WHO/BS/2020.2393
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 19 to 23 October 2020

Proposed 2nd WHO International Standard for insulin-like growth factor-I (IGF-I), recombinant, human

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Summary

A candidate preparation of recombinant human insulin-like growth factor-I (IGF-I) was prepared in ampoules coded 19/166. A collaborative study was organised with 16 laboratories in 11 countries in two phases. In Phase 1, the candidate preparation was value assigned in SI units via HPLC assay against a primary calibrant. Phase 2 was designed to provide confirmatory data on the immunoreactivity of the candidate standard and its suitability to serve as an International Standard for the calibration of immunoassays of human IGF-I. This phase was also designed to assess the commutability of the candidate preparation, 19/166, in IGF-I immunoassays.

Phase 1 study participants were sent ampoules of the candidate standard, 19/166, alongside a primary calibrant, PS01, and the current IS, 02/254. The primary calibrant, PS01, was prepared and assigned a mass value of 1.045 mg/vial during the collaborative study to establish the 1st IS, 02/254 [1]. Participants were asked to provide estimates of the mass content of the candidate IS, 19/166, in terms of the primary calibrant, PS01, via HPLC assay. A total of 23 valid assays were performed across 8 laboratories, giving rise to an overall geometric mean for IGF-I content in the candidate IS, 19/166, of 33.0 µg/ampoule, with expanded uncertainty of 30.5 – 35.6 µg/ampoule (*k=2.36*).

Phase 2 study participants were provided with ampoules of the candidate IS, 19/166, and the current IS, 02/254, alongside human serum and plasma samples. A total of 9 laboratories performed immunoassays, including 9 different methods, some of which were peformed in multiple laboratories (13 different laboratory performed methods in total). One laboratory method was excluded from analysis due to a narrow assay measurement range. All laboratories measured thirteen human serum samples and five human plasma samples in parallel with dilutions of 19/166 and 02/254. The data provided demonstrated the candidate IS, 19/166, to be immunoreactive, and to behave in a similar manner to the 1st IS, 02/254, in the immunoassays included in the study. This indicates that continuity of IGF-I measurements would be achieved following introduction of the replacement IS.

The immunoassay results were also analysed to assess the commutability of the standard with patient samples using a difference in bias approach. Of the 12 laboratory performed methods which demonstrated a consistent patient sample bias, the candidate standard, 19/166, was shown to be fully commutable with patient samples in 9 laboratory methods. In the 3 laboratory methods in which the standard was non commutable, one of these methods (Lab 16C) is the same method as one which was found commutable by a different laboratory (Lab 13). Of the remaining 2 methods (Lab 8A and 8B), the 1st IS, 02/254, was also found to be non commutable, and demonstrated negative bias when samples were reported relative to either 19/166 or 02/254. The all study bias estimates for serum and plasma samples showed a slight improvement in harmonisation (less bias) across the laboratories and methods when reported relative to either the 1st or candidate 2nd International Standards.

In order to predict the long-term stability of the candidate IS, 19/166, samples stored at elevated temperatures for 9 months were analysed by immunoassay. However, no significant loss of activity was observed in samples stored at elevated temperatures, meaning that the predicted loss of activity per annum could not be predicted at this time. Nonetheless, the apparent stability of

the candidate IS, 19/166, during storage at elevated temperatures indicates that the material will have good long-term stability when stored at -20°C.

Taken together, the results from Phase 2 immunoassay estimates and commutability assessment suggest that the candidate IS, 19/166, is suitable as to serve as a replacement for the 1st IS, 02/254, for the continued calibration of immunoassay methods for the measurement of IGF-I.

Therefore it is proposed that the candidate preparation in ampoules coded 19/166 is established as the **2nd International Standard for insulin-like growth factor-I, recombinant, human** with an assigned content of **33.0 µg/ampoule (expanded uncertainty of 30.5 – 35.6 µg/ampoule; *k=2.36*)**.

Introduction

Insulin-like growth factor-I (IGF-I) is a 70 amino acid 7,655 Da protein. It is produced primarily in the liver in response to growth hormone, and is the principal mediator of the effects of growth hormone, eliciting anabolic effects on a variety of cell and tissue types [2]. The measurement of circulating IGF-I is widely established in the diagnosis of growth disorders. There are a variety of commercially available IGF-I immunoassay methods, including manual ELISA and automated platform assays. The majority of these assays are traceable to the WHO International Standard for insulin-like growth factor-I (IGF-I), recombinant, human, coded 02/254. This material was value-assigned in mass units (micrograms per ampoule), reflecting the units of measurement in clinical laboratories. Stocks of 02/254 are close to exhaustion, necessitating the preparation of a replacement IS, to ensure continuous availability of an IS for calibration of IGF-I immunoassay measurements.

With this in mind, a preparation of highly purified, therapeutic grade recombinant human IGF-I has been filled into ampoules (NIBSC code 19/166), following procedures recommended by WHO [3], as a candidate replacement International Standard. This batch of ampoules has been evaluated in a two-phase collaborative study. Phase 1 aimed to assign an IGF-I content to the candidate IS in terms of a primary calibrant IGF-I preparation, PS01. This primary calibrant was prepared and value-assigned during the establishment of the current IS, 02/254 [1]. Recent HPLC and amino-acid analyses demonstrated that this material remained fit for use as a primary calibrant for the candidate replacement IS, 19/166. Phase 2 of the study aimed to demonstrate the suitability of the candidate IS, 19/166, to serve as an International Standard to calibrate human IGF-I immunoassays, by confirmation of the immunoreactivity of the candidate IS, 19/166; demonstratation of the continuity of IGF-I measurements between the candidate IS and current IS, 02/254; and an assessment the commutability of the candidate IS with patient samples. The study also aimed to assess the long-term stability of the candidate IS, 19/166, via an accelerated thermal degradation (ATD) study.

Aims

The aims of the collaborative study were as follows:

1. To assign an IGF-I contant to the candidate IS, 19/166, in micrograms per ampoule, via HPLC assay against a primary calibrant, PS01.
2. To confirm the continuity of IGF-I measurements using both the candidate IS, 19/166, and current IS, 02/254, in immunoassays used for the measurement of patient samples.
3. To assess the commutability of the candidate IS, 19/166, with patient samples.
4. To assess the stability of the candidate IS, 19/166, via an accelerated thermal degradation (ATD) study.

Materials and Methods

Bulk materials and processing

A bulk preparation of highly purified, recombinant human insulin-like growth factor-I (IGF-I) was generously donated to the WHO by Ipsen (Paris, France). The material was provided as four vials each containing 4 mL of a 10 mg/mL solution of formulated drug product (Increlex®, batch N21820). The bulk IGF-I solution was diluted to approximately 60 µg/mL in 40 mM sodium phosphate pH 7.0 containing 20 mg/mL trehalose. This solution was dispensed in 0.5 mL aliquots into 2.5 mL glass ampoules, lyophilised and sealed under nitrogen. This process was carried out at NIBSC on 29th August – 2nd September 2019. Ampoules will be stored at NIBSC at -20^{o}C.

Product charaterisation

A total of 4,578 ampoules were filled. The batch had a mean fill mass of 0.5197 g (CV 0.385%, n=187) and a mean dry weight of 0.0119 g (CV 1.68%, n=6). The mean residual moisture, measured via a manual Karl Fischer coulimetric titration, was 2.03% (CV 17.86%, n=12) and mean oxygen head space 0.22% (CV 41.97%, n=12). Although the residual moisture is higher than typically expected of a WHO IS, this is consistent with moisture levels observed in the current IS, 02/254 (2.15%) [1]. No microbiological contamination was detected.

Collaborative study design

Participants

16 laboratories in 11 countries took part in either the Phase 1 or Phase 2 of the study and are listed alphabetically, by country, in Table 1. Throughout the study, each participating laboratory is referred to by a code number . The code numbers were randomly assigned and do not reflect the order of listing.

Table 1: list of participants in order of country

AUSTRALIA	Tursun Kerim Therapeutic Goods Administration, Biochemistry section, 136 Narrabundah Lane, Symonston, ACT 2609
BELGIUM	Charlotte Delvaux Immunodiagnostic Systems, Rue Ernest Solvay 101, 4000, Liege
CANADA	Barry Lorbetskie, Simon Sauvé Health Canada, Centre for Biologics Evaluation, Sir Frederick Banting DRW (A/L 2201E) Tunney's Pasture, Ottawa, ON, K1A 0K9
CHINA	Liang Chenggang National Institutes for Food and Drug Control, 2 Tiantan Xili, Beijing 100050
GERMANY	Matthias Herkert DRG Instruments, Frauenbergstrasse 18, 35039, Marburg
GERMANY	Martin Bidlingmaier Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, Endocrinology Laboratory, Ziemssenstrasse 1, 80336, Munich
GERMANY	Lutz Pridzun Mediagnost Gesellschaft für Forschung und Herstellung von Diagnostika GmbH, Aspenhaustrasse 25, 72770, Reutlingen
GERMANY	Alessandra Hoppe Roche Diagnositcs GmbH, Nonnenwald 2, 82377, Penzberg
INDIA	Jai Prakash, M. Kalaivani Indian Pharmacopoeial Commission, Biologics Section, Sector 23, Rajnagar, Ghaziabad, 201002
*ITALY	Francesco Donati Federazione Medico Sportiva Italiana, Viale Tiziano 70, 00196, Rome
JAPAN	Yukari Nakagawa, Akiko Ebisawa Pharmaceutical and Medical Device Regulatory Science Society of Japan (PMRJ), 2-1-2 Hiranomachi, Chuo-ku, Osaka 541-0046
REPUBLIC OF KOREA	Joonho Eom Ministry of Food & Drug Safety, National Institute of Food and Drug Safety Evaluation, 187 Osongsaengmyeong 2-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do
UK	Chinwe Duru, Paul Matejtschuk National Institute of Biologicals and Control, South Mimms, Hertfordshire, EN6 3QG
UK	Katherine Partridge, Ben Cowper National Institute of Biologicals and Control, South Mimms, Hertfordshire, EN6 3QG
UK	Gwen Wark UK NEQAS Guildford Peptide Hormones Scheme, Royal Surrey County Hospital, Egerton Rd, Guildford, GU2 7XX

USA	Aleksander Baldys Siemens Healthineers, 511 Benedict Avenue, Tarrytown, NY 10591

*laboratory was unable to complete analysis due to the Covid-19 pandemic and necessitated lockdown

Samples

The collaborative study was organised by NIBSC. The materials provided to participants are summarised in Table 2. Instructions for Use were provided with the samples. Human serum and plasma samples were kindly collected by Dr Gwen Wark (UK NEQAS) or purchased from First Link UK Ltd and TCS Biosciences.

Table 2: samples provided to study participants

Sample ID	Contents
Primary calibrant (PS01)	1.045 mg/vial IGF-I
Candidate 2nd IS for IGF-I, recombinant, human (19/166)	Nominally 30 µg/vial IGF-I, plus 10 mg trehalose and 20 µmoles sodium phosphate
1st IS for IGF-I, recombinant, human (02/254)	8.5 µg/vial IGF-I, plus 10 mg trehalose and 20 µmoles sodium phosphate
Serum1-13	0.5-1.0 mL human serum
Plasma1-5	0.5-1.0 mL human plasma

Methods

A summary of methods provided by participants is provided in Table 3.

Table 3: methods provided by study participants

Study phase	Participants
Phase 1 (HPLC assay)	Laboratories 2, 4, 5, 7, 9, 11, 12, 15
Phase 2 (Immunoassay)	Laboratories 1, 3, 6, 8, 9, 10, 11, 13, 16

Phase 1 (HPLC assay) participants were asked to provide estimates of the IGF-I contents of the candidate IS, 19/166, and the current IS, 02/254, by assaying against the primary calibrant, PS01, by HPLC, using the method provided in the study protocol (Appendix 2). It was requested that three independent assays be performed, using fresh ampoules/vials for each assay. However, due to limited stocks, only a single ampoule of the current IS, 02/254, was provided for inclusion in a single assay. Participants were also requested to provide estimates of the purity of native human IGF-I in the candidate IS, 19/166, from HPLC assay data.

Phase 2 (immunoassay) participants were asked to assay the candidate IS, 19/166, the current IS, 02/254, serum samples 1-13 and plasma samples 1-5 (depending on assay compatability) using the immunoassay routinely in use in their laboratory. It was requested that three independent assays be performed, using fresh ampoules/samples for each assay. Detailed guidelines for sample preparation and analysis were provided in the study protocol (Appendix 2). Across the 9 laboratories performing immunoassays, 9 different methods were used, including a combination of automated and manual ELISA methods: Diasorin Liaison® XL, Roche Elecsys®, Siemens Immulite® and ADVIA Centaur®, Immunodiagnostic Systems iSYS, DRG Instruments 600 ELISA, Mediagnost E20 ELISA and R20 RIA, and R&D Quanitkine ELISA. Some methods were peformed in multiple laboratories, whilst some laboratories performed multiple methods, giving rise to a total of 13 laboratory performed methods.

Stability assessment of the candidate IS, 19/166

An accelerated thermal degradation (ATD) study was carried out to predict the long-term stability of the candidate IS, 19/166. Ampoules were stored at elevated temperatures (+4, +20, +37 and +45^{o}C) for 35 weeks. Samples were then analysed for IGF-I content via immunoassay in comparison with a reference sample stored at -20^{o}C. Due to limited numbers, and availability at a late stage of the collaborative study, ATD sample analysis was only performed in a single laboratory.

Statistical Analysis

Value assignment of 19/166 and 02/254 by HPLC assay

To ensure consistency of mass content estimatations between laboratories, all returned raw data was analysed centrally at NIBSC. If necessary, the reported primary calibrant, PS01, serial dilution concentrations were re-calculated, using its previously assigned mass value of 1.045 mg/vial and the reported vial reconstitution mass. These values, and corresponding peak areas, were entered into GraphPad Prism v8.2.1 for Windows (GraphPad Software, San Diego, California, USA, www.graphpad.com) for standard curve determination and estimation of the mass content of the candidate IS, 19/166, using linear regression.

Results from all valid assays were combined to generate unweighted geometric mean (GM) estimates for each standard and a variance components analysis was performed using log transformed estimates in order to determine intra-lab and inter-lab components of variation, calculated using Minitab 18 software (Minitab Inc. USA). Variability has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\}$ x 100%), where s is the standard deviation of the $\log_{10}$ transformed estimates). The expanded uncertainty on the final HPLC content estimates incorporates uncertainty in the value assigned to primary calibrant, PS01, by amino acid analysis [1].

Estimates of native IGF-I purity in 19/166

Results from all valid assays were combined to generate unweighted geometric mean (GM) estimates of native IGF-I purity for each laboratory and these laboratory means were used to calculate an overall unweighted geometric mean estimate. A variance components analysis was performed using log transformed estimates in order to determine intra-lab and inter-lab

components of variation, calculated using Minitab 18 software (Minitab Inc. USA). Variability has been expressed using geometric coefficients of variation (GCV = $\{10^s-1\}\times100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates).

Assessment of the immunoreactivity of 19/166 and 02/254

Dilutional linearity (parallelism with kits standards) was assessed for each standard in each laboratory by calculating the slope of the fitted regression line for log estimated concentration against log nominal concentration. Results from all valid assays were combined to generate unweighted geometric mean (GM) estimates of IGF-I concentration for 19/166 and 02/254 in each laboratory and these laboratory means were used to calculate overall unweighted geometric mean estimates. A variance components analysis was performed using log transformed estimates in order to determine intra-lab and inter-lab components of variation, calculated using Minitab 18 software (Minitab Inc. USA). Variability has been expressed using geometric coefficients of variation (GCV = $\{10^s-1\}\times100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates).

Assessment of commutability

Commutability of the candidate IS, 19/166, and the current IS, 02/254, was assessed using a difference in bias approach. Geometric mean estimates for serum and plasma samples were calculated from reported estimates, and estimates relative to both 19/166 and 02/254. Median values, calculated from $\log_{10}$ transformed estimates for analysis in order to achieve approximately constant scatter over the range of concentrations used, were used as the study consensus values for each sample in the analysis. Bias values were calculated as the laboratory GM estimate as % of the study median value for the sample. In order to derive an acceptable bias range (for analysis of this study only), the standard deviation of the log transformed bias values was calucalted within each laboratory, and a pooled value, s_P, was calculated across all laboratories. Criteria representing the maximum acceptable bias were then set as $\pm3s_P$. Reference standards were to be concluded as commutable if the observed difference in bias was within the commutability criteria. For this commutability assessment, the bias for plasma and serum samples has been assumed to be constant over the concentration range used.

Assessment of stability

Samples stored at elevated temperatures (+4, +20, +37, +45ºC) and a reference temperature (-20ºC), were analysed via immunoassay, with the intention of fitting an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [5], and thus predict the degradation rates when stored at a range of temperatures. However, no significant loss of activity was observed at elevated temperatures, therefore it was not possible to estimate the rate of degradation at this time.

Results

Data returned & assay validity

Phase 1
HPLC data was returned by 8 laboratories, who in total performed 25 independent assays. All assays satisfied the system suitability criteria in the study protocol, and are therefore valid. However, after central data review at NIBSC, the following assays or HPLC injections were omitted from further analysis:

- Lab 7, injection 3 in assays 1 & 2 was excluded. In both cases the peak area was significantly reduced (by approximately 10%) compared with preceeding injections. Assays 3 and 4 were also excluded altogether, due to inconsistent chromatogram appearance between sample replicates.

Phase 2
Immunoassay data was returned by 9 laboratories, who in total performed 39 independent assays. All assays included kit controls/standards and met the associated acceptance criteria. However, after central analysis at NIBSC, the following runs or assays were excluded from further analysis:

- Lab 9 immunoassays were performed using a R&D Quantikine ELISA kit. This method has a significantly reduced assay range, which does not allow for measurement of the core concentrations requested (25 – 400 ng/mL IGF-I) and does not cover the range of concentrations expected from the serum and plasma samples. The data obtained was therefore not included in the commutability assessment or geometric mean calculations
- Results for candidate IS, 19/166, in assay 2 in Lab 16B were excluded as the intra-lab GCV values for this sample in this lab (28-45%) far exceeded that in all other cases (all values < 25%, with 92% of values < 10%)
- Results for serum sample 1 in Lab 1 was removed from analysis as an outlier.

Estimated content of the candidate IS, 19/166

HPLC content estimates (µg/ampoule) in terms of the primary calibrant, PS01, are summarised in Table 4. The overall geometric mean for the candidate IS, 19/166, across the eight laboratories is 33.0 µg per ampoule. With relative standard uncertainty, this gives rise to a final content estimate of 33.0 µg/ampoule with expanded uncertainty of 30.5 - 35.6 µg/ampoule (with coverage factor of k=2.36 taken to correspond to a 95% level of confidence). The uncertainty comprises intra- and inter-laboratory variability, as well as the uncertainity in the assigned value of the primary calibrant, PS01 [1]. HPLC content estimates from each laboratory were in good agreement, with both intra-lab and inter-lab GCV below 5% (3.2% and 2.8% respectively).

Estimates of the IGF-I content of the current IS, 02/254, in terms of the primary calibrant, PS01, are also summarised in Table 4. Due to limited supply of 02/254 ampoules, this sample was

included in only a single assay by each laboratory. The overall geometric mean across the 8 laboratories is 8.1 µg per ampoule. Including relative standard uncertainty gives rise to a final content estimate of 8.1 µg/ampoule with expanded uncertainty of 7.0 – 9.3 µg/ampoule (with coverage factor of k=2.36 taken to correspond to a 95% level of confidence) and inter-lab GCV 17.4%.

Table 4: individual HPLC assay estimates of IGF-I content of the candidate IS, 19/166, and 1st IS, 02/254, in terms of the primary calibrant, PS01, in µg per ampoule. GM = geometric mean, GCV = geometric coefficient of variation. Uncertainty comprises intra- and inter-laboratory variability, as well as the uncertainity in the assigned value of the primary calibrant, PS01.

Lab	19/166				02/254			
	Assay 1	Assay 2	Assay 3	Lab GM	Assay 1	Assay 2	Assay 3	Lab GM
2	34.268	34.611	32.895	33.917			9.239	9.239
4	32.376	32.270	32.094	32.247	6.917			6.917
5	29.963	34.010	32.212	32.019	7.397			7.397
7	33.539	30.860		32.052	9.977			9.977
9	34.041	32.843	33.687	33.520	8.441			8.441
11	34.274	35.453	35.310	35.008			8.722	8.722
12	32.874	33.099	33.042	33.005		8.744		8.744
15	31.880	32.100	31.840	31.940			6.180	6.180

Summary statistics	19/166	02/254
Overall GM	33.0	8.1
Intra-lab variance component (as GCV)	3.2%	n/a
Inter-lab variance component (as GCV)	2.8%	17.4%
Expanded uncertainty (95% confidence, k=2.36)	30.5 – 35.6	7.0 – 9.3

Estimated purity of native IGF-I in the candidate standard, 19/166

HPLC estimates of the purity (%) of native IGF-I in the candidate IS, 19/166, are summarised in Table 5. The estimated mean across the eight laboratories is 97.7% (Intra-lab GCV 0.6%, Inter-lab GCV 0.4%).

Immunoreactivities of the candidate IS, 19/166, and the current IS, 02/254

Participants were asked to run a set of core dilutions of the candidate IS, 19/166, and the current IS, 02/254, ranging from nominally 25-400 ng/ml, as described in the study protocol (Appendix 2). However, the analysis of immunoassay data for 19/166 and 02/254 was based on the results from nominal concentrations 50 to 400 ng/ml only in order to avoid any lack of dilutional

linearity at the lower concentrations whilst still covering the range of expected concentrations of serum and plasma samples used for commutability assessment.

Table 5: estimates of IGF-I purity of native IGF-I in the candidate IS, 19/166, by HPLC assay. GM = geometric mean, GCV = geometric coefficient of variation.

Lab	19/166			
	Assay 1	**Assay 2**	**Assay 3**	**Lab GM**
2	97.920	98.070	97.920	97.970
4	96.592	96.967	97.279	96.946
5	97.410	97.460	97.480	97.450
7	98.880	96.540		97.703
9	98.550	97.780	98.170	98.166
11	98.814	97.800	98.029	98.213
12	97.600	95.970	97.480	97.014
15	97.940	98.180	98.220	98.113

Summary statistics	**19/166**
Overall GM	97.7
Intra-lab variance component (as GCV)	0.6%
Inter-lab variance component (as GCV)	0.4%
Expanded uncertainty (95% confidence, k=2.36)	97.3 – 98.1

Dilutional linearity (parallelism with kit standards) was assessed for each standard in each laboratory by calculating the slope of the fitted regression line for log estimated concentration against log nominal concentration. Results are shown in Appendix 1, Table A1.1 and indicate broadly acceptable parallelism with all slope ratios in the range of 0.86-1.05 (the majority are within 0.95-1.05 as highlighted, with only laboratories 1 and 8A falling outside this range) and no overall trend for values to be greater or less than 1.0 for either standard. A greater degree of parallelism was observed between the candidate IS, 19/166, and the current IS, 02/254, with slope ratios in the range 0.97-1.04 and an inter-laboratory GCV of only 2.2%.

Across all valid assays, geometric mean laboratory estimates of IGF-I content of the candidate IS, 19/166, and the current IS, 02/254, were determined, and are summarized in Table 6. Laboratory geometric mean estimates for 19/166 ranged from 27.637 µg/ampoule to 52.438 µg/ampoule with an overall **geometric mean estimate of 35.0** µg/ampoule **(95% CI: 30.7 – 39.9, n=35)**. For 02/254, laboratory geometric mean estimates ranged from 7.911 µg/ampoule to 14.305 µg/ampoule with a **geometric mean estimate of 9.8** µg/ampoule **(95% CI: 8.7 – 11.1, n=36).**

The estimated IGF 1 content of 35.0 µg/ampoule for the candidate IS, 19/166, is in very good agreement with the assigned HPLC content of 33.0 µg/ampoule. The geometric mean of 9.8 µg/ampoule for the current IS, 02/254, is slightly larger than the assigned content of 8.5

µg/ampoule (with expanded uncertainty 7.7-9.2 µg per ampoule) [1]. Intra-assay variability was acceptable for the measurement of both 19/166 and 02/254 with reported overall GCV% values of laboratory estimates of 5.1% and 5.3% respectively (Table 6). Inter-lab variability for the measurement of 19/166 and 02/254 was 22.9% and 20.8% respectively (Table 6). These values are enlarged by data provided by Labs 8A, 8B, 11 and 16B (the latter two of which used the same method). These data aside, all reported estimates range from 27.1-33.8 µg/ampoule for 19/166, and 7.8-9.9 µg/ampoule, which are in particularly close agreement with the assigned HPLC contents. Importantly, both standards appear to be behave in a similar manner in each assay.

Table 6: individual laboratory geometric mean immunoassay estimates of IGF-I content (µg/ampoule) of the candidate IS, 19/166, and the current IS, 02/254. GM = geometric mean, GCV = geometric coefficient of variation.

Lab	19/166				02/254			
	Assay 1	Assay 2	Assay 3	Lab GM	Assay 1	Assay 2	Assay 3	Lab GM
1	27.497	27.511	27.904	27.637	7.841	8.155	7.962	7.985
3	32.561	29.755	31.019	31.090	9.138	9.868	9.247	9.412
6	32.745	31.045	33.675	32.470	9.029	9.017	9.082	9.043
8A	48.830	54.034	54.650	52.438	14.074	14.239	14.606	14.305
8B	46.076	43.332	44.757	44.708	12.402	11.674	12.508	12.189
10A	32.654	33.150	33.457	33.085	8.971	9.388	9.500	9.283
10B	29.434	28.834	29.240	29.168	8.176	8.100	8.044	8.106
11	41.673	40.447	40.082	40.728	12.942	11.410	11.478	11.923
13	27.819	32.295	31.982	30.629	8.733	8.920	8.712	8.788
16A	31.529	33.776	33.143	32.802	9.388	9.359	9.517	9.421
16B	41.912		48.759	45.206	12.232	9.689	12.636	11.441
16C	27.867	27.052	31.000	28.590	8.059	7.908	7.768	7.911

Summary statistics	19/166	02/254
Overall GM	35.0	9.8
Intra-lab variance component (as GCV)	5.1%	5.3%
Inter-lab variance component (as GCV)	22.9%	20.8%
Expanded uncertainty (95% confidence, k=2.20)	30.7 – 39.9	8.7 – 11.1

Commutability of the candidate IS, 19/166, and the current IS, 02/254

The commutability of the candidate IS, 19/166, and the current IS, 02/254, with plasma and serum samples was assessed for all methods included in the study, with exception of the R&D Quantikine ELISA method due to its incompatible assay range.

Data used for the assessment of commutability are shown in Appendix 1, Tables A1.2, A1.3 and A1.4. Table A1.2 shows the geometric mean reported estimates for the serum and plasma samples in each laboratory. Geometric mean estimates relative to the candidate IS, 19/166, and the current IS, 02/254, are shown in Tables A1.3 and A1.4 respectively. Median values, calculated using log transformed estimates, are shown for each sample and have been used as the study consensus values for each sample in the analysis. Samples were ranked by increasing level of concentration (Table A.1.2) for the purposes of plotting the data in this order for presentation. Bias values were calculated as the laboratory GM estimate as a % of the study median value for the sample and are shown in Tables 7-9 and Figures 1-3.

The limits for acceptable bias of $\pm 3S_P$ were calculated as described in the Statistical Analysis section, giving ±0.077, or 0.838 to 1.193 on the untransformed scale, i.e. the bias should be demonstrated to be not less than 83.8% and not more than 119.3% to be considered acceptable. Log transformed bias values for estimates relative to a standard are equivalent to the difference in bias between the test sample and the standard, so values within the acceptance criteria can be taken to indicate commutability of the standard with serum and plasma samples for that laboratory.

The bias in laboratory reported estimates in Table 7 is also shown graphically in Figure 1. The shaded cells in Table 7 indicate that the bias for these reported estimates of these samples is outside the limits of acceptable bias of 83.8% to 119.3%. Laboratory 1 reports more negative bias in the majority of samples tested, whereas laboratory 8A, 11 and 16B show more positive bias in reported values.

Bias values for estimates relative to the candidate IS, 19/166, or the candidate IS, 02/254, (Tables 8 and 9 respectively) are equivalent to the difference in bias between the test sample and the standard, so values within the acceptance criteria can be taken to indicate commutability of the standard with serum and plasma samples for that laboratory. This data is also represented graphically in Figures 2 and 3. The shaded cells show where the bias for a particular sample relative to 19/166 or 02/254 is outside the acceptable commutability criteria. For both standards, 8 out of 12 laboratory methods show bias values for all samples are within these limits, indicating that in these 8 methods (Labs 3, 6, 10A, 10B, 11, 13, 16A and 16B), both 19/166 and 02/254 are commutable. In Lab 1, although 3 and 2 of the 13 serum samples are outside the acceptable limits when reported relative to 19/166 and 02/254 respectively, the standards are also deemed commutable with this method. The bias for the majority of serum/plasma samples in laboratories 8A, 8B and 16C are however outside the acceptable range, showing negative bias in Labs 8A and 8B, and positive bias in Lab 16C. This indicates that in the assays performed by Lab 8A, 8B and 16C, the candidate IS, 19/166, is non-commutable. Importantly however, the current IS, 02/254, is also found non-commutable in these methods, indicating that the two standards are behaving in a similar manner in these assays.

Plots showing all bias estimates from Tables 7-9 are shown in Figure 4. Although partly due to unacceptable bias seen in reported results from Labs 1, 8A, 11 and 16B, this figure illustrates that the study data as a whole demonstrates a slight improvement in harmonization, with less bias across these laboratories and methods when the candidate IS, 19/166, or the current IS, 02/254, are used as standards.

Table 7: bias in reported estimates for IGF-I concentrations in serum and plasma samples (lab GM estimate as % of study median value for sample); shaded cells are outside range 83.8 - 119.3%, GM = geometric mean.

Sample	Lab											
	1	**3**	**6**	**8A**	**8B**	**10A**	**10B**	**11**	**13**	**16A**	**16B**	**16C**
Serum1		100.0%	94.8%	121.4%	105.2%	86.9%	87.0%	113.8%	98.5%	104.1%	127.4%	98.5%
Serum2	83.5%	90.9%	98.1%	134.0%	103.9%	100.5%	86.0%	120.1%	97.4%	107.3%	132.4%	99.6%
Serum3	81.0%	90.3%	93.5%	139.4%	136.1%	118.2%	87.8%	119.6%	92.6%	102.2%	126.4%	97.9%
Serum4	87.9%	92.7%	94.2%	127.7%	106.0%	97.7%	85.0%	121.6%	94.7%	102.4%	125.7%	104.3%
Serum5	80.0%	94.1%	96.1%	136.6%	105.3%	98.8%	88.3%	121.0%	97.6%	101.3%	123.2%	110.1%
Serum6	66.0%	100.8%	97.2%	131.7%	96.6%	86.9%	84.6%	121.8%	99.2%	103.5%	131.9%	104.3%
Serum7	92.3%	88.8%	91.4%	128.7%	129.9%	109.9%	86.8%	130.0%	91.5%	98.5%	130.6%	101.6%
Serum8	77.5%	95.0%	95.9%	117.3%	106.0%	86.3%	82.4%	111.6%	97.5%	102.6%	125.3%	109.7%
Serum9	80.1%	92.8%	95.8%	117.1%	104.5%	84.1%	82.5%	109.2%	97.7%	102.4%	124.3%	107.5%
Serum10	69.1%	95.4%	96.5%	122.3%	113.3%	91.3%	87.5%	110.6%	98.1%	102.0%	123.5%	109.2%
Serum11	64.9%	93.7%	96.1%	118.1%	110.4%	89.8%	87.7%	114.4%	97.4%	102.7%	122.9%	106.4%
Serum12	91.2%	93.7%	95.1%	110.7%	106.0%	86.5%	84.9%	110.6%	98.5%	101.5%	124.2%	106.6%
Serum13	81.0%	96.0%	95.1%	124.0%	113.9%	93.1%	87.5%	123.1%	97.9%	102.2%	125.2%	104.6%
Plasma1		94.2%	97.0%	118.2%	109.8%	112.1%	83.3%		92.3%	103.1%	127.0%	90.8%
Plasma2		97.8%	94.7%	121.2%	108.6%	98.0%	87.5%		98.0%	103.7%	124.5%	102.0%
Plasma3		98.0%	95.8%	121.4%	101.8%	91.3%	88.0%		98.2%	102.0%	120.1%	102.1%
Plasma4		97.8%	96.9%	125.7%	102.7%	97.2%	90.1%		98.2%	104.9%	131.9%	101.9%
Plasma5		98.0%	94.9%	112.8%	116.1%	111.2%	85.4%		92.1%	102.0%	116.1%	95.9%

Table 8: bias in estimates for serum and plasma samples relative to 19/166 (lab GM estimate as % of study median value for sample); shaded cells are outside range 83.8 - 119.3%.

Sample	Lab											
	1	**3**	**6**	**8A**	**8B**	**10A**	**10B**	**11**	**13**	**16A**	**16B**	**16C**
Serum1		110.2%	100.0%	79.3%	80.6%	90.0%	102.1%	95.7%	110.1%	108.8%	96.5%	118.0%
Serum2	101.2%	97.9%	101.2%	85.6%	77.8%	101.7%	98.8%	98.7%	106.5%	109.5%	98.1%	116.6%
Serum3	98.5%	97.7%	96.8%	89.4%	102.4%	120.1%	101.3%	98.7%	101.7%	104.7%	94.0%	115.1%
Serum4	107.2%	100.5%	97.7%	82.1%	79.9%	99.5%	98.2%	100.6%	104.2%	105.2%	93.7%	122.9%
Serum5	97.2%	101.7%	99.4%	87.5%	79.1%	100.3%	101.7%	99.7%	107.1%	103.7%	91.5%	129.4%
Serum6	80.8%	109.8%	101.4%	85.0%	73.1%	88.9%	98.2%	101.2%	109.6%	106.8%	98.8%	123.5%
Serum7	112.1%	95.7%	94.4%	82.3%	97.4%	111.4%	99.8%	107.0%	100.2%	100.7%	96.9%	119.1%
Serum8	99.7%	108.6%	104.9%	79.5%	84.3%	92.7%	100.3%	97.4%	113.0%	111.1%	98.5%	136.3%
Serum9	101.2%	104.3%	103.1%	78.0%	81.7%	88.8%	98.8%	93.7%	111.4%	109.0%	96.0%	131.4%
Serum10	87.4%	107.1%	103.8%	81.5%	88.5%	96.3%	104.8%	94.9%	111.8%	108.5%	95.4%	133.3%
Serum11	81.4%	104.6%	102.6%	78.1%	85.6%	94.1%	104.3%	97.4%	110.3%	108.5%	94.3%	129.0%
Serum12	113.0%	103.2%	100.3%	72.3%	81.2%	89.6%	99.7%	93.0%	110.2%	106.0%	94.1%	127.7%
Serum13	98.8%	104.2%	98.7%	79.7%	85.9%	94.9%	101.2%	101.9%	107.7%	105.0%	93.4%	123.3%
Plasma1		101.0%	99.6%	75.2%	81.9%	112.9%	95.2%		100.4%	104.8%	93.7%	105.8%
Plasma2		105.5%	97.8%	77.5%	81.5%	99.3%	100.7%		107.4%	106.0%	92.4%	119.7%
Plasma3		105.6%	98.9%	77.6%	76.3%	92.5%	101.1%		107.4%	104.2%	89.0%	119.6%
Plasma4		103.6%	98.3%	79.0%	75.6%	96.8%	101.7%		105.5%	105.3%	96.1%	117.3%
Plasma5		106.2%	98.5%	72.5%	87.5%	113.2%	98.7%		101.3%	104.8%	86.5%	113.0%

Table 9: bias in estimates for serum and plasma samples relative to 02/254 (lab GM estimate as % of study median value for sample); shaded cells are outside range 83.8 - 119.3%

Sample	Lab											
	1	**3**	**6**	**8A**	**8B**	**10A**	**10B**	**11**	**13**	**16A**	**16B**	**16C**
Serum1		100.0%	98.7%	79.9%	81.2%	88.1%	101.0%	89.8%	105.5%	104.0%	104.8%	117.2%
Serum2	97.6%	90.1%	101.3%	87.4%	79.5%	101.0%	99.0%	94.0%	103.4%	106.2%	108.0%	117.4%
Serum3	94.9%	89.8%	96.7%	91.2%	104.5%	119.1%	101.4%	93.9%	98.6%	101.5%	103.4%	115.8%
Serum4	104.8%	93.8%	99.2%	85.0%	82.8%	100.2%	99.8%	97.1%	102.7%	103.5%	104.6%	125.5%
Serum5	94.2%	94.0%	100.0%	89.8%	81.2%	100.0%	102.4%	95.4%	104.5%	101.1%	101.3%	130.9%
Serum6	78.1%	101.3%	101.7%	87.0%	74.9%	88.5%	98.7%	96.6%	106.7%	103.9%	109.0%	124.7%
Serum7	108.2%	88.3%	94.6%	84.2%	99.8%	110.8%	100.2%	102.0%	97.4%	97.8%	106.8%	120.1%
Serum8	95.8%	99.7%	104.7%	81.0%	85.9%	91.8%	100.3%	92.4%	109.5%	107.5%	108.1%	136.9%
Serum9	99.3%	97.7%	104.9%	81.0%	84.9%	89.7%	100.7%	90.7%	110.0%	107.6%	107.5%	134.6%
Serum10	83.3%	97.4%	102.6%	82.2%	89.4%	94.5%	103.8%	89.2%	107.3%	104.1%	103.8%	132.7%
Serum11	79.0%	96.8%	103.3%	80.3%	88.0%	94.0%	105.2%	93.3%	107.8%	105.9%	104.5%	130.7%
Serum12	108.8%	94.8%	100.2%	73.8%	82.9%	88.8%	99.8%	88.4%	106.8%	102.7%	103.4%	128.4%
Serum13	97.4%	97.9%	100.9%	83.2%	89.7%	96.2%	103.6%	99.1%	106.9%	104.1%	105.1%	126.9%
Plasma1		94.3%	101.1%	77.9%	84.9%	113.8%	96.8%		99.0%	103.2%	104.6%	108.1%
Plasma2		97.3%	98.1%	79.4%	83.5%	98.8%	101.2%		104.5%	103.1%	101.9%	120.8%
Plasma3		98.8%	100.5%	80.5%	79.2%	93.3%	103.0%		106.0%	102.7%	99.5%	122.3%
Plasma4		95.2%	98.2%	80.5%	77.2%	95.9%	101.8%		102.3%	102.0%	105.6%	118.0%
Plasma5		99.3%	100.1%	75.2%	90.8%	114.2%	100.5%		99.9%	103.2%	96.7%	115.6%

Figure 1: bias in reported estimates for serum and plasma samples (lab GM estimate as % of study median value for sample.

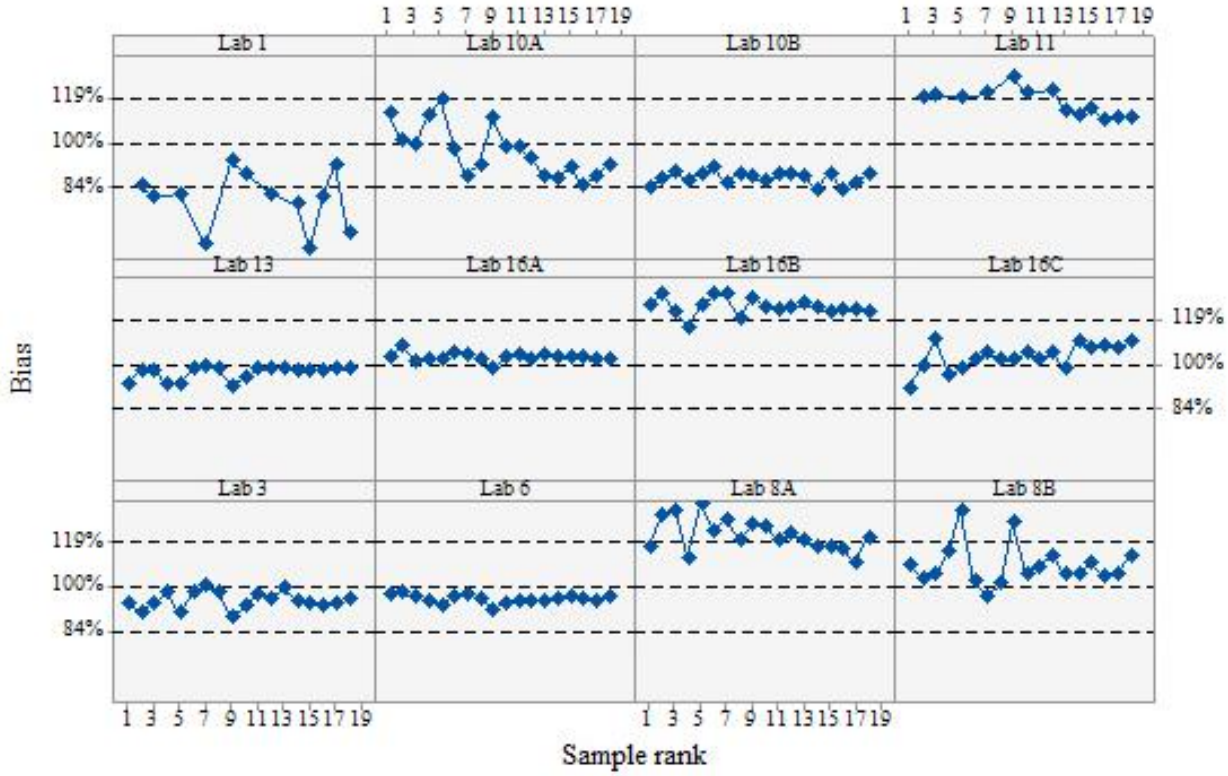


Figure 2: bias in estimates for serum and plasma samples relative to 19/166 (lab GM estimate as % of study median value for sample)

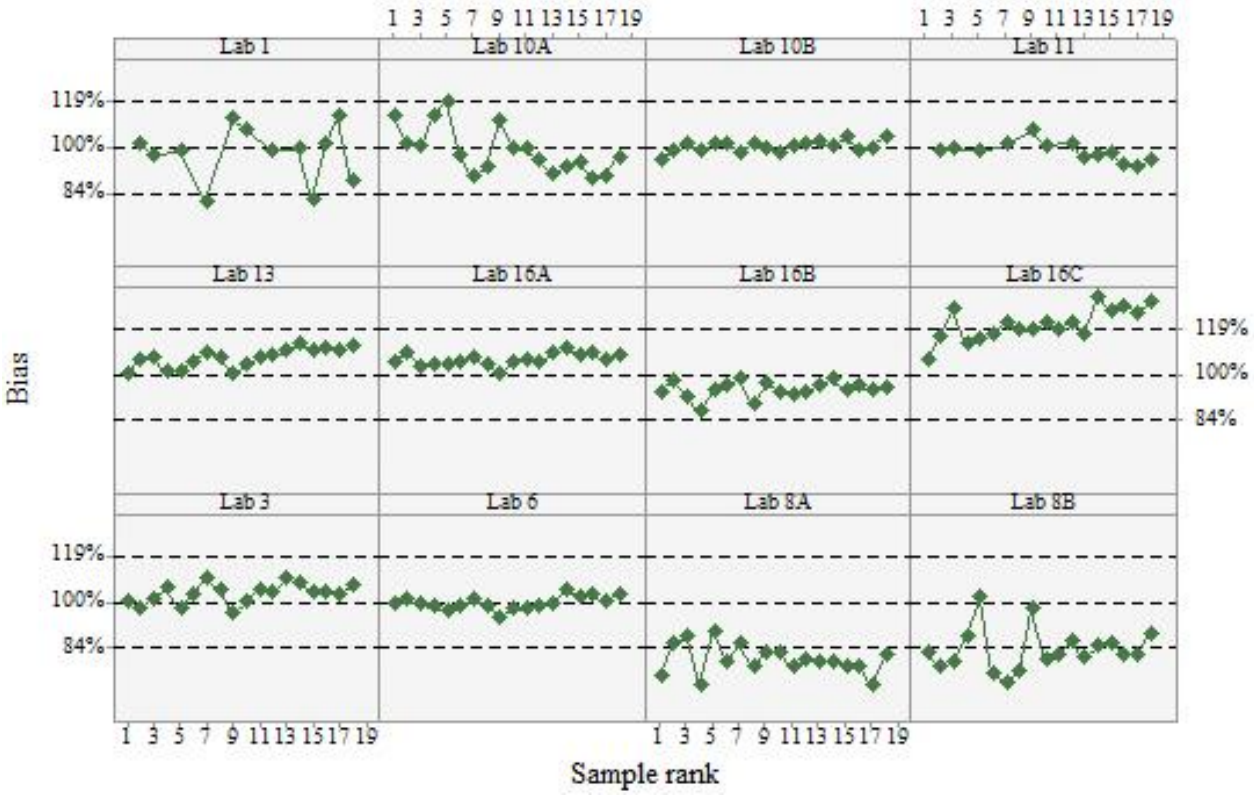


Figure 3: bias in estimates for serum and plasma samples relative to 02/254 (lab GM estimate as % of study median value for sample)

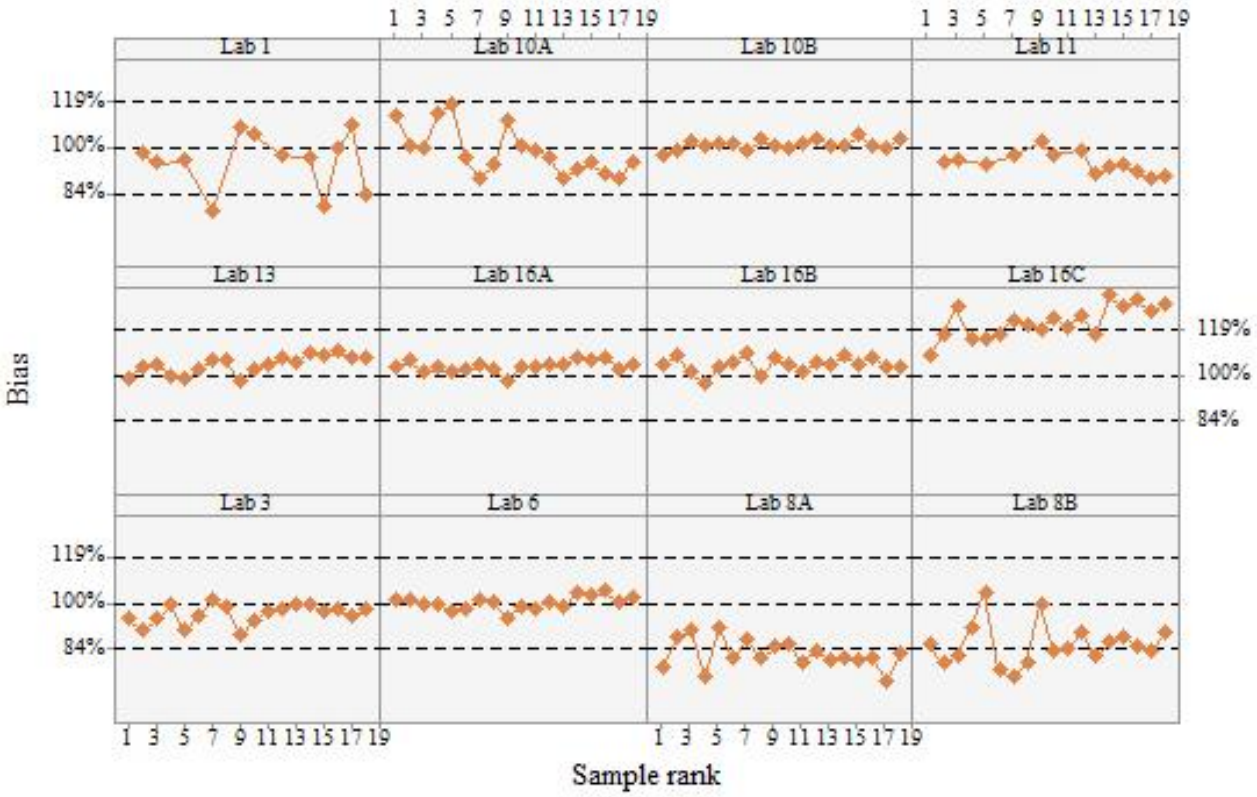
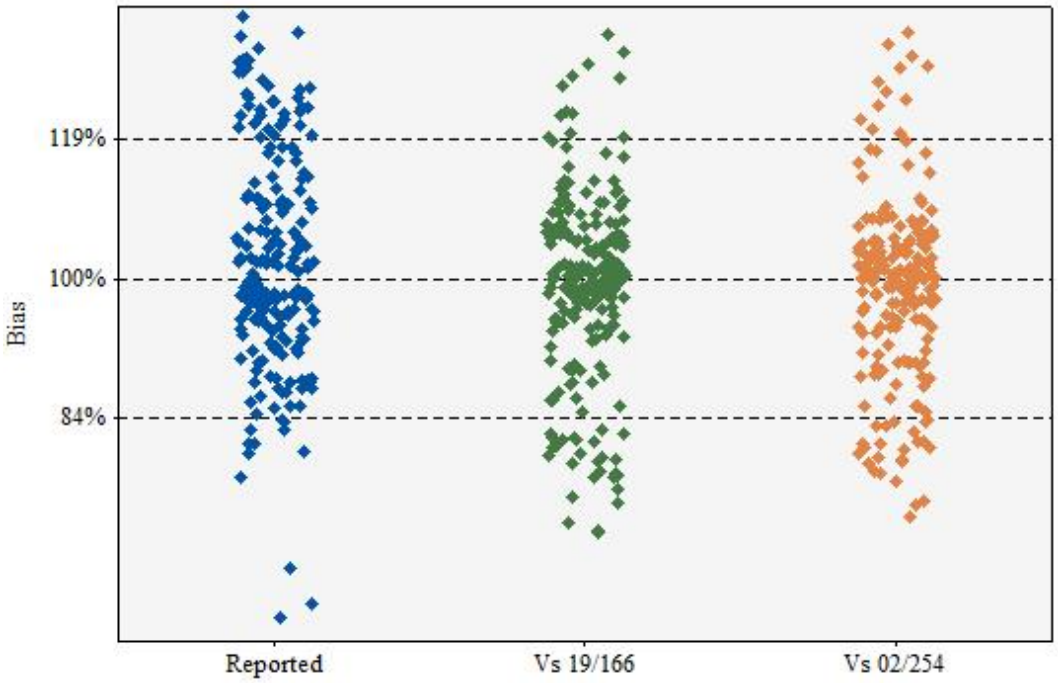
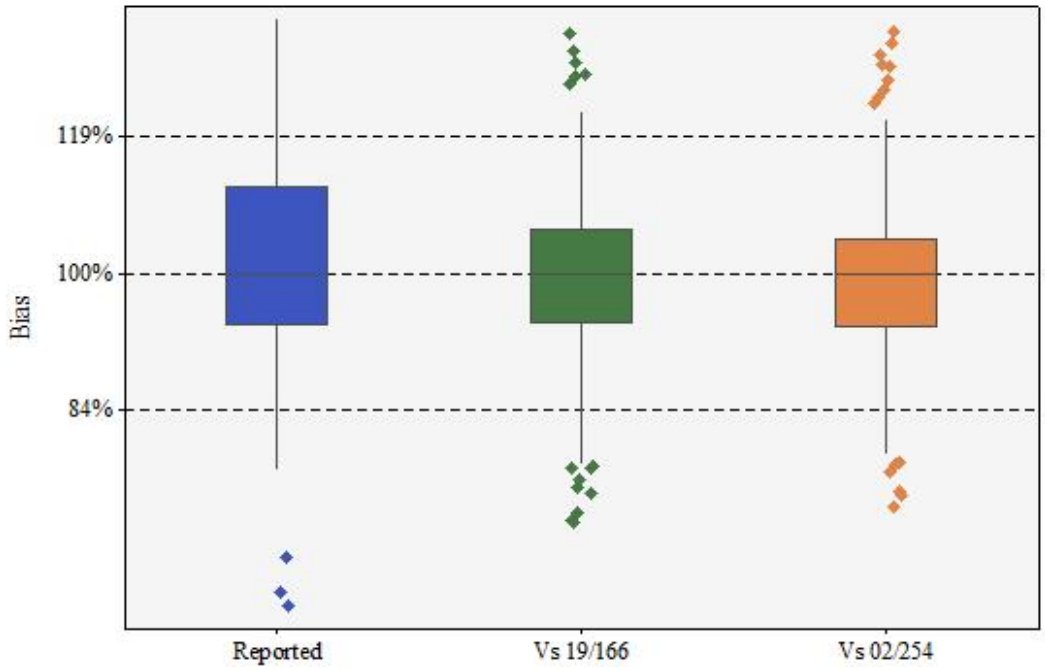


Figure 4: all study bias estimates for serum and plasma samples (reported, relative to 19/166, relative to 02/254) shown as a) individual value plot and b) boxplot

(a)



(b)



To summarise, the candidate IS, 19/166, was found to be commutable with patient samples in 9 laboratory methods (Labs 1, 3, 6, 10A, 10B, 11, 13, 16A and 16B) and non commutable with patient samples in 3 methods (Labs 8A, 8B and 16C). The current IS, 02/254, was also found to be commutable with patient samples in 9 of these 12 methods (Labs 1, 3, 6, 10A, 10B, 11, 13, 16A and 16B) and non commutable with patient samples in 3 laboratories (Labs 8A, 8B and 16C). Of the cases in which 19/166 and 02/254 were non commutable, it is interesting to note that the method used in lab 16C is the same as that used by lab 13, in which both 19/166 and 02/254 were commutable. It is also of note that in all laboratories, the bias values of the candidate IS, 19/166, and the current IS, 02/254, were aligned. For example, although non commutable in labs 8A and 8B, the bias values of 19/166 and 02/254 were comparable, and showed a similar negative bias, suggesting that the candidate IS, 19/166, behaves in a similar manner in these assays in comparison to the current IS, 02/254. It is of note that in two laboratories in which 19/166 and 02/254 were not commutable the immunoassay is not calibrated to the current IS, 02/254.

It is important to note that the commutability criteria for the difference in bias approach have been derived statistically, rather than based on clinical relevance, and are directly related to the bias seen in patient samples in each assay. There is the potential for intra-assay variability between methods to have had an impact on the statistically-derived definitions of commutability/non commutability. This variability may be influenced, for example, by experimental variation caused by a number of external factors such as assay procedures, dilution buffers, different operators etc. However, it is not possible within the scope of a collaborative study to perform the intra- and inter-method comparisons that would be required to further examine this.

Taken together with the results of the immunoassay estimates, which were in good agreement with the assigned values of the standards, the results indicate that the introduction of the candidate IS, 19/166, as a replacement for the current IS, 02/254, is suitable for the continued calibration of immunoassays for human IGF-I.

Stability of the candidate IS, 19/166

The results of immunoassay analysis of candidate IS, 19/166, ampoules stored at elevated temperatures (+4, +20, +37 and +45°C) for approximately 35 weeks alongside reference ampoules stored at -20°C is summarised in Table 11. 3 independent assays were performed.

Table 11: potencies (i.e. IGF-I contents by immunoassay) of ATD samples relative to the reference sample (stored at -20°C).

ATD sample	Relative potency	95% confidence interval
+4 °C	0.956	0.905 – 1.011
+20 °C	0.958	0.907 – 1.013
+37 °C	0.925	0.875 – 0.977
+45 °C	0.978	0.925 – 1.034

Analysis of accelerated thermal degradation (ATD) samples was performed by a single laboratory only. Results are expressed as relative potencies, meaning the geometric mean IGF-I concentration in each ATD sample is expressed relative to that of the reference sample. No significant loss of activity was observed at elevated temperatures (Table 11) therefore it was not possible (at the current time) to predict the rate of degradation during long-term storage at -20^{o}C.

Discussion

Stocks of the 1st International Standard for insulin-like growth factor-I (IGF-I), human, recombinant, coded 02/254, widely used for the calibration of immunoassays for human IGF-I, are nearly exhausted, necessitating production of a replacement IS. A candidate IS, coded 19/166, has been prepared and assessed by international collaborative study in two phases, aimed at assigning the IGF-I content, in SI units, via HPLC assay, and demonstrating immunoreactivity and suitability to serve as an IS for calibration of IGF-I immunoassays.

In study phase 1, 8 expert laboratories performed HPLC assays of the candidate IS, 19/166, against a primary calibrant, PS01. Across all valid assays, mean laboratory estimates of IGF-I content ranged from 29.963 to 35.453 µg/ampoule giving rise to a final content estimate of 33.0 µg/ampoule with expanded uncertainty of 30.5-35.6 µg/ampoule (k=2.36). Laboratory estimates were in good agreement, with an acceptable inter-assay variability of GCV 2.8%. Phase I study participants were also requested to provide estimates of native IGF-I (i.e the main chromatographic peak) purity, giving rise to an overall geometric mean purity of 97.7% (inter-lab variability GCV 0.4%). The nature of the impurities has been previously investigated by LC-MS analysis at NIBSC, showing the main impurities to bear close structural relation to native IGF-I, including oxidised and C-terminally truncated forms (Appendix 1, Figure A1.1). These impurities are assumed to retain immunoreactivity in commercially available immunoassays, therefore content estimates from HPLC assays were calculated using total peak areas, including both the native form (main peak) and related impurities.

The current IS, 02/254, was included in a single HPLC assay by each phase 1 study participant. These assays yielded estimates ranging from 6.180 – 9.977 µg/ampoule, giving rise to an overall content estimate of 8.1 µg/ampoule with expanded uncertainty of 7.0 – 9.3 µg/ampoule (k=2.36). This compares to the assigned content of 8.5 µg per ampoule (7.7-9.2 µg per ampoule when accounting for expanded uncertainity). Although in broad agreement with the assigned value, this lower value is partly attributable to the increased inter-lab variability, GCV 17.4%, seen with 02/254 estimates. It may also be of importance to note that 02/254 contains a much lower IGF-I content, which necessitiated low reconstitution volume (0.5 ml, where fill volume was 1 ml) and can result in variable recovery of the material. This lower IGF-I content also gives rise to low HPLC sample concentrations/peak intensities, which can make accurate peak integration difficult (especially for low abundance impurities).

The immunoreactivity of the candidate IS, 19/166, in current IGF-I immunoassays was assessed in phase 2 of the study, alongside the current IS, 02/254. Laboratory estimates gave an overall geometric mean of 35.0 µg/ampoule for 19/166 which is in very close agreement with the assigned value of 33.0 µg/ampoule by HPLC. Laboratory estimates gave an overall geometric

mean of 9.8 µg/ampoule for 02/254, which is slightly higher than its assigned value of 8.5 µg/ampoule. In addition, there was broadly acceptable parallelism for each material across all assays, with the majority of slope ratios in the range of 0.95-1.05 and no overall trend for values to be greater or less than 1.0 for either standard. A greater degree of parallelism was observed between 19/166 and 02/254. This suggests that the candidate IS, 19/166, behaves similarly to the current IS, 02/254, in the broad range of clinical IGF-I immunoassays included in the study, and that transition from current to replacement IS is unlikely to adversely affect the continuity of clinical IGF-I measurements.

Both the candidate IS, 19/166, and the current IS, 02/254, were analysed in comparison with patient samples by immunoassay to determine the commutability of the standards with patient samples in these assays using a difference in bias approach. Of the 12 different laboratory methods included in this analysis, the candidate IS, 19/166 and the current IS, 02/254, were both commutable with 9 laboratory methods. Of the 3 methods in which they were not commutable, one was a method that was found to be commutable as performed by a different laboratory. This indicates that experimental variation, such as that caused by external factors (e.g. different dilution buffers, operators and procedures) may have had an impact on the commutability as assessed by the difference in bias approach in this study. It is also important to note that within the confines of a collaborative study, where each assay is performed only three times, it is not possible to assess how experimental variation may impact commutability. It therefore should be recommended that each manufacturer performs their own assessment of the behaviour of the candidate IS, 19/166, in their assays in comparison with native samples.

One laboratory performed immunoassays using the R&D Systems Quantikine ELISA method. This assay has a narrow range, of 0.1-6 ng/mL, and thus the data returned was not suitable for inclusion in the linearity, IGF-I content or commutability assessments (performed using core nominal concentrations of 50-400 ng/mL). Nonetheless, the reported data demonstrates that the candidate IS, 19/166, behaves in the same manner as the current IS, 02/254, in this assay (Appendix 1, Figure A1.2), and thus will be suitable for the calibration of this immunoassay.

In order to predict the long-term stability of the candidate IS, 19/166, an accelerated thermal degradation (ATD) study was performed (Table 11). However, no significant loss of activity was observed in sample stored at elevated temperatures for 35 weeks, which does not enable prediction of long-term stability during storage at -20ºC, although the lack of observed degradation does indicate that the candidate IS, 19/166, is highly stable, and suitable for use as an International Standard. However, further studies of ATD samples will be performed by HPLC[1] in the near future, to better predict long long-term stability of 19/166.

In conclusion, the candidate IS, 19/166, is a stable, mass-assigned IGF-I preparation which has been shown in this study to behave in a very similar manner to the current IS, 02/254, in clinical IGF-I immunoassays. This indicates that 19/166 is suitable to serve as a WHO International Standard for insulin-like growth factor-I, and that its replacement of the current IS, 02/254, will ensure continuity of IGF-I immunoassay measurements in the future.

[1]ATD analysis by HPLC could not be performed alongside immunoassays due to disruption caused by the Covid-19 pandemic.

Proposal

It is proposed that the candidate preparation in ampoules coded 19/166 is established as the **2nd International Standard for insulin-like growth factor-I, recombinant, human**, with an assigned content of **33.0 µg/ampoule (expanded uncertainty of 30.5-35.6 µg/ampoule; *k=2.36*)**. [2]

Acknowledgements

We gratefully acknowledge the important contributions of all participants of the collaborative study phases, Ipsen, who kindly donated the IGF-I material, the Standardisation Science Group at NIBSC for preparation of trial materials and the Standards Processing Division at NIBSC for the preparation and dispatch of ampouled materials.

Comments from participants

A copy of this report was sent to all participants involved in both the Phase 1 and Phase 2 of the collaborative study, with responses received from five participants. All those who replied were in agreement with the proposal. Some minor amendments to text were incorporated, either to correct names and addresses of participants, or to clarify statistical methods used for Phase 2.

References

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5. Kirkwood TB. *Predicting the stability of biological standards and products*. Biometrics, 1977, 33(4):736-42

Appendix 1 – additional tables/figures

Table A1.1: fitted slope-ratios for parallelism assessment of different standards; shaded cells are outside range 0.95-1.05

Lab	19/166 vs kit std	02/254 vs kit std	19/166 vs 02/254
1	0.919	0.884	1.040
3	1.006	1.011	0.996
6	0.951	0.971	0.979
8A	0.861	0.884	0.974
8B	1.041	1.045	0.996
10A	0.988	1.016	0.973
10B	1.000	0.996	1.004
11	0.984	0.973	1.011
13	0.983	0.982	1.001
16A	0.973	0.969	1.004
16B	1.013	1.031	0.983
16C	1.024	0.986	1.038
GCV	5.3%	5.4%	2.2%

Table A1.2: geometric mean reported estimates (ng/ml) for serum and plasma samples

Sample	Lab												Median	Sample rank
	1	3	6	8A	8B	10A	10B	11	13	16A	16B	16C		
Serum1		278.7	264.2	338.5	293.2	242.3	242.4	317.1	274.5	290.3	355.0	274.5	278.7	13
Serum2	87.1	94.8	102.4	139.8	108.3	104.8	89.8	125.3	101.6	111.9	138.1	103.8	104.3	2
Serum3	113.2	126.3	130.8	194.9	190.4	165.3	122.9	167.3	129.6	142.9	176.8	136.9	139.9	5
Serum4	178.3	188.1	191.0	259.1	215.0	198.1	172.3	246.6	192.2	207.7	255.0	211.5	202.8	10
Serum5	84.7	99.7	101.9	144.8	111.6	104.6	93.5	128.2	103.5	107.3	130.5	116.7	106.0	3
Serum6	100.3	153.3	147.9	200.2	146.8	132.1	128.7	185.2	150.8	157.4	200.6	158.6	152.1	7
Serum7	173.3	166.6	171.6	241.6	243.9	206.2	163.0	243.9	171.7	184.8	245.1	190.6	187.7	9
Serum8	234.8	287.9	290.5	355.5	321.2	261.6	249.6	338.2	295.2	310.9	379.6	332.3	303.0	14
Serum9	265.8	308.2	318.1	388.8	347.0	279.3	273.8	362.5	324.3	339.9	412.6	357.0	332.0	16
Serum10	286.2	394.8	399.6	506.4	469.2	377.9	362.4	458.0	406.1	422.1	511.1	452.0	414.0	18
Serum11	203.1	293.4	300.7	369.7	345.5	280.9	274.5	358.1	304.9	321.3	384.8	332.9	313.0	15
Serum12	358.3	368.1	373.5	435.1	416.4	340.0	333.7	434.4	387.1	398.9	487.9	418.9	393.0	17
Serum13	184.8	219.1	216.9	282.7	259.7	212.3	199.6	280.7	223.2	233.0	285.7	238.5	228.1	12
Plasma1		61.7	63.5	77.4	71.9	73.4	54.5		60.4	67.5	83.1	59.4	65.5	1
Plasma2		203.4	196.9	252.0	225.9	203.7	182.1		203.9	215.6	258.9	212.1	208.0	11
Plasma3		164.8	161.1	204.1	171.3	153.5	148.1		165.1	171.5	201.9	171.6	168.1	8
Plasma4		148.7	147.3	191.1	156.0	147.7	136.9		149.2	159.4	200.4	154.8	152.0	6
Plasma5		112.9	109.3	129.9	133.7	128.1	98.4		106.1	117.5	133.7	110.5	115.2	4

Table A1.3: geometric mean estimates for serum and plasma samples relative to 19/166

Sample	Lab												Median
	1	3	6	8A	8B	10A	10B	11	13	16A	16B	16C	
Serum1		0.672	0.610	0.484	0.492	0.549	0.623	0.584	0.672	0.664	0.589	0.720	0.610
Serum2	0.236	0.229	0.236	0.200	0.182	0.238	0.231	0.231	0.249	0.256	0.229	0.272	0.234
Serum3	0.307	0.305	0.302	0.279	0.319	0.375	0.316	0.308	0.317	0.327	0.293	0.359	0.312
Serum4	0.484	0.454	0.441	0.371	0.361	0.449	0.443	0.454	0.471	0.475	0.423	0.555	0.451
Serum5	0.230	0.241	0.235	0.207	0.187	0.237	0.241	0.236	0.253	0.245	0.217	0.306	0.237
Serum6	0.272	0.370	0.342	0.286	0.246	0.299	0.331	0.341	0.369	0.360	0.333	0.416	0.337
Serum7	0.470	0.402	0.396	0.346	0.409	0.467	0.419	0.449	0.421	0.423	0.407	0.500	0.420
Serum8	0.637	0.695	0.671	0.508	0.539	0.593	0.642	0.623	0.723	0.711	0.630	0.872	0.640
Serum9	0.721	0.744	0.735	0.556	0.582	0.633	0.704	0.668	0.794	0.777	0.685	0.937	0.713
Serum10	0.777	0.952	0.923	0.724	0.787	0.857	0.932	0.843	0.994	0.965	0.848	1.186	0.889
Serum11	0.551	0.708	0.695	0.529	0.580	0.637	0.706	0.659	0.747	0.735	0.638	0.873	0.677
Serum12	0.972	0.888	0.863	0.622	0.699	0.771	0.858	0.800	0.948	0.912	0.809	1.099	0.860
Serum13	0.501	0.528	0.501	0.404	0.436	0.481	0.513	0.517	0.547	0.533	0.474	0.626	0.507
Plasma1		0.149	0.147	0.111	0.121	0.166	0.140		0.148	0.154	0.138	0.156	0.147
Plasma2		0.491	0.455	0.360	0.379	0.462	0.468		0.499	0.493	0.430	0.556	0.465
Plasma3		0.398	0.372	0.292	0.287	0.348	0.381		0.404	0.392	0.335	0.450	0.376
Plasma4		0.359	0.340	0.273	0.262	0.335	0.352		0.365	0.364	0.332	0.406	0.346
Plasma5		0.272	0.252	0.186	0.224	0.290	0.253		0.260	0.269	0.222	0.290	0.256

Table A1.4: geometric mean estimates for serum and plasma samples relative to 02/254

Sample	Lab												Median
	1	3	6	8A	8B	10A	10B	11	13	16A	16B	16C	
Serum1		0.629	0.621	0.503	0.511	0.555	0.635	0.565	0.664	0.655	0.659	0.737	0.629
Serum2	0.232	0.214	0.241	0.208	0.189	0.240	0.235	0.223	0.246	0.252	0.256	0.279	0.238
Serum3	0.301	0.285	0.307	0.290	0.332	0.378	0.322	0.298	0.313	0.322	0.328	0.368	0.318
Serum4	0.474	0.425	0.449	0.385	0.375	0.454	0.452	0.440	0.465	0.468	0.474	0.568	0.453
Serum5	0.226	0.225	0.239	0.215	0.195	0.240	0.245	0.228	0.250	0.242	0.242	0.313	0.239
Serum6	0.267	0.346	0.347	0.297	0.256	0.302	0.337	0.330	0.365	0.355	0.373	0.426	0.342
Serum7	0.461	0.376	0.403	0.359	0.425	0.472	0.427	0.435	0.415	0.417	0.455	0.512	0.426
Serum8	0.625	0.650	0.683	0.528	0.560	0.599	0.654	0.603	0.714	0.701	0.705	0.893	0.652
Serum9	0.707	0.696	0.747	0.578	0.605	0.639	0.718	0.646	0.784	0.767	0.766	0.959	0.713
Serum10	0.762	0.891	0.939	0.752	0.818	0.865	0.950	0.816	0.982	0.952	0.949	1.214	0.915
Serum11	0.540	0.662	0.707	0.549	0.602	0.643	0.720	0.638	0.737	0.725	0.715	0.894	0.684
Serum12	0.953	0.831	0.878	0.646	0.726	0.778	0.875	0.774	0.936	0.900	0.906	1.125	0.876
Serum13	0.492	0.495	0.510	0.420	0.453	0.486	0.523	0.500	0.540	0.526	0.531	0.641	0.505
Plasma1		0.139	0.149	0.115	0.125	0.168	0.143		0.146	0.152	0.154	0.160	0.148
Plasma2		0.459	0.463	0.374	0.394	0.466	0.477		0.493	0.486	0.481	0.570	0.472
Plasma3		0.372	0.379	0.303	0.299	0.351	0.388		0.399	0.387	0.375	0.461	0.377
Plasma4		0.336	0.346	0.284	0.272	0.338	0.359		0.361	0.359	0.372	0.416	0.352
Plasma5		0.255	0.257	0.193	0.233	0.293	0.258		0.256	0.265	0.248	0.297	0.257

Figure A1.1: UPLC-MS analysis of IGF-I impurities in the candidate IS, 19/166.

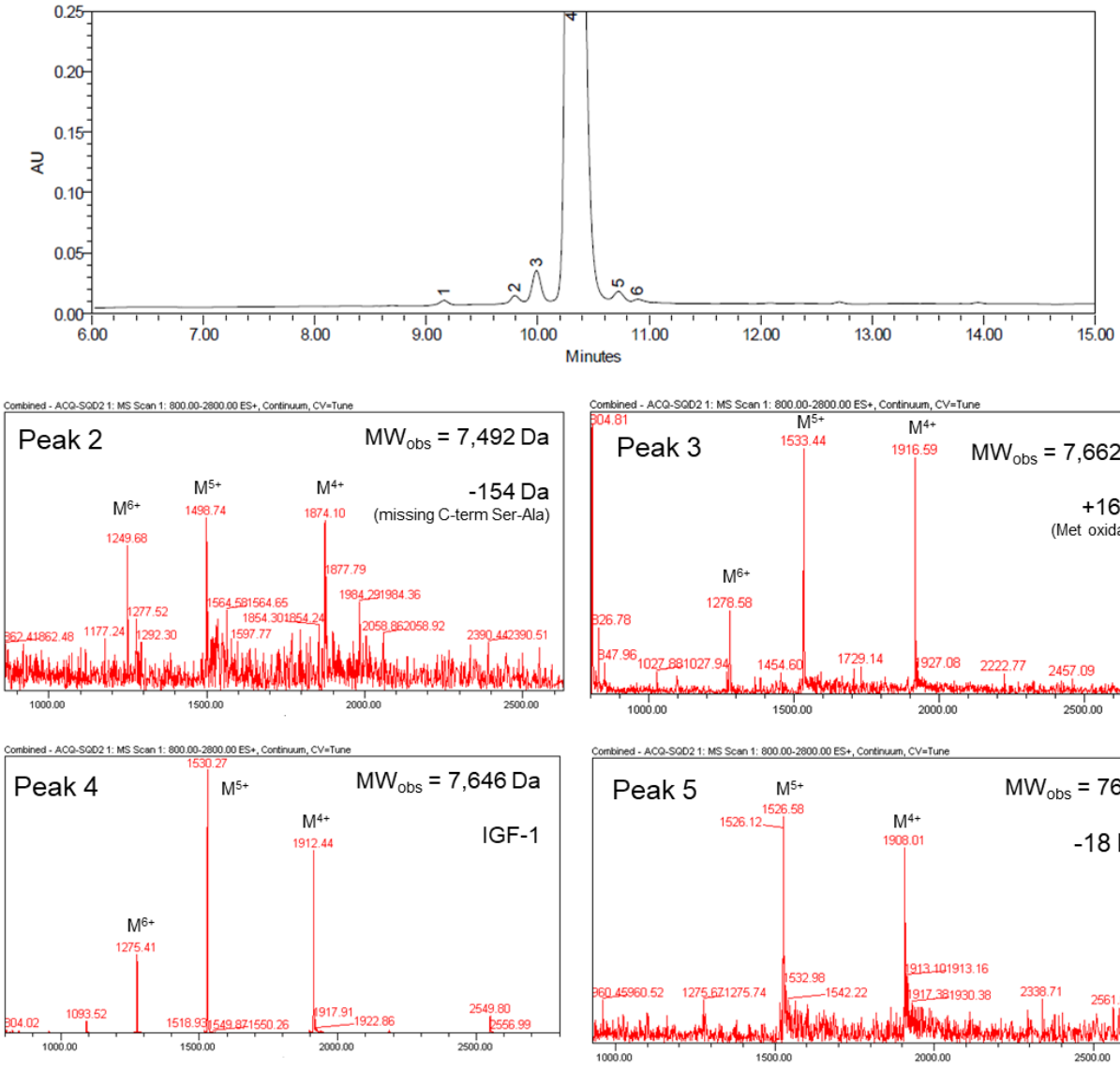
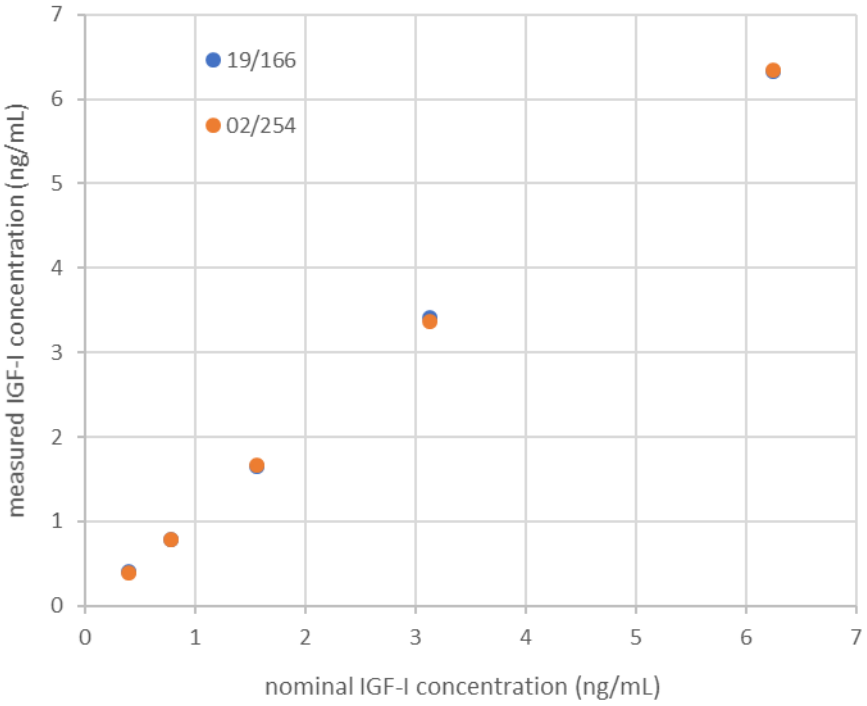


Figure A1.2: plots of mean measured IGF-I concentrations (ng/mL) versus nominal IGF-I concentrations, derived from analysis of the candidate IS, 19/166, and the current IS, 02/254, using the R&D System Quantikine ELISA method



Appendix 2 – study protocol

PHASE 1

HPLC ASSAY OF THE CANDIDATE STANDARD, 19/166, USING THE PRIMARY CALIBRANT, PS01, AS A REFERENCE STANDARD

1. KEY EQUIPMENT & MATERIALS

- A gradient high performance liquid chromatography (HPLC) instrument equipped with a variable wavelength UV detector, loop injection valve, a column oven, and a refrigerated auto-sampler.

- **HPLC column -** Zorbax 300SB-C18 (4.6 x 150 mm, particle size 3.5 µm, pore size 300 Å) or validated equivalent.

- **Mobile phase A -** 0.1% trifluoroacetic acid. E.g. mix 1 mL of HPLC-grade trifluoroacetic acid with 999 mL of HPLC-grade water. Degas.

- **Mobile phase B -** 0.1% trifluoroacetic acid, 90% acetonitrile. E.g. mix 0.5 mL of HPLC-grade trifluoroacetic acid with 450 mL of HPLC-grade acetonitrile and 50 mL of HPLC-grade water. Degas.

2. STANDARD AND SAMPLE PREPARATION

- **Perform <u>three independent HPLC assays</u>, using fresh ampoules/vials for each assay.**

- **Preparation of the standard curve of the primary calibrant PS01.**
  A standard curve of the mean peak area resulting from a minimum of five quantities of rhIGF-1 should be prepared. Dilutions should be prepared <u>in duplicate</u>, using water as the diluent.

  For example, dissolve the contents of a vial of primary calibrant PS01 with 5 mL water to obtain an IGF-1 concentration of approximately 200 µg/mL. Mix gently but thoroughly to ensure complete dissolution of the lyophilised material. Use this stock solution to accurately prepare dilutions (in duplicate) of 100, 50, 25 and 12.5 µg/mL. HPLC injection of 100 µl of each preparation will therefore provide a standard curve with determinations of 20, 10, 5, 2.5 and 1.25 µg rhIGF-1.

  <u>Due to the very fine consistency and adsorbent nature of the lyophilised material contained within PS01 vials, it is recommended that initial reconstitution be achieved by injection of the diluent through the rubber stopper, followed by gentle but thorough mixing of the vial contents to ensure complete dissolution of the lyophilised material.</u>

- **Preparation of the candidate IS, 19/166, ampoules**

Reconstitute ampoule contents in 0.5 ml water to obtain a rhIGF-1 concentration of approximately 60 µg/mL. Mix gently but thoroughly to ensure complete dissolution of the lyophilised material.

- **Preparation of the 1st IS, 02/254, ampoule**
  Reconstitute ampoule contents in 0.5 ml water to obtain a rhIGF-1 concentration of approximately 17 µg/mL. Mix gently but thoroughly to ensure complete dissolution of the lyophilised material.

- **The precise mass of solvent added to each vial or ampoule during reconstitution should be measured using a weighing balance and reported in the provided results sheet, alongside details of primary calibrant PS01 serial dilutions.**

3. SAMPLE ANALYSIS

- **HPLC operating conditions:**
  - Flow rate 1.0 mL/min
  - Injection volume 100 µL
  - Column temperature 40 °C
  - Autosampler 8 °C
  - Run time 50 mins
  - Detection UV at 214 nm
  - Mobile phase gradient elution, as detailed in table A1.

Time (mins)	A (%)	B (%)
0 → 2	75 → 72	25 → 28
2 → 37	72 → 60	28 → 40
37 → 40	60 → 20	40 → 80
40 → 42	20	80
42 → 43	20 → 75	80 → 25
43 → 50	75	25

Table A1: *gradient elution conditions. NB. The approximate retention time of rhIGF-1 is 20 mins. The mobile phase composition may be adjusted in order to obtain the desired retention time. Please report any deviations from the protocol.*

- **System suitability**
  - Perform three injections of primary calibrant, PS01, preparation containing 0.2 mg/mL IGF-I. An example chromatogram is provided in Figure A1.
  - Impurity peak A must be detected. The expected relative retention time (RRT) of impurity peak A (relative to the rhIGF-I main peak) is 0.97.
  - The relative standard deviation (RSD) of the rhIGF-1 main peak area must be ≤1.25%.

- **Sample analysis**
  - Perform one injection per individual primary calibrant PS01 preparation (dilutions are prepared in duplicate, therefore a total of two injections per rhIGF-1 concentration will be performed).
  - Perform three injections per 19/166 preparation (please note that 19/166 ampoules are reconstituted in only 0.5 mL water, meaning that excess sample dead volumes during HPLC injection must be minimised to enable triplicate injections of 100 µl).
  - Perform three injections per 02/254 preparation.
  - Repeat the system suitability steps outlined above following the final injection. If the system suitability criteria are not met, then the test samples may be rejected.

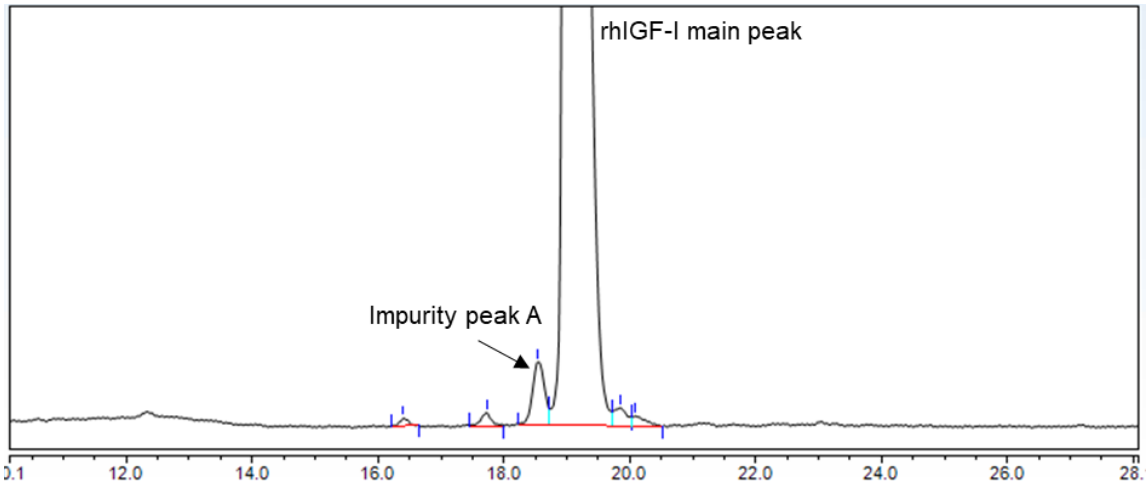


Figure A1. *A typical rhIGF-1 HPLC chromatogram.*

4. DATA ANALYSIS

For each independent assay perform the following steps:

- Perform peak integration as depicted in the example chromatogram provided in Figure A1. Related impurity peaks A-E should be visible and individually integrated/quantified.

- Using the mean combined peak areas of rhIGF-1 main and related impurity peaks measured in the duplicate chromatograms of primary calibrant PS01 dilutions, produce a standard curve of total integrated peak area vs rhIGF-1 concentration.

- Using the mean combined peak areas of rhIGF-1 main and related impurity peaks measured in the triplicate chromatograms of each ampouled preparation, calculate the content of rhIGF-1 and related impurities in each preparation using the standard curve of the primary calibrant, by linear regression analysis.

- **Please record all details of system suitability samples, reconstitution masses, primary calibrant dilutions, and peak areas, concentrations and contents in the Excel spreadsheet provided. Please include copies of all corresponding chromatograms when returning data.**

PHASE 2

STANDARD AND SAMPLE PROCESSING FOR IMMUNOASSAYS

1. RECONSTITUTION AND DILUTION OF THE CANDIDATE STANDARD, 19/166

1. Before opening, ampoules should be brought to room temperature to minimize moisture uptake.
2. Reconstitute each ampoule in 0.5 ml PBS plus 0.1% BSA or appropriate assay buffer.
3. Add 0.5 ml solution from step 2 to 7 ml PBS plus 0.1% BSA or appropriate assay buffer to provide an approximately 4 µg/ml stock solution ("Solution A").
4. Perform a 1:5 dilution of Solution A (e.g. add 400 µl to 1600 µl PBS plus 0.1% BSA or appropriate assay buffer) to provide an 800 ng/ml working stock solution. This will form "Dilution 1" from which serial dilutions should be made:
    - Perform a 1:2 dilution of "Dilution 1" into PBS plus 0.1% BSA or appropriate assay buffer, forming "Dilution 2" (400 ng/ml IGF-I).
    - Perform a 1:2 dilution of "Dilution 2" into PBS plus 0.1% BSA or appropriate assay buffer, forming "Dilution 3" (200 ng/ml IGF-I).
    - Perform at least four further 1:2 serial dilutions as described above. Table B1 below provides the full details of the dilutions and their expected concentrations. To enable comparison across different immunoassays at the same dilution point, participants are asked to include the **6 core concentrations** highlighted in **bold in all assays**. If assay space permits, additional concentrations should be included.

2. RECONSTITUTION AND DILUTION OF THE 1st IS, 02/254

1. Before opening, ampoules should be brought to room temperature to minimize moisture uptake.
2. Reconstitute the ampoule in 1 ml PBS plus 0.1% BSA or appropriate assay buffer.
3. Add 1 ml solution from step 2 to 1.125 ml PBS plus 0.1% BSA or appropriate assay buffer to provide an approximately 4 µg/ml stock solution ("Solution A").
4. Perform a 1:5 dilution of Solution A (e.g. add 400 µl to 1600 µl PBS plus 0.1% BSA or appropriate assay buffer) to provide an 800 ng/ml working stock solution. This will form "Dilution 1" from which serial dilutions should be made:
    - Perform a 1:2 dilution of "Dilution 1" into PBS plus 0.1% BSA or appropriate assay buffer, forming "Dilution 2" (400 ng/ml IGF-I).
    - Perform a 1:2 dilution of "Dilution 2" into PBS plus 0.1% BSA or appropriate assay buffer, forming "Dilution 3" (200 ng/ml IGF-I).

- Perform at least five further 1:2 serial dilutions as described above. Table B1 below provides the full details of the dilutions and their expected concentrations. To enable comparison across different immunoassays at the same dilution point, participants are asked to include the **6 core concentrations** highlighted in **bold in all assays**. If assay space permits, additional concentrations should be included.

Step / dilution	Nominal IGF-I concentration (ng/ml)	
	19/166	**02/254**
Step 2 – ampoule reconstitution	60,000	8,500
Step 3 – dilution to stock concentration ("Solution A")	4,000	
Step 4 - dilution 1 (1:5)	800	
dilution 2 (1:2)	**400**	
dilution 3 (1:2)	**200**	
dilution 4 (1:2)	**100**	
dilution 5 (1:2)	**50**	
dilution 6 (1:2)	**25**	
dilution 7 (1:2)	**12.5**	
dilution 8 (1:2)	6.25	
dilution 9 (1:2)	3.125	

Table B1: *nominal IGF-I concentrations at each dilution step. The six core concentrations to be included in all assays are highlighted in bold.*

3. PREPARATION OF CLINICAL SAMPLES

- Thaw serum samples at room temperature and mix well.
- Thaw plasma samples by placing in a water bath at 37ºC for 5-10 mins to minimise the formation of cryoprecipitates.
- Centrifuge all samples at 1500 rpm for 5 mins and remove supernatant for testing.

4. ASSAY DESIGN AND PLATE LAYOUT

Participants are requested to perform three independent assays, run on different days, using fresh ampoules/samples for each assay where provided.
An independent assay will use a single calibrated kit, integral or plate as required for your method, and should include measurement of the following:

- One set of dilutions prepared from each of the current IS, 02/254, and the candidate standard, 19/166, including the six core IGF-I concentrations (**400, 200, 100, 50, 25 and 12.5** ng/ml) as described above. Additional dilutions should be made if necessary, to ensure that a minimum of five points in the linear part of the dose response curve are measured. Dilutions of the ampouled preparations should be prepared and measured in duplicate.

- One set of serum samples (n=13) and plasma samples (n=5) which have been thawed specifically for that run. Serum/plasma samples should be prepared and measured in duplicate.
- Kit calibrators and in-house standards, where available, for the method(s) of choice, prepared and measured in replicates as per the manufacturers' instructions.
- A blank composed of the sample reconstitution/diluent buffer of choice, prepared and measured in duplicate.

Following preparation, samples should be analysed using the chosen immunoassay method immediately (i.e. as quickly as is practicable).

A suggested plate map for a 96-well manual ELISA plate is provided below:

19/166, dilution 2, replicate 1	02/254, dilution 2, replicate 1	Serum1, replicate 1	Serum9, replicate 1	Plasma4, replicate 1	In-house std 4, replicate 1	19/166, dilution 2, replicate 2	02/254, dilution 2, replicate 2	Serum1, replicate 2	Serum9, replicate 2	Plasma4, replicate 2	In-house std 4, replicate 2
19/166, dilution 3, replicate 1	02/254, dilution 3, replicate 1	Serum2, replicate 1	Serum10, replicate 1	Plasma5, replicate 1	In-house std 5, replicate 1	19/166, dilution 3, replicate 2	02/254, dilution 3, replicate 2	Serum2, replicate 2	Serum10, replicate 2	Plasma5, replicate 2	In-house std 5, replicate 2
19/166, dilution 4, replicate 1	02/254, dilution 4, replicate 1	Serum3, replicate 1	Serum11, replicate 1	Kit std/ctrl 1, replicate 1	In-house std 6, replicate 1	19/166, dilution 4, replicate 2	02/254, dilution 4, replicate 2	Serum3, replicate 2	Serum11, replicate 2	Kit std/ctrl 1, replicate 2	In-house std 6, replicate 2
19/166, dilution 5, replicate 1	02/254, dilution 5, replicate 1	Serum4, replicate 1	Serum12, replicate 1	Kit std/ctrl 2, replicate 1	In-house std 7, replicate 1	19/166, dilution 5, replicate 2	02/254, dilution 5, replicate 2	Serum4, replicate 2	Serum12, replicate 2	Kit std/ctrl 2, replicate 2	In-house std 7, replicate 2
19/166, dilution 6, replicate 1	02/254, dilution 6, replicate 1	Serum5, replicate 1	Serum13, replicate 1	Kit std/ctrl 3, replicate 1	Sample diluent, replicate 1	19/166, dilution 6, replicate 2	02/254, dilution 6, replicate 2	Serum5, replicate 2	Serum13, replicate 2	Kit std/ctrl 3, replicate 2	Sample diluent, replicate 2
19/166, dilution 7, replicate 1	02/254, dilution 7, replicate 1	Serum6, replicate 1	Plasma1, replicate 1	In-house std 1, replicate 1		19/166, dilution 7, replicate 2	02/254, dilution 7, replicate 2	Serum6, replicate 2	Plasma1, replicate 2	In-house std 1, replicate 2	
19/166, dilution 8, replicate 1	02/254, dilution 8, replicate 1	Serum7, replicate 1	Plasma2, replicate 1	In-house std 2, replicate 1		19/166, dilution 8, replicate 2	02/254, dilution 8, replicate 2	Serum7, replicate 2	Plasma2, replicate 2	In-house std 2, replicate 2	
19/166, dilution 9, replicate 1	02/254, dilution 9, replicate 1	Serum8, replicate 1	Plasma3, replicate 1	In-house std 3, replicate 1		19/166, dilution 9, replicate 2	02/254, dilution 9, replicate 2	Serum8, replicate 2	Plasma3, replicate 2	In-house std 3, replicate 2	

5. DATA REPORTING

Estimates of the rhIGF-1 content of the current IS, 02/254, the candidate standard, 19/166, and the serum and plasma samples should be calculated in comparison with the assay kit standard/calibrator and reported in the Excel spreadsheet provided.
Participants are also requested to provide details of the assay method used, including dilution steps, in the reporting sheet provided, together with all raw data e.g. counts for each sample in electronic format if possible.

Appendix 3 – Draft IFU

2nd WHO Internatioal Standard for insunlin-like growth factor-I, recombinant, human 19/166
(version 1, dated XX/XX/XXXX)

1. INTENDED USE

The 2nd International Standard for insulin-like growth factor-I (IGF-I), recombinant, human, coded 19/166, is intended for use in the calibration of IGF-I immunoassays. It replaces the 1st IS, coded 02/254, was is discontinuted. [The 2nd IS was established by the Expert Committee on Biological Standardisation of the World Health Organisation in October 2020].

2. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS OR ANIMALS IN TH HUMAN FOOD CHAIN:

The preparation does not contain material of human origin.

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 sfety preocedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

Each ampoule of the International Standard contains **33.0 µg/ampoule** (with expanded uncertainty of 30.5-35.6 µg/ampoule, *k*=2.36) of IGF-I**.**

4. CONTENTS

Country of original of biological material: France

Each ampoule contains the residue after freze-drying of 0.5 mL of a solution that contained:

IGF-I	approximately 30 µg
Trehalose	10 mg
Sodium phosphate pH 7.0	20 µmoles

5. STORAGE

Unopened ampoules should be stored at -20ºC.
Please note: because if the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

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

hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar. Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. For all practical purposes each ampoule contains the same quantity of the substances listed above. Depending on the intended use, dissolve the total contents of the ampoule in a known volume of a suitable diluent. Users should make their own investigations into the type of diluent suitable for their use. If extensive dilutions are prepared, a carrier protein should be added. The ampoules do not contain bacteriostat and solutions of the material should not be assumed to be sterile.

8. PREPARATION OF AMPOULES AND COLLABORATIVE STUDY

A solution 10 mg/mL recombinant human IGF-I was diluted to approximately 60 µg/mL and formulated with 20 mg/mL trehalose and 40 mM sodium phosphate pH 7.0. The solution was dispensed as 0.5 g aliquots into glass ampoules, lyophilised and sealed. Ampoules were stored at -20ºC.

This batch of ampoules, coded 19/166, was evaluated in a two-phase collaborative study to 1) value assign the standard, in SI units, via a HPLC assay, and 2) to assess its immunoreactivity and suitability to serve as an International Standard by immunoassay in comparison with the 1st IS, 02/254, and a panel of human serum and plasma samples [1].

The results of the Phase 1 study gave an assigned content for 19/166 of 33.0 µg/ampoule (with expanded uncertainty 30.5-35.6 µg/amp). In Phase 2, both 19/166 and 02/254 were found to behave in a similar manner in the immunoassays used. The commutability of 19/166 with patient samples in the immunoassay methods used in the Phase 2 collaborative study was also assessed, using a difference in bias approach. In the 12 different laboratory methods performed, 19/166 was commutable with 9 methods. Of the 3 methods with which 19/166 was non commutable, it is notable that one of these methods was the same as that performed by a different laboratory in which is was found commutable.

It is important to note that the commutability criteria for the difference in bias approach have been derived statistically, rather than based on clinical relevance. It is not possible, within the confines of a collaborative study, to fully assess commutability of the candidate IS, 19/166, in all immunoassay methods. It is therefore recommended that manufacturers make their own assessment of the commutability of 19/166 with their assay method.

A thermally accelerated degradation study was also performed. Data from immunoassay analysis of accelerated thermal degradation samples of 19/166 found no significant loss in activity at

elevated temperatures, indicating that the candidate is sufficiently stable when stored at -20ºC to serve as an International Standard.

In conclusion, the candidate IS, 19/166, was deemed to represent a well characterised, mass assigned standard for human IGF-I, that was shown to behave in a very similar manner to the 1st IS, 02/254, in immunoassays of human IGF-I in terms of immunoreactivity and commutability, and is therefore suitable as a replacement for 02/254 for the continued calibration of these immunoassays.

9. CITATION

In any circumstance where the Recipient publishes a reference to NIBSC materials, it is important that the title of the preparation and any NIBSC code number, and the name and address of NIBSC are cited correctly.

10. LIABILITY AND LOSS

9.1 Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (http://www.nibsc.org/terms_and_conditions.aspx) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference.

9.2 Unless the context otherwise requires, the definitions in the Conditions shall apply.

9.3 Nothing in this document or the Conditions shall limit or exclude NIBSC's liability for fraud or fraudulent misrepresentation, death or personal injury caused by its negligence, or the negligence of its employees. Subject to clause 9.1:

9.3.1 NIBSC shall under no circumstances whatsoever be liable to the Recipient, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, for any loss of data, loss of profit, loss of business or goodwill, or any indirect or consequential loss or damage suffered or incurred by the Recipient arising in relation to the supply of the Materials or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or by any other person; and

9.3.2 NIBSC's total liability to the Recipient in respect of all other losses arising under or in connection with the Contract, whether in contract, tort (including negligence), breach of statutory duty, or otherwise, shall in no circumstances exceed 100% of the fees paid to NIBSC for the Materials.

9.4 The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the

use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

11. REFERENCES

[1] WHO ECBS report to be referenced

12. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	White powder		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	N/A		
Handling:	See caution, section 2		
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 a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			