World Health Organization

WHO/BS/2026.2500
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 20 – 24 April 2026

Collaborative Study for the First WHO International Standard for unfractionated heparin for molecular weight calibration

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NOTE:

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

Product Standards, Specifications and Nomenclature
Department of Medicines and Health Products Policies and Standards
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

Twelve laboratories from seven countries returned data for the collaborative study to verify the broad standard table for the proposed 1st International Standard (IS) for Unfractionated Heparin for Molecular Weight Calibration, 07/324. The laboratories were able to use the calibrant to calibrate their molecular weight systems and calculate the weight average molecular weight (Mw) with high precision, the intra laboratory coefficients of variation (CV) ranged from 0.05 to 3.70% with most results below 1% for the five unknown samples included in the study. When the proportion of material within specific ranges was calculated for the five sample, good precision was also observed within laboratories with two-thirds of values below 2%, the CVs ranged from 0.68 to 38.07% for % below 8000 Da, 0.08 to 7.08% for % between 8000 and 16000 Da, 0.14 to 6.51% for % between 16000 and 24000 Da and 0.36 to 11.65% for % greater than 24000 Da, with the highest CV attributable to one participant. Across the laboratories there was good agreement for the different measurements with the inter laboratory CV for weight average molecular weight being between 0.41 and 0.96%, and for the proportion slices for < 8000 Da the CVs were 0.20 to 1.96%, with one sample showing higher variability at 8.91%, for > 8000 to < 16000 Da the CVs were 0.54 to 1.84%, for > 16000 to < 24000 Da the CVs were 0.66 to 1.12% and for > 24000 Da the CVs were 1.74 to 3.02%.

It is recommended that Sample Cal (NIBSC Code 07/324) be adopted as the 1st International Standard for Unfractionated Heparin for Molecular Weight Calibration, together with the broad-standard table outlined in this report.

INTRODUCTION

Unfractionated heparin is a long-established biological medicine that has been used as an anticoagulant for nearly one hundred years. It is included on the WHO List of Essential Medicines, and its biological activity is currently measured against the WHO 6th International Standard (IS) for Unfractionated Heparin, 07/328. A distinguishing characteristic of unfractionated heparin is that it is a polydisperse, heterogeneous polysaccharide (1).

The polydisperse nature of heparin can be assessed using gel permeation chromatography (GPC). Although absolute molecular weight determination can be achieved using high-performance GPC with light-scattering detection (2), this approach requires specialist equipment and considerable expertise. A simpler and more widely accessible option is to use a broad-standard molecular weight calibrant together with refractive index detection (3). This technique has been used since the 1980s to characterize the molecular weight of low-molecular-weight heparins and is a monograph requirement in several pharmacopoeias.

Molecular weight determination for low-molecular-weight heparin relies on the use of a calibrant, with the 2nd IS (05/112) being the current reference material. The bulk material used to establish this IS was also used to create the European Pharmacopoeia (EP) and United States Pharmacopeia (USP) calibrants, thereby promoting harmonization—although each pharmacopoeia requires use of its own calibrant as specified in its monograph. In 2014, the USP established an unfractionated heparin molecular weight calibrant following an extensive international collaborative study involving experts from NIBSC (4). In 2015, the Chinese Pharmacopoeia (CP) produced its own calibrant, using the USP

material to assign its values (5). With these two broad-standard calibrants in place, the establishment of a WHO International Standard (IS) was considered an important next step to ensure global harmonization, and the proposal was endorsed by the WHO ECBS in 2019.

The 2019 proposal aimed to establish a WHO IS linked to both the USP and CP calibrants as at that time the link between these calibrants was unknown. However, because the CP calibrant was itself value-assigned using the USP standard as published in 2020, it was concluded that adopting the USP standard directly—following a qualification study—would better support harmonization. Under this approach, the broad standard table (BST) developed and verified during the USP study would be used in an international collaborative study to confirm its suitability. The study requested GPC/HPLC-based methods using refractive index detection, with the supplied candidate material used as the calibrant in a broad-standard calibration approach (3).

The primary objective of the study was to verify and qualify whether the candidate calibrant, used with the BST, is suitable for adoption as a WHO International Standard for system calibration by determining the molecular weight of five different unfractionated heparins. A secondary objective—exploring a strategy for future replacement of the current unfractionated heparin molecular weight calibrant—is not discussed in this report.

PARTICIPANTS

Fourteen different laboratories agreed to participate in the collaborative study, with samples sent to twelve laboratories as two were unable to obtain the required import permits. The participants were eight heparin manufacturers, one contract testing laboratory, two from a pharmacopeia and one regulatory authority. The participants were from seven different countries covering three WHO regions and are listed in alphabetical order in Appendix 1.

THE CANDIDATE, NIBSC CODED 07/324

The candidate material was filled in 2007 as a potential replacement to establish the 6th International Standard for Unfractionated Heparin. The fill characteristics are shown in Appendix 4 with the material filled and freeze dried according to guidelines for the production of international biological reference materials (6, 7). This material was not selected at the time to be an IS but was found to have a broad molecular weight profile which made it suitable to be a possible molecular weight calibrant for unfractionated heparin. The material was then established by the USP as their Heparin Sodium Molecular Weight Calibrant in 2014 (4).

SAMPLES

Each participant was supplied with four sets of six samples – a calibrant and five unknown heparin samples.

CS716 – Sample Cal (NIBSC Code 07/324) – see BST in Table 1.
CS716 – Sample A (NIBSC Code 22/102 – a typical sample, approximately Mw 17,000 Da

CS716 – Sample B (NIBSC Code 07/334 – a lower mass sample, approximately Mw 14,500 Da
CS716 – Sample C (NIBSC Code 07/332 – a higher mass sample, approximately Mw 22,000 Da)
CS716 – Sample D (Panel heparin sample – a typical sample, approximately Mw 16,000 Da)
CS716 – Sample E (Panel heparin sample – a typical sample, approximately Mw 16,000 Da)

ASSAY METHOD AND STUDY DESIGN

Each participant was requested to conduct their routine broad standard method using refractive index detection with Sample Cal employed to calibrate the system. The calibrated system would then calculate the weight average molecular weight and proportion of material within defined ranges of the unknown samples, A to E. A generic broad standard method for calibrating systems to measure heparin samples is described in Mulloy & Hogwood 2022 (3). The USP Heparin Sodium monograph method 'D' Molecular Weight Determination is another such example of a method (4). The broad standard table associated with the calibrant used by each participant is shown in Table 1. Each participant was requested to provide details of their molecular weight systems and local method, with this information described in table 11.

Four independent injection series were required, each to be calibrated with a separate ampoule of sample 'Cal' – supplied protocol is shown in appendix 2. The participants were requested to provide the following information for each sample:

Weight average molecular weight (Mw)
% material with MW below 8000 Da
% material with MW between 8000 and 16000 Da
% material with MW between 16000 and 24000 Da
% material with MW above 24000 Da

Each participant was also requested to provide raw chromatogram data to enable local processing at MHRA. Sample reanalysis was conducted for each participant using the basic principle of selecting the same baseline and integration region for all chromatograms for a participant (see figure 1 for example).

RESULTS AND ANALYSIS

The calculated data returned by each laboratory, including system suitability samples, are presented in Appendix 3, Tables 1a–1l. All laboratories performed two injections per sample for each assay sequence. All chromatograms submitted were subsequently re-processed at MHRA to ensure a standardized approach to baseline selection, integration limits, and data handling, thereby minimizing inter-laboratory analytical variability. In most laboratories, each injection sequence contained a single calibrant injection; however, participants 7, 8, and 14 included two calibrant injections per sequence. In these cases, re-analysis was conducted separately for each calibrant injection, with independent calibration curves generated for injections one and two. For every calibration curve produced, the coefficient of determination (R^2) was verified to be greater than 0.999, a criterion that was met in all instances.

The mean of all values (reported and recalculated) was used as the assumption of normally-distributed appeared reasonable, as supported by minimal differences between the mean and median – see table 2 and 3 as examples. The coefficient of variation (standard deviation / mean %) was used to assess intra and inter laboratory variability and Grubbs' test used to determine any outliers.

Weight average molecular weight (Mw)

Returned data: All laboratories performed the molecular weight analysis with high precision as evidenced by the intra laboratory coefficients of variation (%CV) being less than 1% in 75% of cases, and below 2% in 88% of cases (see table 4). One laboratory, 14, showed greater variation in the Mw with all CVs above 4.5%. The inter laboratory CV was below 1% for two samples, B and D, with A and D between 1 and 2%. For sample E, the CV was 2.29%, but laboratory 14 was found to be an outlier, and exclusion reduced the CV to 0.48%.

Re-analysed data: For the reprocessed data, intra laboratory CVs were also low with 72% below 1% and 90% below 2% (see table 5 and figure 2). This re-analysis, when applied to laboratory 14 reduced their CVs to below 4% for all five samples. Within the re-analysis there were no outliers and the inter laboratory CVs were all less than 1%, highlighting excellent agreement between laboratories when a consistent analysis approach was taken.

The difference in Mw between the returned and re-analysed data was low (see table 6) with samples A, B and D showed a slight negative difference -29, -14 and -25, sample C showing a positive difference +88 and sample E either +106 with all data or +1 with the outlier excluded.

Molecular weight ranges

For the molecular weight ranges, the re-analysed data were used for analysis due to the lower inter-laboratory CVs seen for Mw, and the improvement in the data from laboratory 14. A summary comparing the inter-laboratory CVs for submitted and reprocessed data is shown in appendix 3 table 2a to d.

The inter-laboratory CVs for the different molecular weight ranges were more variable than the Mw. For the four slice ranges, the slices representing the lowest and highest molecular weight components of each heparin showed the highest variability.

When calculating the proportion of material below 8 kDa, only 42% (25/60) of intra laboratory CVs were below 2%, with 70% (42/60) below 5% (see table 7). Within this data sample C, which has a high Mw and as a result has a lower proportion of low molecular weight material, the reported inter CVs were much higher with most above 5% (11/12) – when mean values are closer to zero a higher CV is generally observed. Apart from sample C, the inter laboratory CVs were low, A – 0.20%, B – 1.35%, D – 1.96% and E – 1.37% (including the outlier the CV is 2.53%). For sample C, the CV was 8.91% with an outlier excluded (24.64% with the outlier), but it should be noted that the proportion of material within this range for sample C at a calculated mean of 1.27% is much lower than the other samples which range from 7.99% to 11.43%. Sample C is an atypical heparin.

The CVs calculated for proportion of material within the range 8 to 16 kDa within each laboratory were generally low, with 88% (53/60) below 2% and only one sample (Lab 14 sample C) with a CV between 5% and 10% (see table 8). The inter laboratory CVs for all samples were also low, where apart from sample C values were less than 1%, A – 0.54% (with outlier 0.86%), B – 0.63%, C – 1.84%, D – 0.86%, E – 0.90%.

The CVs calculated for proportion of material within the range 16 to 24 kDa within each laboratory were generally low, with 87% (52/60) below 2% and four results with a CV between 5% and 10% (see table 9). The inter laboratory CVs for all samples were also low for all samples with values close to or less than 1%, A – 0.66%, B – 1.12%, C – 0.88% (with outlier 1.48%), D – 0.63%, E – 0.62%.

For the proportion of material calculated to be above 24 kDa, 47% (28/60) of the intra laboratory CVs were less than 2% with 85% below 5% (see table 10). Two CVs were greater than 10%, both from laboratory 14 (Sample A and D). The inter laboratory CVs for each sample were generally more variable than for the above ranges but were 3% or below, A – 1.83% (with outlier 2.71%), B – 3.02%, C – 1.74%, D – 2.22%, E – 2.58%.

STABLILITY STUDY

Samples of the candidate material were stored at a range of temperatures to enable the determination of the stability of the material. An assessment using molecular weight was performed in 2022 with the data shown graphically in Appendix 5. At elevated temperature the profile for the material changed with an increase in the weight average molecular weight (Mw), which suggested that there was some form of aggregation occurring. This change in Mw could also be observed in the proportion ‘slices’ at the elevated temperatures - observable at +37, +45 and +56°C. However, at the current storage temperature, -20°C and at +4°C there was no difference in the Mw or proportions which gives confidence that the material is stable. Furthermore, there have not been any reported issues during the use of the material as the USP CRS.

DISCUSSION

Heparin has an unusual characteristic for a biological medicine in that it is polydisperse and heterogeneous. In the context of activity assignment this can pose a challenge in selecting a suitable material to enable a single value to be assigned for all possible assay methods, as shown in the study to establish the current International Standard, 07/328 (ECBS Report WHO/BS/09.2124). The polydisperse property of heparin means that its molecular weight cannot be expressed as a single number. Average molecular weights, such as weight average (Mw) and number average (Mn) can be measured (3), as can the peak molecular weight (Mp). These three molecular weight values can be calculated by a broad standard method with a suitable broad standard calibrant.

The original proposal was to establish an International Standard, with the intention to use the USP Calibrant to create a broad standard table for the proposed IS therefore ensuring traceability to an established material. It was proposed to use this standard as the material had been well characterized for the molecular weight measurement of heparin using a broad standard method with a broad standard

table (4). This approach would ensure harmonization between the IS and the USP calibrant. Following the publication of the establishment of the CP Calibrant (5), which is tracible to the USP calibrant, it was concluded that adopting a portion of the USP calibrant to be the 1st IS would ensure harmonization with the USP and CP Calibrants. Although it would be possible to establish the IS using data generated from systems calibrated with both the USP and CP standards, this approach would be complex and was therefore not pursued.

To enable the establishment of the 1st IS, this study sought to verify the established broad standard table associated with the calibrant, ensuring its suitability by measuring the molecular weight of five unknown samples. Whilst most participants indicated that they used the USP method, several used modified or in-house methods for generating their chromatograms. Most participants were able to measure consistent molecular weights and percentage portions of each heparin sample, with only a few results showing a lack of consistency. Following reanalysis, whilst not changing the calculated Mw values for each laboratory or their intra CVs greatly (apart from laboratory 14), the inter laboratory CV was reduced. The reanalysis of all submitted chromatograms ensured that a uniform analytical approach was used.

Five different heparin samples were sent for molecular weight analysis - three heparins were considered to have a typical molecular weight, samples A, D and E, one heparin at the lower end sample B and one atypical heparin with a high Mw, sample C. For all samples the Mw inter laboratory CV was below 1% indicating consistency across laboratories and the suitability of the calibrant with associated broad standard table for measuring Mw. For the selected proportion ranges - % < 8000 Da, % > 8000 to < 16000 Da, % >16000 to < 24000 Da and % > 24000 Da – the intra laboratory CVs from most laboratories were below 2%. The inter laboratory CVs did show more variation with the two heparin samples at either Mw end having a higher CV at the inverse of their weight average molecular weight – sample B with a lower Mw had the highest, albeit relatively low CV for the proportion of material greater than 24,000 Da at 3.02%, whilst sample C with the highest Mw had a high CV for the proportion of material below 8,000 Da at 8.91%. The inter laboratory CVs for the proportion ranges that measured the bulk of heparin, % > 8000 to < 16000 Da and % >16000 to < 24000 Da had low variation with values all below 2%.

As shown in this collaborative study, all participants were able to calibrate their molecular weight systems using the proposed IS (NIBSC code 07/324) with the broad standard table to generate consistent molecular weights and proportion slices. Reanalysis, which involved applying a uniform approach to all chromatograms reduced the inter-laboratory variability, with heparin samples that can be viewed as typical, A, D & E, demonstrating good precision. For the atypical samples, B and C, the results were more variable within the proportions where the ‘least’ amount of material was present but for the other proportion slices the variability was comparable to the typical heparin samples.

PROPOSAL

Based on the results of this study, it is recommended that NIBSC Coded 07/324, should be the 1st International Standard for Unfractionated Heparin for Molecular Weight Calibration (please see Appendix 6 for the instructions for use) with the broad standard values as shown in table 1.

PARTICIPANT RESPONSES

All submitted responses from participants agreed with the proposal to establish 07/324 as the 1st International Standard Unfractionated Heparin for Molecular Weight Calibration with the broad standard table as indicted as used in the study.

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Table 1: Broad Standard Table for the Calibrant

Mw (Da)	% below MW	% above MW
6000	3.2	96.8
8000	10.4	89.6
10000	19.8	80.2
12000	31.7	68.3
14000	43.4	56.6
16000	55.5	44.5
18000	66.0	34.0
20000	74.4	25.6
22000	80.3	19.7
24000	84.4	15.6
26000	87.5	12.5
28000	90.1	9.9
32000	93.4	6.6
36000	95.6	4.4
40000	97.0	3.0

Table 2: Example of combining Mw data from one laboratory using mean and median.

Sample	Mean Run 1	Mean Run 2	Mean Run 3	Mean Run 4	Mean	Median	Mean/Median
A	17653	17558	17635	17666	17628	17644	0.999
B	14443	14630	14435	14480	14497	14462	1.002
C	22563	22277	22364	22334	22385	22349	1.002
D	15860	15857	15851	15991	15890	15859	1.002
E	15980	16082	16089	16056	16052	16069	0.999

Table 3: Comparison of mean and median Mw data from all laboratories

	Mean	Median	Mean/Median
A	17343	17454	0.994
B	14458	14454	1.000
C	22433	22419	1.000
D	15823	15821	1.000
E	16137	16050	1.005

Table 4: As returned weight average molecular weight (Mw) for samples A to E.

	A		B		C		D		E	
Lab	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	17125	0.81%	14475	0.80%	22813	0.86%	15913	0.40%	16100	0.33%
2	17496	1.03%	14552	0.45%	22084	0.79%	15791	0.50%	16126	2.04%
3	17412	1.05%	14535	0.51%	22215	0.70%	15823	1.02%	16137	1.93%
4	17625	0.57%	14408	0.08%	22405	0.29%	15792	0.12%	15969	0.19%
5	16834	1.52%	14353	0.98%	22133	2.34%	15691	1.24%	15898	0.90%
6	17581	0.40%	14503	0.19%	22471	0.55%	15885	0.29%	16069	0.32%
7	17691	1.03%	14435	1.22%	22230	0.74%	15709	0.39%	15948	0.50%
8	17628	0.77%	14497	0.91%	22384	0.55%	15890	0.61%	16052	0.62%
9	17009	0.48%	14437	0.24%	22432	0.70%	15818	0.25%	15965	0.62%
11	17619	0.33%	14459	0.35%	22476	0.40%	15937	0.73%	16048	0.23%
12	16947	0.28%	14449	0.20%	22487	0.31%	15886	0.41%	16045	0.27%
14	17149	6.32%	14394	4.86%	23062	5.71%	15738	4.67%	17289	12.59%
Mean	**17343**	**1.79%**	**14458**	**0.40%**	**22433**	**1.24%**	**15823**	**0.52%**	**16137**	**2.29%**
							Excluding Outlier		**16032**	**0.48%**

Highlighted value is an outlier using Grubb's Test

Table 5: Reprocessed data weight average molecular weight (Mw) for samples A to E.

	A		B		C		D		E	
Lab	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	17619	0.67%	14501	1.02%	22806	0.87%	15959	0.33%	16122	0.40%
2	17395	1.61%	14538	0.80%	22022	1.02%	15810	0.74%	16071	1.76%
3	17401	0.82%	14546	0.53%	22227	0.16%	15832	1.11%	16089	0.91%
4	17532	0.48%	14449	0.05%	22492	0.26%	15838	0.13%	16026	0.16%
5	17112	1.81%	14353	1.02%	22097	2.25%	15672	1.21%	15890	0.86%
6	17463	0.57%	14526	0.48%	22422	0.74%	15950	0.27%	16096	0.41%
7	17478	1.01%	14483	1.47%	22244	1.16%	15732	0.63%	15981	0.78%
8	17331	0.57%	14492	0.92%	22319	0.43%	15904	0.58%	16053	0.61%
9	17396	0.44%	14454	0.40%	22517	0.90%	15864	0.56%	16051	0.37%
11	17327	0.28%	14476	0.33%	22413	0.41%	15931	0.59%	16052	0.40%
12	17349	0.24%	14475	0.22%	22423	0.21%	15917	0.25%	16073	0.21%
14	17057	3.40%	14376	2.35%	22161	2.55%	15763	3.70%	15871	2.91%
Mean	**17372**	**0.92%**	**14472**	**0.41%**	**22345**	**0.96%**	**15848**	**0.57%**	**16031**	**0.49%**

Table 6: Comparison of returned and re-analysed Mw data

	A	B	C	D	E
Reported	17343	14458	22433	15823	16137
Re-analysed	17372	14472	22345	15848	16031
Difference	-29	-14	88	-25	106

Table 7a: Re-analysed data showing the proportion (%) of material below 8 kDa

	A		B		C		D		E	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	8.41	5.76%	11.46	4.67%	1.22	8.10%	10.10	0.68%	7.89	1.69%
2	8.38	8.76%	11.31	3.17%	2.35	6.92%	10.56	2.80%	8.54	9.61%
3	8.21	3.02%	11.17	1.67%	1.52	8.32%	9.89	5.74%	7.82	3.12%
4	8.13	1.50%	11.34	1.26%	1.14	5.69%	10.11	1.00%	7.80	1.76%
5	8.55	3.86%	11.80	3.96%	1.33	21.63%	10.55	4.50%	8.13	2.46%
6	8.24	1.93%	11.38	1.86%	1.22	8.68%	10.30	2.15%	8.01	2.84%
7	8.07	1.22%	11.51	1.36%	1.37	16.07%	10.43	1.45%	8.05	1.51%
8	8.38	2.40%	11.50	2.14%	1.28	21.40%	10.19	2.47%	7.95	3.29%
9	8.18	1.83%	11.42	1.00%	1.26	10.33%	10.25	1.08%	7.96	1.03%
11	8.29	1.14%	11.40	1.18%	1.19	5.43%	10.12	1.64%	7.87	1.29%
12	8.26	0.73%	11.35	0.72%	1.19	2.85%	10.09	0.77%	7.80	1.08%
14	8.41	20.93%	11.54	20.10%	1.14	38.07%	10.16	21.76%	8.01	22.03%
Mean	**8.29**	**0.20%**	**11.43**	**1.35%**	**1.35**	**24.36%**	**10.23**	**1.96%**	**7.99**	**2.53%**
					1.27	***8.91%***			***7.94***	***1.37%***

Highlighted value is an outlier using Grubb's Test; Italics data excludes the outlier.

Table 7b: Summary of intra laboratory CVs for proportion (%) of material below 8 kDa

CV range	A	B	C	D	E
0 – 2 %	6	7	0	6	6
2 – 5%	4	4	1	4	4
5 – 10%	1	0	6	1	1
>10%	1	1	5	1	1

Table 8a: Re-analysed data showing the proportion (%) of material between 8 and 16 kDa

	A		B		C		D		E	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	45.63	0.76%	55.43	0.57%	26.71	0.73%	50.37	0.21%	50.47	0.87%
2	45.97	1.09%	54.93	0.54%	28.16	2.11%	50.41	0.78%	49.46	1.67%
3	46.12	0.43%	55.37	0.37%	27.64	0.82%	50.87	0.19%	50.50	0.82%
4	45.88	0.28%	55.79	0.17%	26.62	0.82%	50.67	0.13%	50.74	0.14%
5	46.52	1.48%	55.97	0.71%	26.96	3.15%	50.89	0.93%	51.09	1.04%
6	45.83	0.54%	55.28	0.46%	26.57	0.21%	50.04	0.44%	50.20	0.45%
7	45.64	0.90%	55.70	1.04%	27.10	1.52%	50.91	0.82%	50.82	0.89%
8	45.83	0.46%	55.45	0.68%	26.87	1.07%	50.21	1.08%	50.50	0.69%
9	45.82	0.53%	55.60	0.40%	26.54	1.11%	50.47	0.59%	50.54	0.60%
11	46.01	0.37%	55.49	0.37%	26.72	0.65%	50.22	0.57%	50.47	0.36%
12	46.02	0.08%	55.55	0.20%	26.72	0.42%	50.35	0.20%	50.50	0.20%
14	47.03	3.37%	56.29	2.49%	27.37	7.08%	50.91	3.72%	51.50	2.60%
Mean	**46.03**	**0.86%**	**55.57**	**1.36%**	**27.00**	**1.84%**	**50.53**	**0.62%**	**50.57**	**0.97%**
	45.93	***0.54%***								

Highlighted value is an outlier using Grubb's Test; Italics data excludes the outlier.

Table 8b: Summary of intra laboratory CVs for proportion (%) of material between 8 and 16 kDa

CV range	**A**	**B**	**C**	**D**	**E**
0 – 2 %	11	11	9	11	11
2 – 5%	1	1	2	1	1
5 – 10%	0	0	1	0	0
>10%	0	0	0	0	0

Table 9a: Re-analysed data showing the proportion (%) of material between 16 and 24 kDa

	A		B		C		D		E	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	29.86	1.17%	26.39	1.92%	40.29	0.48%	27.62	0.36%	30.52	0.51%
2	29.92	1.81%	26.87	0.78%	39.33	0.88%	27.46	1.07%	30.52	3.66%
3	30.08	1.08%	26.69	0.91%	40.52	0.33%	27.88	0.73%	30.75	1.01%
4	30.32	0.58%	26.51	0.52%	41.13	0.61%	27.74	0.49%	30.77	0.69%
5	30.03	1.02%	25.99	2.37%	41.49	0.69%	27.46	2.09%	30.42	0.94%
6	30.34	0.49%	26.79	0.31%	41.26	0.79%	27.87	0.46%	30.89	0.58%
7	30.55	0.30%	26.18	0.63%	41.13	0.97%	27.42	0.33%	30.50	0.62%
8	30.22	0.50%	26.52	0.41%	41.05	1.12%	27.85	0.59%	30.74	0.61%
9	30.37	0.35%	26.56	0.54%	40.98	0.60%	27.68	0.39%	30.67	0.43%
11	30.22	0.58%	26.59	0.86%	41.09	0.38%	27.86	0.80%	30.83	0.48%
12	30.27	0.14%	26.60	0.29%	41.08	0.36%	27.83	0.16%	30.85	0.31%
14	30.08	5.23%	25.93	6.51%	41.50	2.11%	27.66	6.26%	30.27	5.27%
Mean	**30.19**	**0.66%**	**26.47**	**1.12%**	**40.90**	**1.48%**	**27.69**	**0.63%**	**30.64**	**0.64%**
					41.03	*0.88%*				

Highlighted value is an outlier using Grubb's Test; Italics data excludes the outlier.

Table 9b: Summary of intra laboratory CVs for proportion (%) of material between 16 and 24 kDa

CV range	**A**	**B**	**C**	**D**	**E**
0 – 2 %	11	10	11	10	10
2 – 5%	0	1	1	1	1
5 – 10%	1	1	0	1	1
>10%	0	0	0	0	0

Table 10a: Re-analysed data showing the proportion (%) of material greater than 24 kDa

	A		B		C		D		E	
	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
1	16.09	1.69%	6.72	4.37%	31.79	0.96%	11.90	1.01%	11.11	2.12%
2	15.74	4.11%	6.89	4.39%	30.16	2.10%	11.59	2.57%	11.32	6.33%
3	15.59	1.93%	6.77	2.35%	30.31	0.37%	11.38	2.82%	10.94	3.09%
4	15.67	1.21%	6.37	1.29%	31.11	0.55%	11.49	0.63%	10.69	0.56%
5	14.90	5.48%	6.23	4.83%	30.22	4.53%	11.10	4.96%	10.36	4.48%
6	15.58	1.56%	6.54	2.42%	30.94	1.06%	11.79	1.07%	10.90	1.82%
7	15.75	2.63%	6.61	9.92%	30.41	2.22%	11.25	2.82%	10.63	3.95%
8	15.57	1.73%	6.53	5.93%	30.81	0.97%	11.76	3.41%	10.81	3.07%
9	15.63	1.59%	6.42	3.04%	31.23	1.58%	11.60	2.47%	10.83	2.15%
11	15.47	0.77%	6.53	1.59%	30.99	0.82%	11.79	1.90%	10.84	1.89%
12	15.47	0.55%	6.50	1.65%	31.01	0.36%	11.74	1.01%	10.86	0.84%
14	14.48	11.63%	6.25	9.15%	29.99	5.90%	11.27	11.65%	10.22	9.28%
Mean	**15.49**	**2.71%**	**6.53**	**3.02%**	**30.75**	**1.74%**	**11.55**	**2.22%**	**10.79**	**2.73%**
	15.59	***1.83%***								

Highlighted value is an outlier using Grubb's Test; Italics data excludes the outlier.

Table 10b: Summary of intra laboratory CVs for proportion (%) of material greater than 24 kDa

CV range	A	B	C	D	E
0 – 2 %	8	3	8	5	4
2 – 5%	2	6	3	6	6
5 – 10%	1	3	1	0	2
>10%	1	0	0	1	0

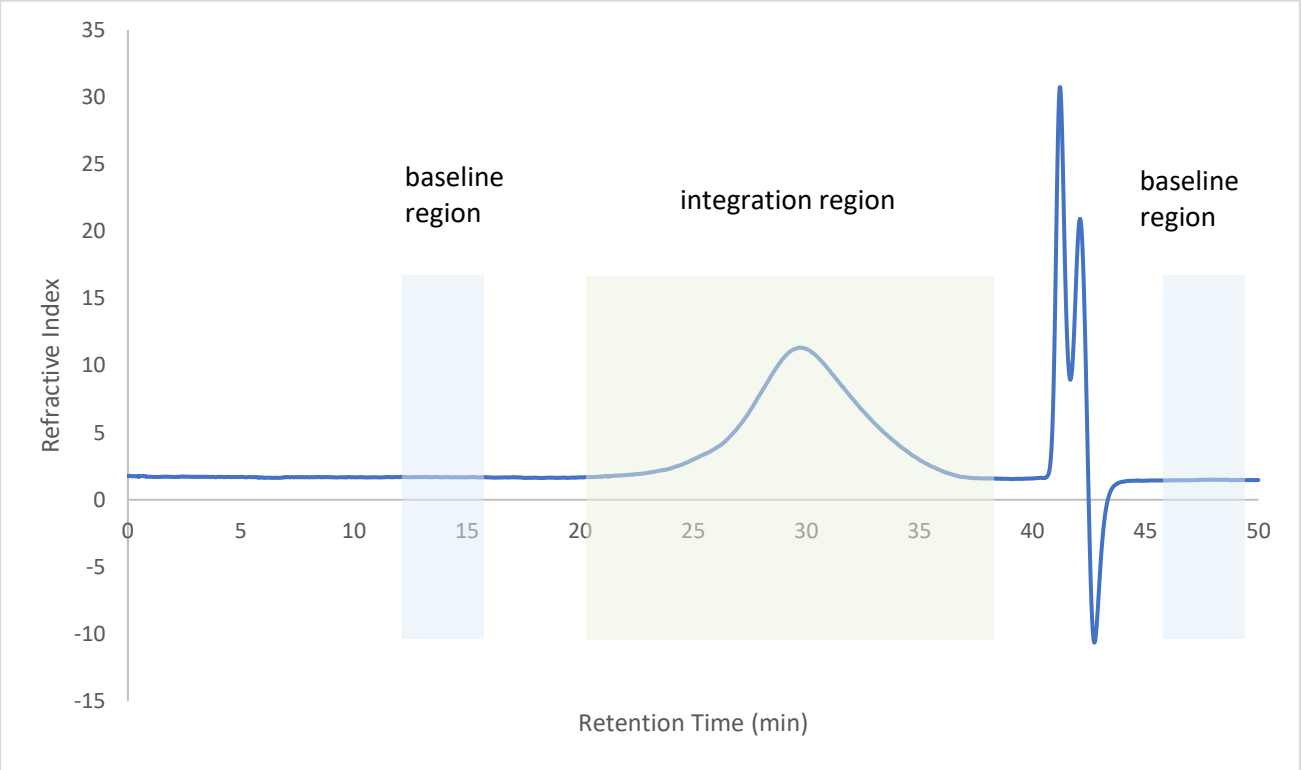
Table 11: Details of molecular weight system and method as provided by the participants.

Lab	System	Columns	Buffer	[Sample]	Flow Rate	Run Time[1]	Additional Information
1	Agilent 1260 Infinity II Isocratic with RID detector	Pre-column 6mmx40cm; 7µm (TSK Guard SWXL Column 7,8mmx30cm; 8µm (TSK G4000 SWXL)) Column 7,8mmx30cm; 5µm (TSK G3000 SWXL)	Ammonium Acetate Solution (1M) and Sodium Azide Solution (1%)	5 mg/mL	0,6 mL/min	50min	Software Empower ® 3.6.1
2	Acquisition system: OmniSEC Resolve/Reveal v.11.40; Elaboration system: Clarity v.8.8.1.16	TSKGel G4000 7.8mm*30cm, 8µm packing L59 in series with TSKGel G3000 7.8mm*30cm, 5µm packing L59, Guard column 6.0mm*4cm, 7µm packing L59.	Ammonium acetate 0.1 M		0.6 mL/min	Variable, 35, 36 and 42 min	Solvent peak as FRM
3	HPLC Alliance Waters 2695 RI Detector 2414	TSKgel G4000 SWXL, 7.8 mm × 30 cm, 8 µm TSKgel G3000 SWXL, 7.8 mm × 30 cm, 5 µm TSK SWXL, 6 mm × 4 cm, 7 µm	0.1M Ammonium acetate solution and sodium azide 0.02%.	10mg (±5%) / 2mL running buffer	0.6 mL/min	50 min	USP Hearin Sodium Monograph, IDentification test D. Molecular Weight
4	Empower3	TSK guard column SWxl, TSK G4000 SWxl, TSK G3000 SWxl	0.1 M ammonium acetate solution-0.02% sodium azide solution	5 mg/mL	0.6 mL/min	60 min	USP Heparin Sodium 'D Molecular Weight determination'
5	WATERS GPC system Arc Premier + RI detector 2414	Precolumn TSK gel SWXL+TSK gel G4000 SWXL+TSK gel G3000 SWXL	Ammonium acetate 0.1M + sodium azide 0.02%	5mg/ml	0.6 mL/min	50 min	Molecular weight determination by Broad Standard Integral calibration method according to the USP Heparin sodium monograph
6	HPLC WATERS Alliance 2695 + RI 2414 (Waters)	Phenomenex Yarra 3u SEC-3000 (CODE ARTICLE 00H-4513-K0) + Yarra 3u SEC-4000 (CODE ARTICLE 00H-4514-K0)	0.1 M Ammonium acetate / 0.02% Sodium azide	5 mg/mL	0.6 mL/min	55 min	Salt peak as FRM, Application of the USP Monographs: Heparin Sodium / Empower 3 Chromatography Data Software
7	Waters Alliance HPLC - Waters 2414 RI	Tosoh TSKgel G4000sw.xl + Tosoh TSKgel G3000sw.xl	Ammonium acetate 0.1M	5 mg/mL	0.6 mL/min	52 min	USP Heparin Sodium Monograph Id. D

8	Agilent GPC50	Guard TSK gel SWXL+TSK gel G4000 SWXL+TSK gel G3000 SWXL	0.1 M Ammonium acetate / 0.02% Sodium azide	5mg/ml	0.6 mL/min	50 min	Alpha-cyclodextrose as FRM BST method similar to USP
9	(HPLC)Waters e2695	(guard column) : TSKSWXL/502FA00763G (analytical column) : TSKG4000SWXL/003KA00378M andTSKG3000SWXL/008MA04837M	(ammonium acetate-sodium azide solution)	5mg/ml	0.6 mL/min	50 min	USP<Heparin Sodium> and MOLECULAR WEIGHT DETERMINATION
11	Agilent 1260 Infinity HPLC system with refractive index detector	Tosoh Biosciences TSK-Gel SWXL, Tosoh Biosciences TSK-Gel G4000SWXL, Tosoh Biosciences TSK-Gel G3000SWXL,	0.1 M Ammonium acetate / 0.02% Sodium azide	~5 mg/mL	0.6 mL/min	60 min	Heparin Sodium, Identification D, Molecular Weight Determinations,
12	Agilent 1260 Infinity HPLC system with refractive index detector	Tosoh Biosciences TSK-Gel SWXL, Tosoh Biosciences TSK-Gel G4000SWXL, Tosoh Biosciences TSK-Gel G3000SWXL,	0.1 M Ammonium acetate / 0.02% Sodium azide	~5 mg/mL	0.6 mL/min	60 min	Heparin Sodium, Identification D, Molecular Weight Determinations,
14	Agilent LC & LC/MS 1100 Series/ GPC/SEC SW OpenLab 1.4	TSKgel G2000SWxl Guard column 40x6mm 7µm - TSKgel G4000SWxl 300x7.8mm 8µm - TSKgel G3000SWxl 300x7.8mm 5µm	0.1 M ammonium acetate and 0.02% sodium azide solution	5mg/ml	0.6 mL/min	50 min	According to USP NF 2024.

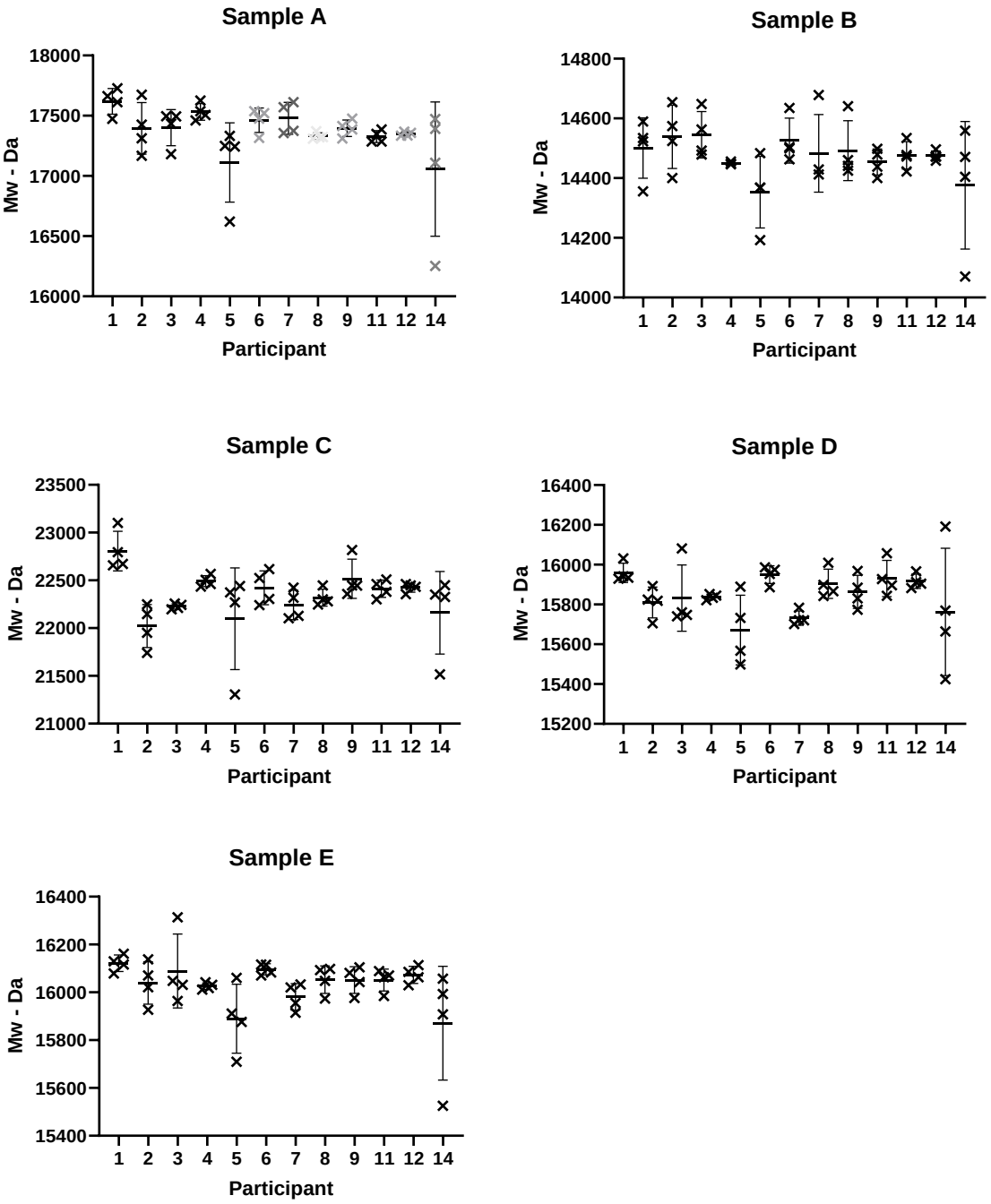
[1] Runtime is either from supplied information or extracted from the chromatograms.

Figure 1: Example Chromatogram with baseline and integration ranges highlighted.



Please note that this is an example chromatogram that indicates the broad approach used for the reanalysis of data, for each re-analysis the same baseline and integration regions were selected for all chromatograms for each participants data where feasible.

Figure 2: Scatterplot of the mean (from two injections) weight average molecular weight (Mw) for each chromatogram set with associated standard deviation from the processed data



Appendix 1: Participants – Alphabetically by Organisation Name

Leonardo Valderrama
ADESTE Industria de Produtos Animais Ltda, BRAZIL

Sophie Rousseau
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Mónica Camino de la Blanca, Guillermo Franco Rodríguez, Mª Teresa Gómez Ochoa
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SANOFI Heparin Process Characterisation Laboratory, FRANCE

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USP: ADL-Rockville, USA

Mandy Alger, Hee Tran, Andrew Schmidt
USP RSL-Rockville, USA

Appendix 2: Study protocol

CS716 – Protocol

Verification of the proposed WHO 1st International Standard for Unfractionated Heparin Molecular Weight Calibration

Background

The method for this study should be GPC/HPLC based using the supplied candidate, with its Broad Standard Table (BST), as the calibrant to calibrate the system (see Mulloy & Hogwood, Methods Mol Bio 2303: 227 – 240 or USP Heparin Sodium 'D Molecular Weight determination') for molecular weight measurement. Whilst other HPLC methods can be used, the primary focus in this study will be to confirm the supplied BST is suitable to establish the candidate as an International Standard across different methods that use a broad standard table to calibrate a system for measuring heparin molecular weights.

The design of the study will require four independent runs, preferably on different days, using the calibrant to determine the weight average molecular weight (Mw) of several heparin samples provided in the study. The primary objective of this study is to verify that the candidate calibrant, with BST, is suitable to be adopted as an International Standard. A secondary objective is to investigate the strategy to replace the unfractionated heparin molecular weight calibrant.

Samples for the study

Provided for the study

CS716 – Sample Cal – see BST below.
CS716 – Sample A
CS716 – Sample B
CS716 – Sample C
CS716 – Sample D
CS716 – Sample E

The contents of each ampoule or tube are approximately 10 mg (±5%) which enables the samples to be reconstituted with the same volume of running buffer. It is not expected that these slight differences in concentration will influence the measurement of molecular weight using a broad standard method.
Please note if you wish to include your own heparin samples within this study for comparison (such as a molecular weight control/system suitability), please do so and modify the results sheet to report this data. Include your expected values for your own heparin samples in the result sheet.

Method

Four independent injection series should be run using sample 'Cal' as the calibrant (see BST) to calibrate your molecular weight system are requested. Each injection series should be preferably performed on a different day with a fresh set of samples.

The broad standard method that is in routine use within your laboratory should be employed to calibrate the system and from this the molecular weights of the unknown samples calculated. A generic broad standard method for calibrating systems to measure heparin samples is described in Mulloy & Hogwood, Methods Mol Bio 2022; 2303: 227-240. The USP Heparin Sodium monograph method 'D' Molecular Weight Determination is another such example of a method. Please record details of your molecular weight system and method details in the results sheet.

If your routine method involves two injections within a run sequence, please report both sets of data for each sample.
Please note that if feasible the testing order should be varied for each set of samples. As an example, the proposed testing order can be used:

Day 1 – Cal, A, B, C, D, E
Day 2 – A, C. E. Cal, B, D
Day 3 – E, D, C, B, A, Cal
Day 4 – C, E, Cal, A, D, B

Broad standard table

MW (Da)	% below MW	% above MW
6000	3.2	96.8
8000	10.4	89.6
10000	19.8	80.2
12000	31.7	68.3
14000	43.4	56.6
16000	55.5	44.5
18000	66.0	34.0
20000	74.4	25.6
22000	80.3	19.7
24000	84.4	15.6
26000	87.5	12.5
28000	90.1	9.9
32000	93.4	6.6
36000	95.6	4.4
40000	97.0	3.0

Requested sample calculations.
For each heparin sample please calculate the following from each independent run series and report the values in the excel reporting sheet:

Weight average molecular weight (Mw)
% MW below 8000 Da
% MW between 8000 and 16000 Da
% MW between 16000 and 24000 Da
% MW above 24000 Da

Data submission
The reporting workbook contains three spreadsheets – system details, Mw results and file names. Please complete all details where possible in the supplied spreadsheet.

For each molecular weight sample tested, including the calibrant, please provide the data in the. AIA/**.CDF** file format and include with the spreadsheet with the return of results.

Please send completed data to john.hogwood@mhra.gov.uk by 31st January 2025.

If you have any queries please do contact, using the above email address.

Appendix 3: Participants returned data.

Table 1a: Participant 1

	Sample	Injection	Mw (Da)	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17200	8	46	30	15
		2	17200	8	46	30	15
	B	1	14500	11	56	27	7
		2	14500	11	56	26	7
	C	1	23100	1	27	40	32
		2	23000	1	27	40	32
	D	1	16000	10	50	27	12
		2	16000	10	50	28	12
	E	1	16200	8	51	30	11
		2	16000	8	52	30	11
	SST Start	1	16000	6	52	34	9
		2	15900	6	52	34	9
	SST End	1	15900	6	52	34	9
		2	15900	6	52	33	9
Set/Run 2	A	1	16900	8	47	30	15
		2	17000	8	47	30	15
	B	1	14500	11	55	27	7
		2	14500	11	55	27	7
	C	1	22500	1	27	41	31
		2	22600	1	27	41	31
	D	1	15900	10	50	28	12
		2	15900	10	50	28	12
	E	1	16100	8	50	31	11
		2	16100	8	50	31	11
	SST Start	1	15900	6	52	34	8
		2	15900	6	52	34	9
	SST End	1	15900	6	52	34	9
		2	15900	6	52	34	9
Set/Run 3	A	1	17200	8	46	30	15
		2	17200	8	46	30	15
	B	1	14200	13	56	25	6
		2	14500	11	55	27	7
	C	1	22800	1	27	40	32
		2	22900	1	27	40	32
	D	1	15900	10	51	28	12
		2	15900	10	50	28	12
	E	1	16100	8	51	30	11
		2	16100	8	51	31	11
	SST Start	1	15900	6	51	34	9
		2	16000	6	51	34	9
	SST End	1	16000	6	51	34	9
		2	Invalid				
Set/Run 4	A	1	17300	8	46	30	16
		2	17000	9	46	30	15
	B	1	14600	11	55	27	7
		2	14500	12	55	26	7
	C	1	22800	1	27	40	32
		2	22800	1	27	40	32
	D	1	15800	10	51	28	12
		2	15900	10	51	28	12
	E	1	16100	8	50	31	11
		2	16100	8	50	31	11
	SST Start	1	15900	6	51	34	9
		2	15900	6	51	34	9
	SST End	1	15900	6	52	34	9
		2	16000	6	51	34	9

Table 1b: Participant 2

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17395	8.74	48.94	29.48	12.84
		2	17565	8.07	48.92	29.91	13.10
	B	1	14558	10.97	57.36	25.92	5.75
		2	14571	11.17	57.00	25.98	5.85
	C	1	22158	1.41	31.35	41.18	26.06
		2	22318	2.19	30.81	40.57	26.43
	D	1	15844	9.94	53.19	27.26	9.60
		2	15809	10.26	53.15	27.03	9.56
	E	1	16096	8.35	52.38	29.87	9.39
		2	16048	8.26	52.65	29.84	9.25
Set/Run 2	A	1	17278	9.06	48.98	29.20	12.76
		2	17446	7.79	47.13	30.77	14.31
	B	1	14508	11.38	56.26	26.52	5.84
		2	14468	11.73	56.08	26.30	5.89
	C	1	22177	1.93	30.47	41.02	26.54
		2	22278	1.64	30.37	41.30	26.68
	D	1	15831	10.63	51.80	27.69	9.88
		2	15844	10.39	52.27	27.39	9.95
	E	1	16080	8.68	51.57	30.23	9.52
		2	16060	8.29	51.65	30.53	9.53
Set/Run 3	A	1	17360	8.01	49.01	30.04	12.94
		2	17446	8.67	48.25	29.80	13.27
	B	1	14512	11.44	56.50	26.19	5.87
		2	14582	10.97	56.62	26.50	5.92
	C	1	22029	1.67	30.54	41.18	26.62
		2	21969	2.24	30.45	40.89	26.43
	D	1	15787	10.46	52.37	27.35	9.82
		2	15703	10.22	52.81	27.37	9.60
	E	1	16023	8.10	51.96	30.53	9.41
		2	16082	8.52	51.70	30.26	9.52
Set/Run 4	A	1	17636	8.66	47.84	29.91	13.45
		2	17842	8.17	47.71	30.15	13.83
	B	1	14686	10.75	56.13	26.86	6.26
		2	14528	11.28	56.57	26.21	5.95
	C	1	21914	1.71	31.37	40.90	26.03
		2	21832	1.83	31.74	40.61	25.82
	D	1	15642	10.66	53.05	26.71	9.57
		2	15866	10.28	52.49	27.33	9.90
	E	1	15734	9.39	52.78	28.79	9.04
		2	16886	6.77	49.41	32.45	11.37

Table 1c: Participant 3

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17125	7.87	46.71	30.42	15.00
		2	17496	7.86	46.23	30.17	15.74
	B	1	14665	11.27	54.86	26.74	7.13
		2	14627	10.94	55.31	26.71	7.03
	C	1	22427	2.27	27.47	39.74	30.52
		2	22476	1.64	27.33	40.27	30.77
	D	1	15913	9.65	50.94	27.87	11.54
		2	16193	8.74	51.15	28.02	12.09
	E	1	16325	7.67	49.88	30.90	11.55
		2	16850	7.84	49.16	30.43	12.57
	SS	1	16014	5.98	50.54	34.73	8.75
		2	16044	5.94	50.58	34.70	8.77
Set/Run 2	A	1	17225	8.38	46.33	30.12	15.17
		2	17285	8.38	46.24	30.12	15.26
	B	1	14531	11.42	55.15	26.56	6.87
		2	14546	11.29	55.16	26.66	6.89
	C	1	22209	1.43	27.68	40.62	30.26
		2	22220	1.62	27.71	40.38	30.30
	D	1	15767	10.18	50.65	27.87	11.30
		2	15747	10.36	50.60	27.76	11.29
	E	1	16019	8.11	50.53	30.49	10.87
		2	15988	8.14	50.50	30.56	10.80
	SS	1	17225	8.38	46.33	30.12	15.17
		2	17285	8.38	46.24	30.12	15.26
Set/Run 3	A	1	17672	8.32	46.01	29.91	15.77
		2	17539	8.40	46.04	29.94	15.62
	B	1	14473	11.43	55.45	26.46	6.66
		2	14487	11.41	55.39	26.47	6.73
	C	1	22075	1.55	27.93	40.66	29.86
		2	22081	1.56	27.93	40.65	29.86
	D	1	15727	10.29	50.81	27.71	11.20
		2	15736	11.20	50.82	27.76	11.20
	E	1	15978	8.10	50.61	30.57	10.72
		2	15983	8.02	50.68	30.62	10.68
	SS	1	15832	6.18	51.22	34.43	8.17
		2	15814	6.22	51.24	34.41	8.14
Set/Run 4	A	1	17480	8.52	46.10	29.76	15.62
		2	17477	8.46	46.13	29.79	15.63
	B	1	14470	11.47	55.50	26.34	6.70
		2	14480	11.44	55.48	26.35	6.73
	C	1	22117	1.55	27.90	40.44	30.12
		2	22112	1.57	27.94	40.42	30.08
	D	1	15747	10.31	50.83	27.54	11.32
		2	15753	10.33	50.81	27.54	11.33
	E	1	15967	8.14	50.78	30.36	10.73
		2	15986	8.13	50.75	30.34	10.78
	SS	1	15837	6.25	51.24	34.21	8.30
		2	15802	6.24	51.38	34.19	8.18

Table 1d: Participant 4

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17521	8	46.1	30.3	15.6
		2	17526	8	46.1	30.4	15.5
	B	1	14403	11.2	56.1	26.4	6.3
		2	14422	11.2	56.1	26.5	6.3
	C	1	22326	1.2	26.6	41.3	30.9
		2	22326	1.1	26.6	41.4	30.9
	D	1	15774	10.1	50.9	27.7	11.4
		2	15810	10.1	50.8	27.6	11.5
	E	1	15939	7.8	50.9	30.8	10.5
		2	16017	7.7	50.8	30.8	10.7
	SS	1	15862	5.7	51.4	34.8	8.1
		2	15881	5.7	51.3	34.8	8.1
Set/Run 2	A	1	17674	8.4	45.7	30.2	15.7
		2	17835	8.2	45.6	30.3	16.0
	B	1	14394	11.6	55.6	26.6	6.3
		2	14394	11.6	55.6	26.6	6.3
	C	1	22499	1.2	26.5	41.3	31
		2	22478	1.3	26.5	41.2	31
	D	1	15784	10.4	50.4	27.8	11.4
		2	15812	10.4	50.4	27.8	11.5
	E	1	15977	8.2	50.5	30.7	10.7
		2	16012	8.1	50.5	30.7	10.7
	SS	1	15920	6	50.8	34.9	8.3
		2	15909	5.9	50.7	35.1	8.3
Set/Run 3	A	1	17609	8.3	45.8	30.1	15.7
		2	17657	8.3	45.7	30.2	15.8
	B	1	14410	11.7	55.6	26.3	6.4
		2	14404	11.7	55.7	26.3	6.4
	C	1	22405	1.2	26.8	41	31
		2	22402	1.3	26.8	40.9	31
	D	1	15775	10.4	50.6	27.5	11.5
		2	15767	10.4	50.6	27.5	11.5
	E	1	15941	8.2	50.8	30.4	10.6
		2	15966	8.1	50.8	30.5	10.7
	SS	1	15894	6	51	34.6	8.3
		2	15912	6	50.9	34.7	8.4
Set/Run 4	A	1	17580	8.3	45.9	30.3	15.6
		2	17594	8.3	45.9	30.3	15.6
	B	1	14424	11.6	55.6	26.4	6.5
		2	14415	11.6	55.6	26.4	6.4
	C	1	22365	1.3	26.9	41.2	30.7
		2	22437	1.2	26.9	41.2	30.8
	D	1	15803	10.4	50.5	27.7	11.5
		2	15807	10.4	50.5	27.7	11.5
	E	1	15945	8.1	50.6	30.7	10.6
		2	15958	8.1	50.6	30.6	10.6
	SS	1	15900	5.9	50.8	35	8.3
		2	15911	5.9	50.8	35	8.3

Table 1e: Participant 5

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17049	8.24	46	31	14.84
		2	17039	8.23	46	31	14.81
	B	1	14493	11.58	55	26	6.7
		2	14511	11.55	55	26	6.74
	C	1	22490	1.11	26	41	31.31
		2	22474	1.11	26	41	31.28
	D	1	15901	10.39	50	28	11.86
		2	15911	10.36	50	28	11.87
	E	1	16077	8.06	50	31	11.03
		2	16064	8.11	50	31	10.99
	SS		16000				
Set/Run 2	A	1	16403	8.92	48	30	13.03
		2	16486	8.74	48	30	13.07
	B	1	14371	11.37	56	26	6.08
		2	14257	12.41	56	26	6.13
	C	1	21251	2.75	28	41	28.27
		2	21352	1.85	28	42	28.2
	D	1	15449	10.54	51	28	10.27
		2	15580	10.67	51	28	10.87
	E	1	15630	8.62	52	30	9.63
		2	15822	8.52	51	30	10.31
	SS		15689				
Set/Run 3	A	1	16940	8.36	46.86	30.23	14.55
		2	17013	8.22	46.64	30.42	14.722
	B	1	14405	11.61	55.67	26.3	6.42
		2	14435	11.62	55.57	26.26	6.55
	C	1	22318	1.13	26.82	41.37	30.68
		2	22328	1.11	26.78	41.45	30.67
	D	1	15770	10.55	50.61	27.33	11.51
		2	15808	10.51	50.5	27.37	11.62
	E	1	15958	7.93	51.21	30.27	10.6
		2	15885	8.5	50.9	30	10.61
	SS		15938				
Set/Run 4	A	1	16777	8.7	47.45	29.77	14.08
		2	16967	8.25	46.8	30.41	14.54
	B	1	14105	12.81	56.55	24.69	5.95
		2	14243	12.35	56.19	25.29	6.17
	C	1	22379	1.13	26.41	41.34	31.12
		2	22473	1.09	26.21	41.39	31.31
	D	1	15701	10.72	50.8	27.17	11.3
		2	15408	11.81	51.5	25.96	10.73
	E	1	15876	8.48	50.97	30.05	10.5
		2	15872	8.4	51	30.11	10.49
	SS		16148				

Table 1f: Participant 6

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17664	8.193	45.737	30.201	15.870
		2	17696	8.189	45.655	30.190	15.967
	B	1	14549	11.311	55.197	26.784	6.708
		2	14545	11.273	55.246	26.822	6.659
	C	1	22653	1.203	26.275	41.014	31.508
		2	22684	1.218	26.264	40.968	31.551
	D	1	15931	10.245	50.136	27.810	11.809
		2	15980	10.075	50.181	27.864	11.881
	E	1	16149	7.911	49.951	30.966	11.172
		2	16152	7.986	50.058	30.819	11.137
	SS Start	1	16021	5.899	50.452	34.975	8.674
		2	16029	5.897	50.396	35.004	8.703
	SS End	1	16045	5.843	50.509	34.925	8.723
		2	16068	5.900	50.304	34.969	8.827
Set/Run 2	A	1	17487	8.317	45.847	30.315	15.522
		2	17511	8.288	45.877	30.225	15.610
	B	1	14488	11.506	55.265	26.679	6.550
		2	14489	11.377	55.351	26.748	6.525
	C	1	22392	1.229	26.572	41.197	31.003
		2	22418	1.285	26.567	41.118	31.030
	D	1	15870	10.236	50.211	27.877	11.676
		2	15874	10.244	50.208	27.860	11.688
	E	1	16041	8.091	50.225	30.811	10.872
		2	16055	8.054	50.215	30.893	10.839
	SS Start	1	15840	6.118	50.942	34.787	8.153
		2	15900	6.033	50.810	34.835	8.322
	SS End	1	15983	5.952	50.645	34.861	8.541
		2	15969	5.917	50.561	35.051	8.470
Set/Run 3	A	1	17576	8.31	45.695	30.267	15.727
		2	17565	8.401	45.761	30.1	15.738
	B	1	14494	11.49	55.207	26.742	6.562
		2	14475	11.5	55.244	26.744	6.512
	C	1	22441	1.284	26.548	41.208	30.961
		2	22429	1.245	26.52	41.257	30.978
	D	1	15850	10.376	50.167	27.852	11.605
		2	15855	10.323	50.172	27.883	11.622
	E	1	16033	8.098	50.282	30.847	10.774
		2	16031	8.115	50.281	30.831	10.773
	SS Start	1	15928	6.049	50.75	34.841	8.36
		2	15929	6.104	50.57	34.881	8.445
	SS End	1	15955	6.045	50.704	34.804	8.447
		2	15957	6.069	50.709	34.756	8.465
Set/Run 4	A	1	17584	8.291	45.711	30.264	15.735
		2	17562	8.334	45.819	30.142	15.705
	B	1	14497	11.451	55.281	26.691	6.577
		2	14485	11.445	55.33	26.697	6.528
	C	1	22379	1.268	26.629	41.146	30.957
		2	22372	1.296	26.67	41.115	30.919
	D	1	15859	10.34	50.195	27.804	11.661
		2	15864	10.257	50.241	27.91	11.592
	E	1	16043	8.086	50.239	30.883	10.791
		2	16047	8.077	50.201	30.91	10.813
	SS Start	1	15923	6.009	50.676	34.943	8.371
		2	15929	5.94	50.847	34.822	8.391
	SS End	1	15958	6.029	50.633	34.889	8.448
		2	15944	6.023	50.63	34.936	8.41

Table 1g: Participant 7

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17969	8.054	45.264	30.281	16.4
		2	17761	8.037	45.482	30.364	16.118
	B	1	14354	11.748	55.791	26.069	6.392
		2	14414	11.621	55.693	26.11	6.576
	C	1	22224	1.459	27.077	40.938	30.526
		2	21952	1.889	27.519	40.751	29.841
	D	1	15741	10.62	50.701	27.184	11.495
		2	15628	10.599	51.019	27.3	11.082
	E	1	15903	8.303	50.852	30.24	10.605
		2	16094	8.114	50.464	30.263	11.159
	SS 1	1	17421	8.686	47.156	28.894	15.263
		2	17467	8.758	47.069	28.96	15.213
	SS 2	1	16403	10.941	46.537	28.679	13.843
		2	16321	11.22	46.511	28.579	13.69
Set/Run 2	A	1	17751	7.928	45.59	30.371	16.111
		2	17775	8.012	45.519	30.406	16.063
	B	1	14864	11.168	55.163	25.763	7.906
		2	14407	11.755	55.714	25.99	6.541
	C	1	22349	1.247	27.094	40.813	30.845
		2	22278	1.21	27.06	41.059	30.671
	D	1	15746	10.497	50.919	27.157	11.426
		2	15816	10.484	50.649	27.183	11.658
	E	1	16040	8.185	50.772	30.11	10.933
		2	15907	8.101	50.935	30.453	10.511
	SS 1	1	17487	8.709	47.112	28.856	15.323
		2	17359	8.767	47.103	28.947	15.183
	SS 2	1	16482	11.151	46.307	28.403	14.138
		2	16460	11.058	46.391	28.516	14.035
Set/Run 3	A	1	17641	8.137	45.648	30.458	15.757
		2	17348	7.985	45.867	30.79	15.358
	B	1	14384	11.532	55.817	26.295	6.356
		2	14349	11.689	55.939	26.066	6.306
	C	1	22473	1.356	26.767	41.041	30.836
		2	22336	1.41	26.97	41.093	30.527
	D	1	15704	10.429	50.919	27.438	11.215
		2	15721	10.359	50.839	27.542	11.26
	E	1	15959	8.004	50.734	30.643	10.619
		2	15927	8.059	50.916	30.511	10.514
	SS 1	1	17136	8.648	47.3	29.298	14.754
		2	17540	8.573	46.956	29.06	15.412
	SS 2	1	16473	11.05	46.318	28.643	13.99
		2	16437	11.109	46.422	28.565	13.904
Set/Run 4	A	1	17550	8.253	45.836	30.374	15.537
		2	17730	8.252	45.405	30.322	16.022
	B	1	14360	11.69	55.903	26.081	6.327
		2	14344	11.789	55.948	25.921	6.342
	C	1	22103	1.338	27.326	41.301	30.034
		2	22125	1.461	27.368	41.161	30.011
	D	1	15657	10.411	51.114	27.42	11.056
		2	15660	10.51	51.001	27.418	11.072
	E	1	15885	8.203	51.031	30.361	10.405
		2	15872	8.138	51.069	30.421	10.372
	SS 1	1	17509	8.737	47.154	28.932	15.177
		2	17674	8.699	47.026	28.93	15.345
	SS2	1	16398	11.007	46.627	28.603	13.764
		2	16384	11.071	46.598	28.62	13.71

Table 1h: Participant 8

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17654	8.56	45.62	29.92	15.9
		2	17652	8.12	45.57	30.42	15.9
	B	1	14538	11.65	55.22	26.4	6.75
		2	14348	11.33	55.87	26.87	5.91
	C	1	22604	1.1	26.71	40.86	31.34
		2	22521	1.46	26.66	40.72	31.17
	D	1	15812	10.35	50.32	27.75	11.59
		2	15908	9.8	50.37	28.16	11.65
	E	1	15882	8.06	50.83	30.76	10.36
		2	16077	7.71	50.73	30.84	10.7
	SS	1	17048	5.25	45.76	37.86	11.14
		2	17003	5.17	45.82	38.08	10.93
Set/Run 2	A	1	17539	8.42	45.94	30.11	15.55
		2	17577	8.12	45.71	30.34	15.8
	B	1	14479	11.4	55.87	26.3	6.42
		2	14781	11.27	55.01	26.63	7.09
	C	1	22283	0.78	27.02	41.88	30.29
		2	22270	1.16	26.34	41.6	30.89
	D	1	15740	10.01	51.01	27.89	11.09
		2	15974	10.24	49.89	27.96	11.91
	E	1	16093	7.63	51	30.81	10.56
		2	16071	7.94	50.19	30.93	10.95
	SS	1	16634	4.87	47.01	38.52	9.6
		2	16716	4.94	46.44	38.63	9.99
Set/Run 4	A	1	17634	8.26	45.71	30.23	15.81
		2	17635	8.26	45.750	30.270	15.72
	B	1	14423	11.5	55.57	26.62	6.33
		2	14447	11.670	55.47	26.45	6.41
	C	1	22425	1.18	26.81	41.1	30.94
		2	22303	1.4	27	40.95	30.63
	D	1	15825	10.07	50.62	27.86	11.44
		2	15876	10.69	50.13	27.48	11.71
	E	1	16084	7.75	50.51	30.88	10.85
		2	16094	8.22	50.18	30.62	10.98
	SS	1	16721	4.8	46.71	38.47	10.01
		2	16758	4.87	46.63	38.33	10.2
Set/Run 4	A	1	17428	8.77	45.74	30.05	15.44
		2	17904	8.6	45.05	29.89	16.46
	B	1	14547	11.93	54.72	26.410	6.94
		2	14413	3.41	49.64	37.55	9.38
	C	1	22288	1.6	27.26	40.47	30.66
		2	22379	1.35	26.87	41.04	30.74
	D	1	16041	10.57	49.18	27.77	12.47
		2	15940	10.35	49.74	27.98	11.93
	E	1	16188	8.37	49.92	30.37	11.32
		2	15923	7.92	50.75	30.88	10.42
	SS	1	16984	4.98	46.27	37.87	10.89
		2	17013	4.76	46.2	38.15	10.89

Table 1i: Participant 9

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	16818	8.211	46.671	30.832	14.286
		2	17070	7.862	46.631	30.743	14.764
	B	1	14442	11.405	55.577	26.598	6.42
		2	14497	11.238	55.676	26.501	6.584
	C	1	22694	1.121	26.242	41.055	31.581
		2	22412	1.122	26.699	41.283	30.896
	D	1	15853	10.158	50.445	27.795	11.602
		2	15885	10.22	50.388	27.639	11.753
	E	1	16061	7.915	50.45	30.741	10.894
		2	15987	8.045	50.747	30.493	10.714
	SS	1	15992	5.776	50.66	35.041	8.523
		2	15879	5.906	50.87	35.04	8.184
Set/Run 2	A	1	17026	8.359	46.278	30.558	14.805
		2	17063	8.307	46.149	30.653	14.89
	B	1	14409	11.77	55.49	26.277	6.463
		2	14401	11.502	55.75	26.402	6.346
	C	1	22358	1.284	26.906	41.089	30.72
		2	22497	1.249	26.437	41.122	31.192
	D	1	15818	10.439	50.407	27.643	11.512
		2	15792	10.53	50.572	27.455	11.444
	E	1	15968	8.19	50.612	30.498	10.701
		2	16102	8.129	50.153	30.678	11.04
	SS	1	15888	5.97	50.895	34.872	8.263
		2	16002	5.901	50.367	35.162	8.571
Set/Run 3	A	1	17056	8.183	46.343	30.7	14.774
		2	16987	8.175	46.437	30.773	14.614
	B	1	14477	11.385	55.448	26.65	6.518
		2	14408	11.425	55.732	26.503	6.34
	C	1	22419	1.296	26.57	41.299	30.835
		2	22586	1.258	26.392	41.172	31.177
	D	1	15839	10.192	50.501	27.773	11.535
		2	15805	10.212	50.629	27.742	11.417
	E	1	15986	8.059	50.75	30.569	10.622
		2	15828	7.954	51.031	30.833	10.182
	SS	1	15883	5.977	50.943	34.903	8.177
		2	15940	5.873	50.804	34.993	8.33
Set/Run 4	A	1	17032	8.281	46.606	30.382	14.73
		2	17017	8.255	46.586	30.429	14.729
	B	1	14427	11.493	55.645	26.423	6.439
		2	14431	11.502	55.744	26.297	6.457
	C	1	22250	1.33	27.037	40.965	30.669
		2	22242	1.705	27.049	40.595	30.651
	D	1	15761	10.416	50.723	27.437	11.425
		2	15790	10.328	50.716	27.475	11.48
	E	1	15966	7.945	50.743	30.591	10.72
		2	15818	7.898	51.295	30.679	10.128
	SS	1	15938	5.931	50.876	34.85	8.343
		2	15852	6.013	51.174	34.647	8.166

Table 1j: Participant 11

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	17561	8.28	45.99	30.1	15.63
		2	17598	8.23	45.87	30.21	15.7
	B	1	14473	11.35	55.42	26.67	6.55
		2	14451	11.35	55.57	26.62	6.46
	C	1	22436	1.07	26.57	41.32	31.03
		2	22425	1.13	26.57	41.29	31.01
	D	1	15859	10.24	50.33	27.75	11.68
		2	15881	10.14	50.34	27.81	11.72
	E	1	16043	7.88	50.41	30.87	10.84
		2	15975	7.78	50.64	31	10.58
	SS	1	15927	5.81	50.91	34.92	8.37
		2	15940	5.87	50.7	34.98	8.45
Set/Run 2	A	1	17611	8.38	45.91	30	15.71
		2	17632	8.39	45.94	29.96	15.71
	B	1	14427	11.55	55.62	26.4	6.44
		2	14465	11.31	55.55	26.69	6.46
	C	1	22505	1.15	26.84	41	31.01
		2	22573	1.11	26.55	41.05	31.29
	D	1	16185	10.05	49.6	28.04	12.31
		2	15992	10.12	49.87	28.08	11.94
	E	1	16067	7.95	50.4	30.74	10.91
		2	16054	7.95	50.47	30.71	10.87
	SS	1	15941	5.8	50.82	35.01	8.38
		2	15933	5.9	50.93	34.75	8.42
Set/Run 3	A	1	17637	8.28	45.9	30.05	15.77
		2	17532	8.31	46.05	30.06	15.57
	B	1	14387	11.62	55.73	26.32	6.34
		2	14418	11.53	55.69	26.38	6.41
	C	1	22323	1.22	27.01	41.12	30.64
		2	22422	1.17	26.83	41.1	30.91
	D	1	15873	10.31	50.35	27.67	11.68
		2	15824	10.36	50.6	27.48	11.56
	E	1	16050	7.84	50.38	30.92	10.86
		2	16060	7.87	50.44	30.81	10.89
	SS	1	15970	5.71	50.75	35.1	8.44
		2	15906	6.07	50.7	34.89	8.33
Set/Run 4	A	1	17718	8.13	45.57	30.32	15.98
		2	17661	8.08	45.64	30.44	15.84
	B	1	14549	11.19	55.16	26.99	6.67
		2	14499	11.34	55.24	28.86	6.56
	C	1	22589	1.18	26.53	40.95	31.35
		2	22531	1.12	26.59	41.04	31.25
	D	1	15900	10.01	50.23	28.05	11.71
		2	15980	9.9	50.16	28.03	11.9
	E	1	16027	8.1	50.45	30.61	10.85
		2	16105	7.88	50.19	30.91	11.02
	SS	1	16122	5.64	50.18	35.28	8.9
		2	15961	5.78	50.56	35.19	8.47

Table 1k: Participant 12

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	16934	8.29	46.63	30.51	14.57
		2	16838	8.33	46.81	30.52	14.34
	B	1	14472	11.43	55.41	26.58	6.58
		2	14459	11.54	55.5	26.41	6.55
	C	1	22547	1.02	26.75	40.99	31.24
		2	22553	0.98	26.8	40.97	31.25
	D	1	15898	10.17	50.31	27.77	11.75
		2	15877	10.27	50.28	27.73	11.72
	E	1	16056	7.98	50.37	30.72	10.93
		2	16050	7.75	50.45	30.92	10.88
	SS	1	15910	5.85	51.01	34.79	8.35
		2	15920	5.8	50.95	34.86	8.39
Set/Run 2	A	1	16953	8.33	46.5	30.57	14.6
		2	16977	8.35	46.51	30.46	14.68
	B	1	14406	11.52	55.67	26.44	6.37
		2	14433	11.45	55.61	26.5	6.44
	C	1	22470	1.14	26.64	41.05	31.17
		2	22433	1.08	26.66	41.15	31.11
	D	1	15849	10.3	50.44	27.64	11.62
		2	15922	10.21	50.24	27.74	11.81
	E	1	16014	8.03	50.61	30.58	10.78
		2	15996	8	50.61	30.65	10.74
	SS	1	15913	5.79	51.04	34.85	8.32
		2	15947	5.84	50.89	34.84	8.43
Set/Run 3	A	1	16980	8.27	46.67	30.38	14.68
		2	16989	8.26	46.57	30.49	14.68
	B	1	14472	11.36	55.52	26.56	6.56
		2	14463	11.39	55.45	26.65	6.51
	C	1	22553	1.07	26.6	41.08	31.25
		2	22531	1.16	26.67	41	31.17
	D	1	16000	9.89	50.23	27.9	11.98
		2	15930	10.19	50.24	27.73	11.84
	E	1	16092	7.92	50.25	30.84	10.99
		2	16121	7.83	50.27	30.87	11.03
	SS	1	15956	5.75	50.74	35.06	8.45
		2	15993	5.74	50.62	35.08	8.56
Set/Run 4	A	1	16950	8.4	46.55	30.5	14.55
		2	16952	8.23	46.66	30.53	14.58
	B	1	14477	11.34	55.6	26.49	6.57
		2	14409	11.4	55.76	26.52	6.32
	C	1	22364	1.2	26.73	41.24	30.83
		2	22442	1	26.6	41.39	31.01
	D	1	15819	10.29	50.37	27.79	11.55
		2	15796	10.18	50.61	27.74	11.47
	E	1	16007	7.84	50.62	30.86	10.68
		2	16023	7.95	50.47	30.79	10.79
	SS	1	15917	5.8	50.96	34.93	8.31
		2	15934	5.82	50.85	34.92	8.41

Table 1l: Participant 14

	Sample	Injection	Mw	% < 8k	% 8k to 16k	% 16k to 24k	% >24k
Set/Run 1	A	1	15193	12.3	50.2	28.0	8.7
		2	15942	12.1	48.3	27.1	10.4
	B	1	13376	16.1	57.2	22.0	4.6
		2	13417	16.0	57.2	22.1	4.6
	C	1	20917	2.0	32.1	39.8	20.9
		2	20944	2.1	31.9	39.8	20.9
	D	1	14846	14.3	52.0	24.0	8.4
		2	14713	14.4	52.1	24.2	8.2
	E	1	14937	11.5	53.4	26.6	7.5
		2	14892	11.5	53.7	26.4	7.5
Set/Run 2	A	1	18025	6.7	43.1	32.7	14.1
		2	17917	6.6	43.4	32.9	13.9
	B	1	14508	11.2	55.6	26.6	6.1
		2	14458	11.1	55.8	26.7	6.0
	C	1	23653	0.8	22.4	41.6	26.6
		2	23756	0.8	22.3	41.5	26.6
	D	1	15802	10.1	50.7	27.9	9.8
		2	15835	10.3	50.3	27.8	9.9
	E	1	16843	6.3	47.0	33.9	11.1
		2	16865	6.3	46.9	33.9	11.2
Set/Run 3	A	1	17682	6.7	43.6	33.0	13.8
		2	18068	6.5	43.3	32.8	13.9
	B	1	15213	9.1	52.7	30.1	7.5
		2	15252	9.1	52.6	30.2	7.5
	C	1	23856	0.8	22.1	41.5	26.8
		2	23804	0.7	22.2	41.6	26.8
	D	1	16713	8.1	47.7	30.6	11.4
		2	16697	8.2	47.7	30.5	11.4
	E	1	16849	6.4	46.8	33.8	11.2
		2	16853	6.3	46.9	33.9	11.2
Set/Run 4	A	1	17655	7.91	45.88	30.29	12.4
		2	16711	8.63	47.77	29.78	11.41
	B	1	14500	11.8	55.35	26	6.17
		2	14429	11.56	55.65	26.25	6.1
	C	1	23784	0.85	22.09	41.48	26.81
		2	23784	0.72	21.97	41.69	26.91
	D	1	15509	10.82	51.72	26.78	9.2
		2	15789	10.14	50.73	27.77	9.74
	E	1	20619	6.12	46.78	33.95	11.25
		2	20457	6.13	46.88	33.98	11.23

Table 2a: Intra laboratory CVs for each sample for the proportion of material less than 8 kDa calculated from the reported (by lab) and re-analysed (by MHRA) data.

	A		B		C		D		E	
	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA
1	4.35%	5.76%	6.54%	4.67%	0.00%	8.10%	0.00%	0.68%	0.00%	1.69%
2	5.28%	8.76%	2.79%	3.17%	15.48%	6.92%	2.27%	2.80%	8.84%	9.61%
3	3.14%	3.02%	1.53%	1.67%	15.77%	8.32%	6.96%	5.74%	2.13%	3.12%
4	1.75%	1.50%	1.78%	1.26%	5.77%	5.69%	1.35%	1.00%	2.30%	1.76%
5	3.35%	3.86%	4.44%	3.96%	42.54%	21.63%	4.37%	4.50%	3.07%	2.46%
6	0.58%	1.93%	0.24%	1.86%	1.73%	8.68%	0.48%	2.15%	0.20%	2.84%
7	1.49%	1.22%	1.74%	1.36%	14.76%	16.07%	0.86%	1.45%	1.14%	1.51%
8	2.84%	2.40%	27.38%	2.14%	20.34%	21.40%	2.87%	2.47%	3.23%	3.29%
9	1.85%	1.83%	1.32%	1.00%	14.09%	10.33%	1.33%	1.08%	1.32%	1.03%
11	1.33%	1.14%	1.28%	1.18%	4.07%	5.43%	1.54%	1.64%	1.21%	1.29%
12	0.66%	0.73%	0.63%	0.72%	7.37%	2.85%	1.28%	0.77%	1.23%	1.08%
14	28.99%	20.93%	22.56%	20.10%	53.31%	38.07%	22.32%	21.76%	32.12%	22.03%

Table 2b: Intra laboratory CVs for each sample for the proportion of material between 8 and 16 kDa calculated from the reported (by lab) and re-analysed (by MHRA) data.

	A		B		C		D		E	
	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA
1	0.60%	0.76%	0.63%	0.57%	0.74%	0.73%	0.69%	0.21%	0.89%	0.87%
2	1.50%	1.09%	0.78%	0.54%	1.70%	2.11%	0.94%	0.78%	2.04%	1.72%
3	0.49%	0.43%	0.40%	0.37%	0.85%	0.82%	0.34%	0.19%	1.12%	0.82%
4	0.42%	0.28%	0.41%	0.17%	0.63%	0.82%	0.36%	0.13%	0.31%	0.14%
5	1.39%	1.48%	0.88%	0.71%	2.79%	3.15%	0.98%	0.93%	0.83%	1.04%
6	0.12%	0.54%	0.10%	0.46%	0.26%	0.21%	0.07%	0.44%	0.08%	0.45%
7	0.45%	0.90%	0.46%	1.04%	0.89%	1.52%	0.31%	0.82%	0.38%	0.89%
8	0.57%	0.46%	3.79%	0.68%	1.03%	1.07%	1.12%	1.08%	0.75%	0.69%
9	0.41%	0.53%	0.21%	0.40%	1.15%	1.11%	0.26%	0.59%	0.68%	0.60%
11	0.36%	0.37%	0.37%	0.37%	0.68%	0.65%	0.62%	0.57%	0.25%	0.36%
12	0.22%	0.08%	0.21%	0.20%	0.27%	0.42%	0.26%	0.20%	0.30%	0.20%
14	6.06%	3.37%	3.19%	2.49%	18.47%	7.08%	3.55%	3.72%	6.36%	2.60%

Table 2c: Intra laboratory CVs for each sample for the proportion of material between 16 and 24 kDa calculated from the reported (by lab) and re-analysed (by MHRA) data.

	A		B		C		D		E	
	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA
1	0.61%	1.17%	2.24%	1.92%	0.50%	0.48%	0.77%	0.36%	1.09%	0.51%
2	1.56%	1.81%	1.17%	0.78%	0.65%	0.88%	1.06%	1.07%	3.39%	3.45%
3	0.74%	1.08%	0.60%	0.91%	0.75%	0.33%	0.60%	0.73%	0.58%	1.01%
4	0.29%	0.58%	0.45%	0.52%	0.40%	0.61%	0.43%	0.49%	0.46%	0.69%
5	0.88%	1.02%	2.41%	2.37%	0.81%	0.69%	2.13%	2.09%	0.79%	0.94%
6	0.28%	0.49%	0.11%	0.31%	0.15%	0.79%	0.16%	0.46%	0.12%	0.58%
7	0.52%	0.30%	0.59%	0.63%	0.44%	0.97%	0.53%	0.33%	0.56%	0.62%
8	0.64%	0.50%	13.98%	0.41%	1.12%	1.12%	0.72%	0.59%	0.60%	0.61%
9	0.53%	0.35%	0.50%	0.54%	0.54%	0.60%	0.53%	0.39%	0.39%	0.43%
11	0.55%	0.58%	3.11%	0.86%	0.32%	0.38%	0.79%	0.80%	0.42%	0.48%
12	0.18%	0.14%	0.29%	0.29%	0.35%	0.36%	0.26%	0.16%	0.38%	0.31%
14	7.72%	5.23%	11.71%	6.51%	1.98%	2.11%	8.96%	6.26%	10.70%	5.27%

Table 2d: Intra laboratory CVs for each sample for the proportion of material greater than 24 kDa calculated from the reported (by lab) and re-analysed (by MHRA) data.

	A		B		C		D		E	
	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA	By lab	By MHRA
1	2.34%	1.69%	5.14%	4.37%	1.46%	0.96%	0.00%	1.01%	0.00%	2.12%
2	4.01%	4.11%	2.56%	4.39%	1.20%	2.10%	1.72%	2.57%	7.51%	5.73%
3	1.87%	1.93%	2.45%	2.35%	1.04%	0.37%	2.59%	2.82%	5.98%	3.09%
4	0.99%	1.21%	1.17%	1.29%	0.36%	0.55%	0.40%	0.63%	0.70%	0.56%
5	5.29%	5.48%	4.74%	4.83%	4.40%	4.53%	5.14%	4.96%	4.15%	4.48%
6	0.09%	1.56%	0.46%	2.42%	0.08%	1.06%	0.76%	1.07%	0.17%	1.82%
7	2.16%	2.63%	8.19%	9.92%	1.30%	2.22%	1.98%	2.82%	2.55%	3.95%
8	1.93%	1.73%	15.47%	5.93%	1.07%	0.97%	3.44%	3.41%	2.99%	3.07%
9	1.25%	1.59%	1.27%	3.04%	1.05%	1.58%	0.97%	2.47%	3.00%	2.15%
11	0.81%	0.77%	1.59%	1.59%	0.75%	0.82%	2.00%	1.90%	1.14%	1.89%
12	0.77%	0.55%	1.53%	1.65%	0.47%	0.36%	1.41%	1.01%	1.15%	0.84%
14	16.22%	11.63%	18.19%	9.15%	10.72%	5.90%	12.56%	11.65%	16.54%	9.28%

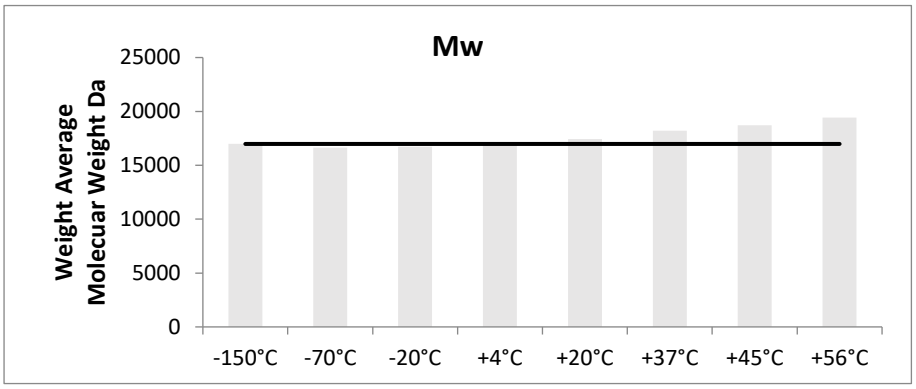
Appendix 4: Product characteristics for the proposed 3rd IS Unfractionated Heparin for Molecular Weight Calibration, 07/324

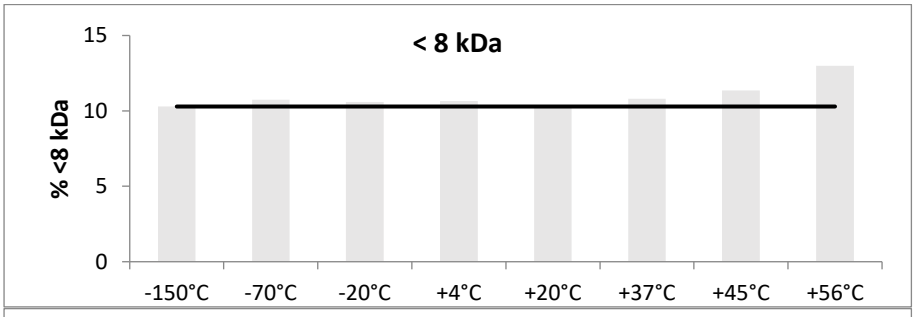
	07/324
Presentation	Sealed, siliconized glass 3 ml DIN ampoules
Number of Ampoules available	~4500
Liquid filling weight (g)	1.0050
CV of fill mass (%) – target < 0.25[1]	0.122 (n = 970)
Homogeneity by functional activity (24 ampoules)[2]	GCV = 0.59% (ANOVA = 0.879)
Mean dry weight (g, n = 6)	0.0116 (CV = 4.57%)
Mean head space oxygen by Orbisphere (%, n = 12) – target <1.13%[3]	0.72 (CV = 17.82%)
Residual moisture by KF analysis (%, n = 12) – target < 1%[4]	0.597 (CV = 22.88%)
Manufacturing site	MHRA, South Mimms
Custodian	MHRA, South Mimms
Storage temperature	-20 °C

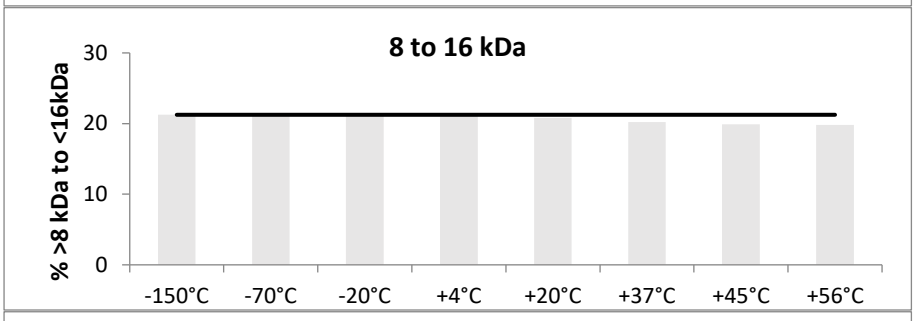
CV = coefficient of variation; GCV = geometric coefficient of variation; [1] the low CV across the fill as measured by the liquid distribution is considered acceptable for this material; [2] the material was filled as a candidate for the 6th IS UFH activity where biological homogeneity was measured across the fill – this value is considered very low and demonstrates acceptable distribution and freeze drying of the material; [3] Oxygen content considered acceptable; [4] Residual moisture level is slightly higher than ideal for heparin which has probably contributed to stability profile of the material.

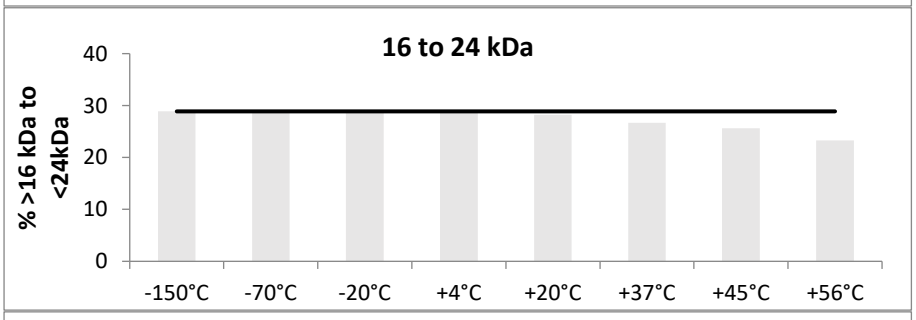
Appendix 5: Stability

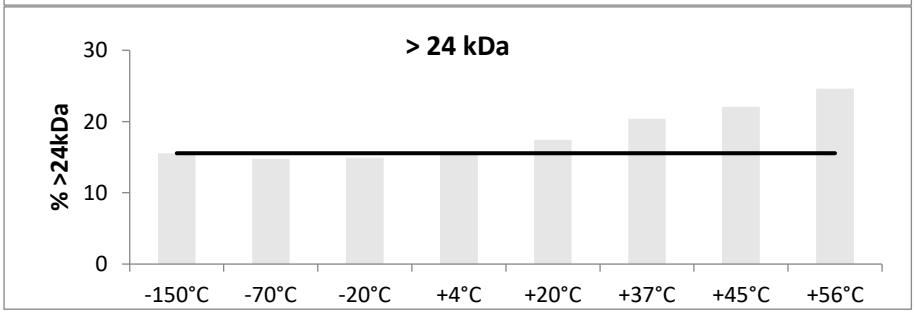
Calculations as shown in the figures below were based on using the -150°C sample as the molecular weight calibrant. The line is the value assigned to the -150°C sample.











Appendix 6: Instructions for Use

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

WHO International Standard
1st International Standard for Unfractionated Heparin for Molecular Weight Calibration
NIBSC code: 07/324
Instructions for use
(Version [Q-DOCS_Version], Dated [Q-DOCS_Date_Published])

9

1. INTENDED USE

The 1st International Standard Unfractionated Heparin for Molecular Weight Calibration consists of ampoules, coded 07/324, containing aliquots of a freeze-dried material prepared from porcine mucosa. This preparation was established as the 1st International Standard Unfractionated for Molecular Weight Calibration by the Expert Committee on Biological Standardisation of the World Health Organisation in 2026.
The material is intended for use as a broad standard calibrant to measure the weight average molecular weight of unfractionated heparin using site exclusion / gel permeation chromatography systems using refractive index detection (1).

2. CAUTION

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

There is no assigned unitage associated with this standard. The standard was qualified by 12 laboratories in 7 countries, to demonstrate the suitability of the Broad Standard Table as shown in Appendix 1

4. CONTENTS

Country of origin of biological material: Italy.
In 2008, 251.6 mg bulk material was dissolved in 25 litres water for injection. The solution was distributed into 25,000 ampoules (CV for volume of fill 0.122% (n=970)), coded 07/324. The contents of the ampoules were then freeze-dried under the conditions normally used for international biological standards (2, 3). The mean dry weight of the freeze-dried plug was 10.3 mg, with a water content of 0.60%

5. STORAGE

Unopened ampoules should be stored in the dark 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

Din Ampoule

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution
The calibrant is intended for use in the determination of the molecular weight distribution of unfractionated heparins by size exclusion chromatography (SEC, also sometimes known as gel permeation chromatography (GPC)). It may be used to calibrate a chromatography system by broad standard calibration, using the molecular weight distribution information as listed in the table in Appendix 1. For each molecular weight (M) in the Table, the percent of sample above M (%>M) and the percent of sample below M (%<M) are given. The use of specialised SEC computer software for calibration of the chromatography system and for calculation of the molecular weights of unfractionated heparin samples is strongly recommended.

This material is not suitable for use to calibrate a system intended to measure the molecular weight of low molecular weight heparins - please use 05/112 for this purpose.

8. STABILITY

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

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

Stability studies have indicated that the material is inhertently stable when stored at -20°C, and suffers no loss in performance following a short shipment period at ambient temperatures

9. REFERENCES

1 - Chromatographic molecular weight measurements for heparin, its fragments and fractions, and other glycosaminoglycans. Mulloy B, Hogwood J. Methods Mol Biol. 2015;1229:105-18. doi: 10.1007/978-1-4939-1714-3_11.PMID: 25325948
2 - Campbell PJ. International biological standards and reference preparations. 1. Preparation and presentation of materials to serve as standards and reference preparations. J Biol Standardisation 1974; 2: 249-267
3 - Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO TRS, No. 932, 2006, Annex 2. pp.114-119 (section A.7)

10. ACKNOWLEDGEMENTS

All participants in the international collaborative study and manufacturers for their kind donation of heparin samples for this study:

Aspen Notre-Dame-de-Bondeville, France

Bioiberica, SA, Plaza Francesc Macià , 7 Barcelona, Spain

Opocrin SPA, 3, V. Pacinotti 41043 Corlo di Formaigine (MO), Italy

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:

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

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

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 solid		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	Yes	Irritant:	No
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contains material of porcine origin		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation:	Seek medical advice		
Ingestion:	Seek medical advice		
Contact with eyes:	Wash with copious amounts of water. Seek medical advice		
Contact with skin:	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.			

15. LIABILITY AND LOSS

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

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 10.3 mg
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.

APPENDIX 1 – BROAD STANDARD TABLE FOR 07/324

Point	M (Da)	% below MW	% above MW
1	6000	3.2	96.8
2	8000	10.4	89.6
3	10000	19.8	80.2
4	12000	31.7	68.3
5	14000	43.4	56.6
6	16000	55.5	44.5
7	18000	66.0	34.0
8	20000	74.4	25.6
9	22000	80.3	19.7
10	24000	84.4	15.6
11	26000	87.5	12.5
12	28000	90.1	9.9
13	**32000**	93.4	6.6
14	**36000**	95.6	4.4
15	**40000**	97.0	3.0

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UK Official Medicines Control Laboratory

World Health Organization