PLEASE RETURN YOUR RESULTS BY 5th FEBRUARY 2021

APPENDIX

Concentrations below are given in $\mu\text{g/ml}$ which is equivalent to mg/L .

**Comparison of Sample S (94/572) and Sample A over the concentration range
0.5 – 0.03 $\mu\text{g/ml}$** **Comparison of Sample S (94/572) and Sample B over the concentration range
1.4 – 0.13 $\mu\text{g/ml}$** 

**Comparison of Sample A and Sample C over the concentration range
1.0 – 0.1 µg/ml**



Appendix 5



Medicines & Healthcare products
Regulatory Agency

**WHO International Standard
Ferritin (Human, Recombinant)
NIBSC code: 19/118
Instructions for use
(Version 1.00, Dated)**

1. INTENDED USE

The standard 19/118 is for use in immunoassays for human serum ferritin.

The recombinant ferritin preparation, 19/118, was evaluated by 12 laboratories in 11 countries for its suitability as an International Standard (IS). The preparation was assayed in a wide range of commercial immunoassays against the 3rd IS for ferritin (94/572) (1). The recombinant ferritin was immunologically similar to the 3rd IS in the majority of assays, and demonstrated adequate stability in accelerated degradation studies. On the basis of these results, and with the overall agreement of the participants of the collaborative study, the World Health Organization (WHO) Expert Committee on Biological Standardization established 19/118 as the 4th IS for ferritin, recombinant, with an ASSIGNED CONTENT OF 10.5 MICROGRAMS/AMPOULE.

Uncertainty: the expanded uncertainty limits are 10.2-10.8 µg/ampoule (95% confidence; k=2.23).

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

Each ampoule contains 10.5 micrograms ferritin

4. CONTENTS

Country of origin of biological material: United Kingdom.
Recombinant human ferritin was expressed in an *E. Coli* strain transformed with the plasmid pET-21a which encodes the full human ferritin L-chain amino acid sequence. Details of its purification and characterization are described in references 2-4.

The recombinant ferritin was diluted in human AB serum (pooled from individual donations which had been tested and found negative for HBsAg, anti-HIV and anti-HCV) before distribution into ampoules and lyophilization.

5. STORAGE

Store unopened ampoules at or below -20°C.
Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

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



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.

Reconstitute the contents of each ampoule with 1.0 ml distilled water. Store at 4°C (short term only since the preparation does not contain sodium azide).

THE FERRITIN CONCENTRATION OF THE RECONSTITUTED MATERIAL IS 10.5 MICROGRAMS/ML.

8. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. Accelerated degradation studies have indicated that this standard is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the standard is withdrawn or replaced. These studies have also shown that the standard is suitably stable for shipment at ambient temperature without any effect on the assigned values.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

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

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

9. REFERENCES

1. S J Thorpe, D Walker, P Arosio, A Heath, J Cook and M Worwood. Clin Chem (1997) 43:1582-1587.
2. Levi et al. Biochem J (1992) 288:591-596.
3. Santambrogio et al. J Biol Chem (1993) 268:12744-12748.
4. Levi et al. J Biol Chem (1994) 269:30334-30339.

10. ACKNOWLEDGEMENTS

We thank the participants of the collaborative study to assign unitage.

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:



World Health
Organization



Medicines & Healthcare products
Regulatory Agency

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: Lyophilisate	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains material of human origin	
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of 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.

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



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: 0.08g
Toxicity Statement: Toxicity not assessed
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

http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (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.

