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# EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
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# 4th WHO International Standard for thyroid-stimulating hormone (TSH), human, pituitary

Ben Cowper*[1], Katherine Partridge[1], Peter Rigsby[1], Alicia Lyle[2], Hubert Vesper[2], Katleen Van Uytfanghe[3] and Chris Burns[1]

[1]*Medicines and Healthcare Products Regulatory Agency (MHRA), Blanche Lane, South Mimms, Hertfordshire, EN6 3QG, UK*
[2]*Centres for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA*
[3]*Ghent University, Laboratory of Toxicology, Ref4u, Ottergemsesteenweg 460, 9000 Gent, Belgium*

*study co-ordinator (ben.cowper@mhra.org; +441707641246)

NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **2 October 2023** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

# Summary

A candidate replacement World Health Organisation (WHO) International Standard for Thyroid-Stimulating Hormone (TSH), coded 81/615, has been evaluated in an international collaborative study, organised in collaboration by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) National Institute for Biological Standards & Control (NIBSC), acting on behalf of WHO, and the International Federation of Clinical Chemistry & Laboratory Medicine (IFCC) Committee for Standardisation of Thyroid Function Tests (C-STFT), which is simultaneously working to replace its "panel" for harmonisation of TSH measurements, and the US Centres for Disease Control and Prevention (CDC).

This collaborative study, comprising 17 participating laboratories (regulatory laboratories and manufacturers of commercial TSH immunoassays), has assigned the TSH contents, in milli-International Units (mIU) per ampoule, of the candidate IS, 81/615, and has evaluated the overall suitability (including long-term stability and commutability) of the preparation to serve as an IS for calibration of TSH immunoassays.

15 laboratories analysed the candidate IS, 81/615, alongside the current 3rd IS, 81/565, in their chosen immunoassay (including 15 different methods in total). A total of 15 assays were performed in total, of which two were excluded from subsequent statistical analysis due to outlying results. The remaining data was used to estimate an overall geometric mean content of 11.7 mIU per ampoule for the candidate IS, 81/615, which is in good agreement with previous analyses of this preparation.

The IFCC C-STFT sample panel was also analysed alongside the WHO preparations, enabling an assessment of their commutability with patient samples. Using a "calibration effectiveness" approach, both the candidate IS, 81/615, and the current IS, 81/565, were found to be non-commutable in the majority of immunoassays. This confirms long held suspicions of non-commutability of the WHO International Standard for TSH. However, the impact of non-commutability is mitigated by the existence of the IFCC C-STFT panel for harmonisation of TSH measurements.

To predict the long-term stability of the candidate IS, 81/615, samples stored at elevated temperatures for 2.5 years were analysed by immunoassay by two participating laboratories, the results of which, allied with real-time data for historical TSH standards, indicate that the preparation will have good long-term stability when stored at -20°C.

Taken together, the results of the study indicate that the candidate IS, 81/615, is suitable to serve as a replacement for the 3rd IS, 81/565.

Therefore, it is proposed that the candidate preparation in ampoules coded 81/615 is established as the **4th International Standard for Thyroid Stimulating Hormone, Human, for Immunoassay** with an assigned content of **11.7 mIU per ampoule.**

# Introduction

Measurements of serum thyroid-stimulating hormone (TSH) by immunoassay are an important component in the diagnosis of thyroid disorders and the subsequent initiation and monitoring of therapy. TSH values in patient samples are typically measured by immunoassay and reported in milli-International Units per litre (mIU/L). A WHO reference preparation for pituitary TSH has been available since 1974 to standardise TSH immunoassays (currently the 3rd WHO International Standard (IS), coded 81/565 [1]), which defines the mIU of TSH activity. However, there are known concerns within the clinical chemistry community regarding the commutability of the WHO IS, 81/565, and its predecessors, in current TSH immunoassays, which are believed to have resulted in a lack of harmonisation of TSH measurements between methods/laboratories [2,3].

The Committee for the Standardisation of Thyroid Function Tests (C-STFT) was established by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to address this issue. The C-STFT have focused their efforts on a multi-assay method comparison study using a panel of approximately 100 serum samples covering a range of TSH concentrations [4]. This panel has co-existed with the WHO IS in recent years, with the latter continuing to define the mIU of TSH activity, and providing a consistent reference material with projected long-term stability and availability (10+ years), albeit with concerns over its commutability, which can be mitigated by use of the C-STFT panel to ensure harmonisation of TSH measurements between methods/laboratories.

As of 2020, stocks of both the 3rd WHO IS, 81/565, and the IFCC C-STFT panel were almost depleted, necessitating the replacement of both materials (81/565 was subsequently depleted in late 2022). The WHO, via the UK Medicines and Healthcare Products Regulatory Agency (MHRA) National Institute for Biological Standards & Control (NIBSC), already possessed a candidate replacement (4th) IS, coded 81/615. This was prepared in the early 1980's, using the same batch of pituitary extract used to the prepare the current 3rd IS, 81/565, and its predecessor, 80/558. It also has the same formulation as the current 3rd IS, 81/565, and has previously been shown to have near-identical TSH content in several multi-laboratory studies [1,5]. Considering the similarities of 81/615 to its predecessors it was anticipated that the concerns over commutability would persist following its establishment as the 4th IS, necessitating the continued availability of a IFCC C-STFT harmonisation panel.

Following discussions between colleagues at MHRA/NIBSC and IFCC C-STFT, it was agreed that the establishment of the candidate 4th IS, 81/615, and a new serum panel should be performed in tandem. Although the candidate IS, 81/615, had already been extensively evaluated (including value-assignment) in previous WHO/NIBSC studies [1,5], it was deemed necessary to re-confirm its suitability (including value-assignment, in mIU per ampoule, relative to the current IS, 81/565) to serve as a WHO IS for current TSH immunoassay methods. Current and candidate WHO materials were therefore distributed to numerous TSH immunoassay manufacturers for analysis, alongside the existing IFCC C-STFT panel, enabling, for the first time, assessment of the commutability of the current and candidate IS, 81/565 and 81/615, with patient samples across a large number of commercial immunoassays.

In addition, an accelerated thermal degradation (ATD) study was planned, involving a limited number of laboratories, to predict the long-term stability of the candidate IS, 81/615.

# Aims

It should be noted that although this project was carried out in collaboration with IFCC C-STFT, the aims stated below, and the contents of the following report, are restricted to matters concerning the establishment of the candidate IS, 81/615, as the 4$^{th}$ WHO IS for TSH. The IFCC C-STFT panel will be referred to in the context of commutability assessment of the current and candidate IS, 81/565 and 81/615, but details of the establishment of a new panel will be reported separately by IFCC C-STFT.

Therefore, the aims of this study are as follows:

- To demonstrate the suitability of the candidate IS, 81/615, to serve as an IS for TSH measurements.
- To value-assign the TSH contents of the candidate IS, 81/615, in IU/ampoule, in terms of the current IS, 81/565, by immunoassay.
- To assess the commutability of the candidate IS, 81/615, and the current IS, 81/565, with patient samples in clinical immunoassays.
- To predict the long-term stability of the candidate IS, 81/615, via accelerated thermal degradation (ATD) study.

# Materials & Methods

## Preparation and characterisation of 81/615

A preparation of Thyroid-Stimulating Hormone pituitary extract batch (AFP 2473B) was prepared in the laboratory of Dr A. F. Parlow (Harbor-UCLA Medical Center, USA) and donated to WHO through Dr S. Raiti of the National Pituitary Agency, USA. Approximately 2 µg pituitary extract was dissolved in 0.2% (w/v) human serum albumin and 1% (w/v) lactose, then filtered (0.45 µm) and distributed in 0.5 mL aliquots into ampoules. Ampoules were then lyophilised, sealed under nitrogen and stored in the dark at -20$^{o}$C.

A total of 3,204 ampoules are available for establishment as an International Standard. The product batch has a mean fill mass of 0.501 g (CV 0.35%, n=77), and a mean residual moisture content of 0.2% (n=3). Residual oxygen contents are not available, as this analysis was not routinely performed at the time of the production fill.

## Collaborative study design

## Participants

17 laboratories in 8 countries participated in the study, and are listed alphabetically, by country, in Table 1. Throughout the report, each participant is referred to by a code number, which was randomly assigned and does not reflect the order of listing in Table 1.

**Table 1:** list of participants in order of country

| | |
|---|---|
| CHINA | Zengming Jin<br>Autobio Diagnostics Co. Ltd, No. 199, 15th Ave, National Eco & Tech Development Area, Zhengzhou 450016, China |
| CHINA | Tao Yang<br>Maccura Biotechnology Co. Ltd, 8#, Anhe 2nd Road, Hi-Tech Industrial Development Zone, Chengdu 611730, China |
| CHINA | Xie Jian, Laiwen Quan<br>Shenzhen Mindray Bio-Medical Electronics Co. Ltd, Mindray Building, Keji 12th Road South, High Tech Industrial Park, Nanshan, Shenzhen 518057, China |
| FRANCE | Nathalie Ripoll<br>BioMerieux SA, 376 Chemin de l'Orme, Marcy l-Etoile 69280, France |
| GREECE / CHINA | Alexander Haliassos, Kostas Makris<br>Diamedica PC Laboratories, Thasou 38A, GR-166 72 Vari, Greece<br>Huijun Cui<br>Shenzhen New Industries Biomedical Engineering Co. Ltd, Room 501, Building B1, Qinglan 2nd Road, Pingshan District, Shenzhen 518000, China |
| GERMANY | Adriana Rykovska-Blume<br>Diasorin Deutschland GmbH, Von-Hevesy-Strasse 3, Dietzenback 63128, Germany |
| GERMANY | Michael Rottmann, Martin Weiss, Claudia Bruecklmayr<br>Roche Diagnostics GmbH, Building 751, Nonnenwald 2, Penzberg D-82377, Germany |
| IRELAND | Davina Flanagan, Helen Hamilton<br>Abbott Diagnostics, Lisnamuch, Longford, Ireland, N39 E932 |
| JAPAN | Hisao Tsukamoto<br>Tosoh Corporation, 2743-1, Hayakawa, Ayase-shi, Kanagawa 232-1123, Japan |
| JAPAN | Ling Yao<br>Sysmex Corporation, 1-3-2 Murotani, Nishi-ku, Kobe 651-2241, Japan |
| JAPAN | Yuka Imai<br>Fujirebio Inc., 51 Komiya-machi, Hachioki-shi, Tokyo 192-0031, Japan |
| JAPAN | Yusuke Nakaarai, Kenichiro Yamashita<br>FUJIFILM Wako Pure Chemical Corporation, 6-1, Takata-cho, Amagasaki-shi, Hyogo 661-0963, Japan |
| UNITED KINGDOM | Ben Cowper, Katherine Partridge<br>Medicines & Healthcare products Regulatory Agency |
| UNITED KINGDOM | Christopher Thomas<br>QuidelOrtho Corporation, Felindre Meadows, Pencoed, Bridgend, CF35 5PZ, UK |

| | |
|---|---|
| UNITED STATES | Carole Dauscher, David Kiaei<br>Siemens Healthcare Diagnostics, 511 Benedict Avenue, Tarrytown, NY 10591, USA |
| UNITED STATES | Julie Medina, Ryan Masica<br>Beckman Coulter, 312 Lake Hazeltine Drive, Chaska, MN 55318, USA |
| UNITED STATES | Alicia Lyle, Hubert Vesper<br>Centres for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA |

## Samples

The materials provided to participants are summarised in Table 2. The current and candidate IS were shipped from MHRA (UK) to Centres for Disease Control and Prevention (Atlanta, GA, USA) for onward shipment alongside the IFCC C-STFT panel. Instructions for Use were provided with the current and candidate IS.

**Table 2:** samples provided to study participants

| ID | Contents |
|---|---|
| Candidate 4th IS, 81/615 | Nominally 11.5 mIU TSH<br>1 mg human serum albumin<br>5 mg lactose |
| 3rd IS, 81/565 | 11.5 mIU TSH<br>1 mg human serum albumin<br>5 mg lactose |
| IFCC C-STFT panel | Approximately 100 human serum samples |

## Methods

All participants performed a single immunoassay, using their chosen method, including serial dilutions at six specified concentrations of the current IS, 81/565, the candidate IS, 81/615, alongside the IFCC C-STFT panel samples. Detailed guidelines for preparation of dilutions of the current IS, 81/565, the candidate IS, 81/615 were provided in the study protocol (Appendix 2).

## Stability assessment of the candidate IS, 81/615

An accelerated thermal degradation (ATD) study was carried out to predict the long-term stability of the candidate IS, 81/615. Ampoules were stored at elevated temperatures (+4, +20, +37 and +45°C) for 2.6 years. Samples were then analysed for TSH contents via immunoassay in comparison with a reference sample stored at -20°C. ATD sample analysis was performed in two laboratories, each using a different immunoassay method. A total of 30 ampoules of the candidate IS were used in total for the ATD study.

## Statistical analysis

### Value assignment of the candidate IS, 81/615

For all assays the potency of candidate IS, 81/615, relative to current IS, 81/565, was estimated by parallel-line analysis with a log transformation of the assay response. Calculations were performed using the EDQM software, CombiStats Version 6.1 [6]. Analysis was accepted if the correlation r-value exceeded 0.99 and the 90% confidence interval for the slope ratio was within the range 0.90-1.11. Relative potency estimates from all valid assays were combined to generate overall unweighted and semi-weighted geometric mean (GM) estimates for the candidate standard. Variability between assays, has been expressed using geometric coefficient 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 WHO preparations with patient samples was assessed by a "calibration effectiveness" approach [7] using data reported for the IFCC C-STFT sample panel. The analysis uses bias values calculated from reported estimates and from estimates recalculated relative to 81/565 or 81/615 using the fitted dose-response data for the WHO preparations. Bias was defined as the laboratory geometric mean sample estimate as a % of the study median value for the sample (used as sample target values for the purposes this analysis).

Samples from the C-STFT panel were excluded from the analysis if they gave a median result outside of the concentration range used for value-assignment of the candidate IS, 81/615 (<0.781 mIU/L or >50 mIU/L), or if the sample was analysed by fewer than half of the participants. These exclusions resulted in a collection of 50 samples used for commutability analysis.

### Assessment of stability

Immunoassay data from accelerated thermal degradation (ATD) study samples was used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [8], and thus predict the degradation rates when stored at -20ºC.

# Results

## Data returned & assay validity

All 17 laboratories returned data; 15 laboratories provided data used for value-assignment and commutability assessment, and two laboratories provided data used for stability assessment. All assays were valid.

Each of the 15 laboratories returning data for value-assignment and commutability assessment performed a different immunoassay method, giving rise to 15 different methods in total. These include TSH immunoassays designed for use with ARCHITECT i2000, AccuraSeed®, AutoLumo A2000 Plus, Access 2, LIAISON® XL, LUMIPULSE® G1200,

VIDAS®, i3000, CL-6000i, cobas® e801, VITROS® XT Integrated System, ADVIA Centaur® XP, MAGLUMI X3, HISCL-5000 and AIA-2000 analysers.

One laboratory (Lab P) encountered partial shattering of the candidate IS, 81/615, ampoule during opening. This caused potential loss of material, reflected by the reporting of significantly underestimated values for dilutions of the candidate IS compared to the current IS, 81/565 (Table A1.1). Another laboratory (Lab G) also reported significantly underestimated values for the candidate IS compared to the current IS, although the cause in this instance was not explained.

Data from both Lab G and P was excluded from the analysis due to the outlying lack of equivalence between results for the candidate and current IS. Both laboratories were provided with further ampoules of the candidate IS, 81/615, for repeat analysis, to provide assurances of the suitability of the candidate IS in these immunoassays, but this data was collected for information only and was not included in the analysis.

It should also be noted that Lab I performed an additional 5-fold dilution of the candidate IS, 81/615, and the current IS, 81/565 (Table A1.1). For example, a nominal concentration of 10 mIU/L was prepared instead of 50 mIU/L, 2.5 mIU/L instead of 12.5 mIU/L, etc.

## Value assignment of the candidate IS, 81/615

Parallel-line analysis (log transformation of assay response) of the candidate IS, 81/615, relative to the current IS, 81/565, was performed using nominal concentrations of 50, 12.5, 3.125 & 0.781 mIU (for Lab I the concentrations were 10, 2.5, 0.625 & 0.156 mIU due to the additional dilution performed). Lower concentrations (0.195 and 0.049 mIU/L) appeared to be outside linear range in some laboratories and were excluded from analysis. Results are summarised in Table 3 and Figure 1.

Linear and parallel dose-response relationships were observed in all the laboratories shown. There was no evidence of deviations from normality in log-transformed potency estimates ($p=0.586$ in Anderson-Darling test), so no alternative calculations of overall mean potency were performed. Both unweighted and semi-weighted geometric mean (GM) potencies of **11.7 mIU per ampoule** were calculated, with an inter-lab geometric coefficient of variance (GCV) of 4.5% (n=13). This value is in good agreement with previous evaluations of the candidate IS, 81/615, which yielded estimates of 11.5 mIU/ampoule [1] and 11.3 mIU/L [3].

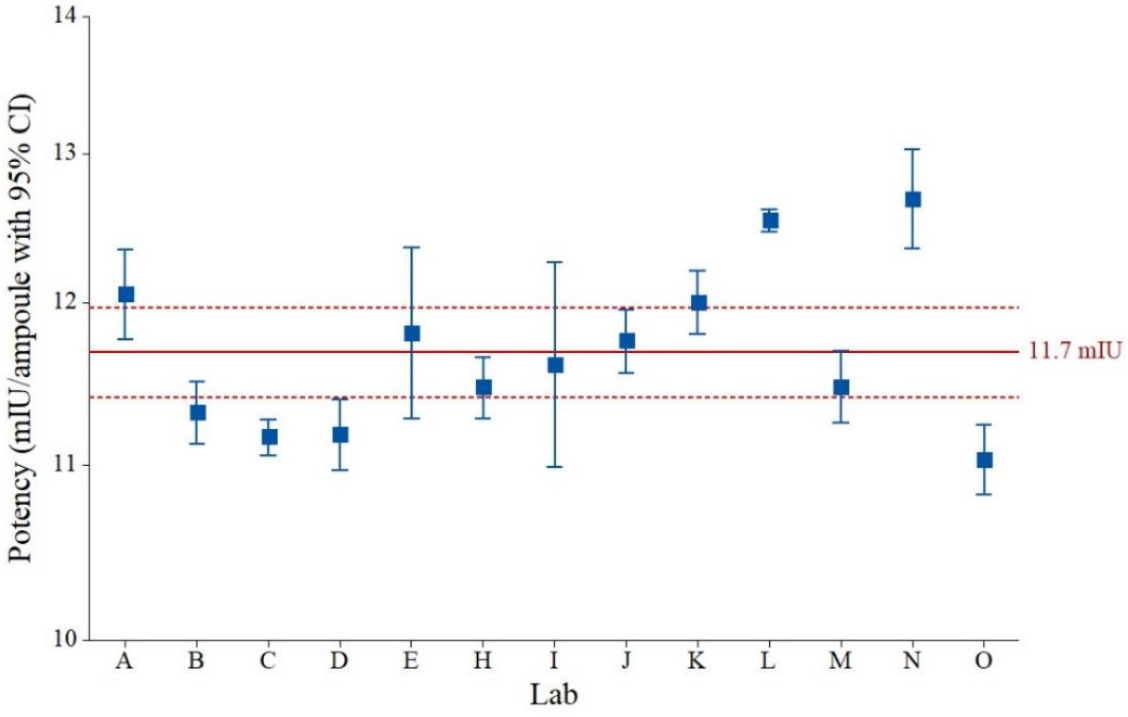
## Commutability assessment of the candidate IS, 81/615, and the current IS, 81/565

The commutability of the candidate IS, 81/615, and the current IS, 81/565, with 50 serum samples from the IFCC C-STFT panel was assessed using data returned by 13 out of 15 participants. Data from Lab G and P was excluded due to outlying results for the WHO preparations.

**Table 3: Value-assignment of the candidate IS, 81/615, relative to the current IS, 81/565**

| Lab | Correlation (r) | Slope ratio (90% CL) | Potency (mIU/ampoule) | | |
|---|---|---|---|---|---|
| | | | 95% LCL | Estimate | 95% UCL |
| A | 0.9999 | 0.9960 (0.9836 to 1.0086) | 11.7608 | 12.0468 | 12.3400 |
| B | 0.9999 | 1.0194 (1.0105 to 1.0284) | 11.1176 | 11.3060 | 11.4976 |
| C | 0.9999 | 0.9976 (0.9925 to 1.0027) | 11.0440 | 11.1534 | 11.2639 |
| D | 0.9999 | 1.0140 (1.0041 to 1.0241) | 10.9605 | 11.1704 | 11.3843 |
| E | 0.9997 | 0.9765 (0.9534 to 1.0001) | 11.2690 | 11.7990 | 12.3540 |
| H | 0.9999 | 1.0047 (0.9963 to 1.0134) | 11.2706 | 11.4565 | 11.6453 |
| I | 0.9997 | 1.0153 (0.9865 to 1.0449) | 10.9752 | 11.5988 | 12.2579 |
| J | 0.9999 | 1.0067 (0.9977 to 1.0157) | 11.5494 | 11.7489 | 11.9518 |
| K | 0.9999 | 1.0114 (1.0025 to 1.0203) | 11.7956 | 11.9966 | 12.2009 |
| L | 0.9999 | 1.0000 (0.9969 to 1.0031) | 12.4621 | 12.5364 | 12.6112 |
| M | 0.9999 | 1.0027 (0.9927 to 1.0128) | 11.2439 | 11.4619 | 11.6841 |
| N | 0.9999 | 1.0050 (0.9912 to 1.0191) | 12.3494 | 12.6838 | 13.0274 |
| O | 0.9984 | 0.9893 (0.9797 to 0.9989) | 10.8167 | 11.0202 | 11.2276 |
| **Unweighted GM** | | | **11.3757** | **11.6803** | **11.9931** |
| **Semi-weighted GM** | | | **11.3988** | **11.6778** | **11.9637** |
| **Inter-lab GCV (n)** | | | **4.5% (13)** | | |

**Figure 1: Value-assignment of the candidate IS, 81/615, relative to the current IS, 81/565**



Data used for the assessment of commutability are summarised in Appendix 1, Tables A1.2 & A1.3, which show geometric mean estimates of TSH contents relative to the candidate IS, 81/615, and the current IS, 81/565, respectively. Median values are included for each sample and have been used as the study consensus values for each sample in the analysis. Samples are identified 1-50 by increasing median TSH concentration for presentation purposes; these identifiers differ from those given to each sample in IFCC C-STFT panel.

Bias values were calculated as the laboratory geometric mean estimate as a % of the study median value for the sample. Bias values when reported estimates are expressed relative to the candidate IS, 81/615, and the current IS, 81/565, are shown in Tables 4-5 and graphically represented in Figures 2-3. A constant bias of 100% indicates optimal commutability of the standard with patient samples, whereas a bias value which deviates from 100%, or shows a significant slope with increasing sample concentration indicates poor, or non-, commutability (depending on the extent of the deviation) of the standard.

For some laboratories/methods, bias values close to 100% are consistently observed for estimates calculated relative to either the candidate IS 81/615 or the current IS 81/565, (e.g. Lab B, D, J and K), indicative of good commutability of each preparation with patient samples in those immunoassays. Yet overall, the results of the assessment indicate that the commutability of the WHO preparations with patient samples is generally poor, with median bias values for estimates calculated relative to the standards showing significant deviations from 100% in many instances. Across all laboratories, median bias values range from 78.4% (Lab M) to 124.4% (Lab E) for the candidate IS, 81/615, and 77.5% (Lab O) and 126.4% (Lab E), indicative of poor/non-commutability for numerous laboratories/methods. Furthermore, significant slopes were observed for two laboratories (Lab A and E), indicative of variable commutability across the range of TSH concentrations tested. For example, Lab E exhibits strong non-commutability (bias values of 140-150%) at low TSH concentrations (e.g. samples ID 1-10) but better commutability (bias values of approximately 100%) at higher TSH concentrations (sample ID 37-46). These slopes are most easily visualised in the graphical representations of commutability data in Figures 2 and 3.

These findings are consistent with common opinion within the clinical community that WHO preparations for TSH have historically been poorly commutable, leading to a lack of harmonisation of TSH measurements between commercial immunoassays [2,3]. It is this situation that led to the efforts of the IFCC C-STFT to develop a TSH sample panel to ensure better harmonisation of TSH measurements [4]. Bias values for serum samples as reported by laboratories (i.e. relative to kit standards/calibrators) are shown in Tables 6 and graphically represented in Figure 4. It is notable that bias values overall indicate better harmonisation between laboratories/immunoassays under these circumstances, with a narrower range of median bias values observed (ranging from 89.4% for Lab F, to 112.1% for Lab C), whilst the slopes previously observed for Lab A and Lab E are significantly reduced. This improvement in harmonisation has occurred recently, and is a direct result of the efforts of the IFCC C-STFT to improve harmonisation through provision of a serum sample panel.

**Table 4:** Bias in estimates for serum samples expressed relative to the candidate IS, 81/615 (lab GM estimate as % of study median value for sample). “x” indicates that a result was not reported or excluded as an outlier for the sample.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 78.1 | 100.0 | 120.6 | 95.3 | 144.2 | 94.8 | 109.1 | 118.8 | 98.3 | 113.1 | 85.4 | 112.3 | 84.5 |
| 2 | 77.9 | 100.0 | 107.6 | 93.0 | 142.4 | 90.4 | 108.9 | 105.1 | 94.7 | 112.6 | 80.9 | 102.3 | 79.0 |
| 3 | 82.3 | 102.0 | 113.6 | 96.8 | 147.2 | 91.0 | 104.3 | 87.5 | 100.0 | 106.9 | 87.5 | 100.9 | 86.3 |
| 4 | 85.6 | 100.0 | 110.6 | 92.4 | 142.5 | 95.4 | 104.6 | 101.9 | 97.4 | 111.0 | 78.4 | 102.1 | 84.3 |
| 5 | 78.1 | 100.0 | 111.0 | 94.7 | 135.0 | 93.5 | 109.5 | 100.7 | 101.6 | 104.5 | 76.1 | 93.1 | 86.6 |
| 6 | 67.4 | 100.0 | 114.0 | 96.2 | 149.5 | 96.1 | 101.1 | 112.4 | 95.3 | 108.1 | 77.2 | 103.9 | 80.4 |
| 7 | 82.2 | 100.0 | 115.1 | 97.4 | 132.5 | 95.2 | 106.7 | 101.3 | 98.1 | 116.6 | 71.3 | 103.4 | 87.7 |
| 8 | 79.4 | 98.7 | 122.6 | 100.6 | 145.6 | 93.2 | 113.4 | x | 99.4 | 116.4 | 78.5 | 119.3 | 88.0 |
| 9 | 82.6 | 101.0 | 100.0 | 97.1 | 138.0 | 92.3 | 109.5 | 108.2 | 96.8 | 111.2 | 63.5 | 106.0 | 87.5 |
| 10 | 82.2 | 100.9 | 113.6 | 96.6 | 133.5 | 97.0 | 117.3 | 96.0 | 99.1 | 124.5 | x | 106.9 | 85.7 |
| 11 | 83.4 | 103.0 | 114.3 | 95.5 | 135.7 | 94.2 | 111.3 | x | 99.7 | 100.3 | 72.3 | 101.7 | 87.1 |
| 12 | 78.9 | 100.0 | 122.3 | 96.6 | 131.6 | 93.4 | 109.0 | 111.3 | 99.4 | 111.0 | 72.2 | 101.3 | 88.7 |
| 13 | 78.8 | 98.7 | 125.8 | 100.0 | 131.3 | 93.1 | 118.4 | 89.7 | 100.6 | 110.6 | 86.1 | 104.5 | 84.3 |
| 14 | 74.7 | 86.9 | 107.9 | 136.1 | 120.2 | 133.3 | 100.0 | 91.2 | 90.3 | 101.1 | 64.1 | 137.8 | 76.2 |
| 15 | 89.2 | 97.9 | 118.9 | 98.3 | 131.4 | 96.3 | 109.3 | 100.0 | 100.4 | 114.5 | 81.4 | 110.0 | 90.1 |
| 16 | 84.8 | 98.9 | 120.8 | 100.0 | 130.6 | 95.2 | 108.2 | 108.1 | 99.6 | 117.3 | 76.3 | 109.7 | 81.9 |
| 17 | 87.7 | 100.0 | 118.2 | 98.1 | 133.3 | 96.1 | 107.7 | 108.1 | 97.2 | 117.4 | 76.6 | 115.7 | 87.8 |
| 18 | 83.4 | 100.0 | 121.9 | 94.4 | 129.2 | 98.3 | 110.4 | 101.9 | 99.2 | 114.4 | 78.4 | 108.0 | 88.5 |
| 19 | 90.7 | 102.4 | 111.0 | 96.5 | 130.8 | 91.2 | 105.9 | 101.3 | 98.7 | 104.8 | 78.7 | x | 92.3 |
| 20 | x | 100.4 | 108.3 | 98.3 | 128.9 | 99.2 | 110.8 | 99.6 | 96.6 | 114.6 | 73.6 | 107.9 | 87.7 |
| 21 | 85.4 | 100.0 | 121.0 | 95.9 | 123.5 | 97.2 | 118.4 | 106.4 | 97.9 | 115.7 | 77.6 | 104.9 | 88.0 |
| 22 | 90.0 | 96.7 | 112.3 | 94.4 | 126.0 | 95.8 | 110.1 | 104.3 | 100.0 | 117.2 | 78.0 | 109.1 | 85.7 |
| 23 | 81.1 | 99.5 | 118.4 | 100.0 | 126.6 | 95.1 | 114.5 | 90.1 | 100.9 | 106.6 | 76.9 | 100.1 | 90.4 |
| 24 | 93.8 | 93.8 | 125.3 | 103.5 | 132.5 | 95.5 | 110.6 | x | 98.5 | 101.5 | 83.2 | 102.3 | 98.0 |
| 25 | 84.7 | 102.4 | 120.6 | 96.0 | 120.5 | 93.4 | 112.5 | 100.0 | 96.2 | 111.9 | 74.5 | 104.0 | 87.1 |
| 26 | 89.1 | 100.0 | 111.1 | 94.0 | 116.0 | 88.3 | 106.4 | 100.3 | 93.4 | 100.5 | 67.6 | 104.2 | 85.6 |
| 27 | 78.8 | 99.5 | 121.8 | 101.8 | 114.0 | 94.4 | 117.7 | 98.0 | 100.9 | 100.0 | 78.3 | 106.6 | 91.0 |
| 28 | 75.2 | 94.8 | 124.3 | 95.8 | 125.9 | 94.8 | 111.7 | 106.8 | 100.0 | 105.5 | 77.9 | 102.1 | 90.2 |
| 29 | 77.2 | 82.5 | 119.8 | 96.6 | 125.3 | 100.0 | 110.8 | 106.4 | 84.1 | 119.3 | 84.1 | 121.4 | 92.1 |
| 30 | 90.0 | 100.0 | 111.9 | 95.8 | 117.5 | 90.7 | 106.4 | 104.6 | 99.6 | 109.1 | 63.0 | 109.2 | 91.6 |
| 31 | 85.6 | 90.0 | 113.2 | x | 116.1 | 96.5 | 103.5 | 105.9 | 96.4 | 110.3 | 76.8 | 107.9 | 90.2 |
| 32 | 109.8 | 98.0 | 72.5 | x | 131.7 | 88.4 | 103.2 | 100.4 | 91.1 | 123.0 | 80.0 | 99.6 | 100.9 |
| 33 | 90.6 | 101.1 | 117.6 | 100.0 | 120.2 | 97.8 | 111.6 | 97.0 | 97.8 | 107.8 | 78.6 | 107.4 | 96.3 |
| 34 | 91.5 | 89.7 | 114.9 | 97.2 | 101.6 | 96.9 | 112.3 | 100.0 | 102.0 | 104.1 | 67.9 | 102.5 | 89.1 |
| 35 | 101.3 | 100.0 | 120.9 | 99.3 | 109.9 | 93.0 | 111.4 | 95.6 | 95.4 | 108.4 | 81.0 | 104.7 | 100.0 |
| 36 | 99.1 | 100.9 | 107.1 | 97.3 | 108.6 | 97.3 | 109.5 | 95.4 | 98.9 | 109.4 | 76.2 | 102.3 | x |
| 37 | 99.2 | 97.3 | 102.1 | 100.8 | 104.9 | 93.6 | 107.7 | 98.1 | 94.1 | 106.3 | x | 102.7 | 88.9 |
| 38 | x | 104.1 | x | 96.1 | 113.7 | 89.7 | x | x | 99.4 | 106.0 | x | x | 100.0 |
| 39 | 100.2 | 100.0 | 100.8 | 101.1 | 102.4 | 93.7 | 106.6 | 96.9 | 94.0 | 111.1 | 81.7 | 96.5 | 95.8 |
| 40 | 100.0 | 101.2 | 95.0 | 101.0 | 92.0 | 96.2 | 111.3 | 86.4 | 97.0 | 108.0 | 83.7 | 100.7 | 100.9 |
| 41 | 103.3 | 93.7 | 121.4 | 99.7 | 100.0 | 86.7 | 101.9 | 101.3 | 88.9 | 103.9 | 84.0 | 104.9 | 86.3 |
| 42 | 114.1 | 92.1 | 122.2 | 96.8 | 100.0 | 94.6 | 107.2 | 105.2 | 96.4 | 106.8 | 84.6 | 111.2 | 95.7 |
| 43 | 107.1 | 100.0 | 127.8 | 92.2 | 97.7 | 90.4 | 104.2 | 105.9 | 91.6 | 116.8 | 82.6 | 108.7 | 98.9 |
| 44 | 107.9 | 96.4 | 116.1 | 100.5 | 91.0 | 87.9 | 105.6 | 99.5 | 87.9 | 103.4 | x | 101.3 | 69.2 |
| 45 | 116.4 | 98.7 | x | 103.9 | 101.3 | 95.0 | 107.8 | 98.3 | 96.2 | 118.3 | 83.9 | 114.2 | 96.6 |
| 46 | 109.9 | 97.5 | 126.1 | 95.7 | 90.4 | 95.4 | 100.0 | 104.4 | 93.9 | 103.5 | 86.4 | 111.0 | 102.1 |
| 47 | 123.6 | 100.6 | 118.9 | x | 86.6 | 89.5 | 103.2 | 99.4 | 91.9 | 108.8 | 83.6 | 101.1 | 90.7 |
| 48 | 135.2 | 104.8 | 128.9 | 99.9 | 89.5 | 97.2 | 112.4 | 92.8 | 100.0 | 123.2 | 82.7 | 109.5 | 99.3 |
| 49 | 131.1 | x | 100.0 | 117.5 | 92.5 | 106.4 | x | x | 98.7 | 120.2 | 64.6 | 92.7 | x |
| 50 | 126.1 | 100.0 | 116.6 | 102.3 | 86.2 | 94.7 | 98.2 | 102.0 | 94.4 | 104.3 | 84.7 | 103.9 | 77.3 |
| | | | | | | | | | | | | | |
| Median | 88.4 | 100.0 | 116.3 | 97.2 | 124.4 | 94.8 | 109.0 | 100.7 | 97.9 | 109.8 | 78.4 | 104.6 | 88.6 |
| GM | 91.4 | 98.4 | 114.2 | 98.7 | 118.2 | 94.9 | 108.5 | 100.8 | 96.7 | 110.3 | 77.6 | 105.9 | 89.2 |
| 95% LCL | 87.2 | 97.1 | 111.1 | 96.9 | 113.0 | 93.3 | 107.1 | 98.8 | 95.6 | 108.5 | 75.7 | 103.9 | 87.1 |
| 95% UCL | 95.8 | 99.7 | 117.5 | 100.5 | 123.5 | 96.6 | 109.9 | 102.8 | 97.8 | 112.1 | 79.6 | 107.9 | 91.2 |
| Slope | 0.127 | -0.001 | 0.003 | 0.014 | -0.132 | -0.003 | -0.013 | -0.011 | -0.012 | -0.001 | 0.016 | -0.007 | 0.022 |

**Table 5:** Bias in estimates for serum samples expressed relative to the current IS, 81/565 (lab GM estimate as % of study median value for sample). “x” indicates that a result was not reported or excluded as an outlier for the sample.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 81.7 | 92.0 | 115.2 | 90.5 | 152.0 | 93.2 | 107.8 | 130.8 | 100.0 | 121.8 | 85.3 | 121.3 | 74.8 |
| 2 | 84.6 | 95.6 | 106.8 | 91.7 | 155.8 | 92.3 | 111.9 | 120.6 | 100.0 | 125.9 | 83.9 | 114.7 | 72.6 |
| 3 | 88.3 | 96.4 | 111.3 | 94.3 | 159.0 | 91.8 | 105.8 | 100.0 | 104.4 | 118.1 | 89.7 | 111.7 | 78.2 |
| 4 | 90.1 | 93.3 | 106.4 | 88.5 | 150.3 | 94.6 | 104.4 | 112.5 | 100.0 | 120.5 | 78.8 | 111.2 | 75.0 |
| 5 | 81.1 | 92.2 | 105.4 | 89.5 | 140.4 | 91.4 | 107.9 | 109.4 | 103.0 | 111.9 | 75.5 | 100.0 | 75.9 |
| 6 | 72.5 | 95.5 | 112.1 | 94.3 | 160.6 | 97.3 | 103.0 | 125.8 | 100.0 | 119.8 | 79.3 | 115.6 | 72.9 |
| 7 | 85.8 | 92.9 | 109.9 | 92.7 | 138.4 | 93.7 | 105.8 | 110.4 | 100.0 | 125.6 | 71.1 | 111.9 | 77.2 |
| 8 | 84.1 | 93.1 | 118.7 | 97.2 | 153.9 | 93.0 | 114.0 | x | 102.8 | 127.2 | 79.4 | 131.0 | 78.6 |
| 9 | 90.2 | 98.5 | 100.0 | 96.9 | 150.5 | 95.1 | 113.7 | 122.7 | 103.4 | 125.4 | 66.3 | 120.1 | 80.6 |
| 10 | 83.6 | 91.8 | 105.7 | 89.7 | 135.4 | 93.0 | 113.5 | 101.4 | 98.6 | 130.7 | x | 112.8 | 73.4 |
| 11 | 87.9 | 97.1 | 110.2 | 91.9 | 142.4 | 93.6 | 111.5 | x | 102.9 | 109.0 | 72.8 | 111.2 | 77.4 |
| 12 | 81.1 | 92.1 | 115.1 | 90.8 | 134.6 | 90.5 | 106.6 | 117.9 | 100.0 | 117.7 | 70.9 | 108.1 | 76.8 |
| 13 | 84.6 | 94.9 | 123.6 | 98.1 | 140.2 | 94.2 | 121.0 | 100.0 | 105.8 | 122.5 | 88.2 | 116.4 | 76.3 |
| 14 | 78.5 | 81.8 | 103.8 | 131.2 | 125.4 | 132.1 | 100.0 | 98.9 | 92.9 | 109.6 | 64.3 | 150.5 | 67.5 |
| 15 | 90.6 | 89.4 | 110.7 | 91.4 | 132.7 | 92.3 | 105.9 | 104.8 | 100.0 | 120.2 | 79.0 | 116.2 | 77.1 |
| 16 | 86.9 | 91.0 | 113.3 | 93.7 | 132.8 | 91.9 | 105.5 | 113.7 | 100.0 | 124.1 | 74.6 | 116.7 | 70.7 |
| 17 | 92.0 | 94.4 | 113.7 | 94.3 | 138.9 | 95.2 | 107.7 | 116.5 | 100.0 | 127.2 | 76.7 | 126.3 | 77.6 |
| 18 | 85.7 | 92.6 | 114.8 | 88.8 | 131.8 | 95.4 | 108.2 | 107.6 | 100.0 | 121.5 | 76.9 | 115.4 | 76.6 |
| 19 | 95.8 | 97.6 | 107.5 | 93.4 | 137.2 | 91.0 | 106.7 | 110.0 | 102.4 | 114.4 | 79.4 | x | 82.1 |
| 20 | x | 93.3 | 102.3 | 92.9 | 131.5 | 96.5 | 108.9 | 105.2 | 97.7 | 122.0 | 72.4 | 115.7 | 76.0 |
| 21 | 88.8 | 94.0 | 115.3 | 91.5 | 127.3 | 95.4 | 117.5 | 113.0 | 100.0 | 124.4 | 77.0 | 113.6 | 77.0 |
| 22 | 91.6 | 88.9 | 104.8 | 88.1 | 127.0 | 92.1 | 106.9 | 108.5 | 100.0 | 123.3 | 75.8 | 115.6 | 73.4 |
| 23 | 87.9 | 97.6 | 117.6 | 99.4 | 135.5 | 97.4 | 118.5 | 100.0 | 107.4 | 119.4 | 79.5 | 112.9 | 82.3 |
| 24 | 97.8 | 88.5 | 119.8 | 99.1 | 136.3 | 94.1 | 110.1 | x | 100.9 | 109.4 | 82.7 | 111.1 | 85.8 |
| 25 | 89.5 | 98.2 | 116.9 | 93.2 | 125.9 | 93.3 | 113.7 | 107.6 | 100.0 | 122.3 | 75.1 | 114.6 | 77.4 |
| 26 | 97.0 | 98.8 | 110.9 | 94.0 | 124.8 | 90.8 | 110.7 | 111.0 | 100.0 | 113.1 | 70.3 | 118.2 | 78.3 |
| 27 | 79.4 | 91.0 | 112.5 | 94.2 | 113.5 | 89.8 | 113.4 | 100.4 | 100.0 | 104.1 | 75.2 | 111.9 | 77.0 |
| 28 | 76.4 | 87.6 | 115.8 | 89.4 | 125.9 | 91.0 | 108.5 | 109.6 | 100.0 | 110.8 | 75.5 | 108.2 | 76.9 |
| 29 | 81.7 | 79.1 | 116.3 | 93.9 | 130.5 | 100.0 | 112.2 | 113.7 | 87.5 | 130.5 | 84.8 | 134.0 | 81.8 |
| 30 | 91.7 | 92.9 | 104.6 | 89.8 | 118.1 | 87.4 | 103.8 | 107.7 | 100.0 | 115.1 | 61.4 | 116.2 | 78.4 |
| 31 | 88.2 | 84.5 | 107.0 | x | 117.7 | 94.0 | 102.1 | 109.9 | 97.9 | 117.6 | 75.5 | 116.1 | 78.0 |
| 32 | 116.2 | 94.9 | 70.5 | x | 136.6 | 88.6 | 104.8 | 107.1 | 95.2 | 134.9 | 80.8 | 110.2 | 89.6 |
| 33 | 93.9 | 95.9 | 111.9 | 95.5 | 122.1 | 95.9 | 110.8 | 101.1 | 100.0 | 115.6 | 77.7 | 116.3 | 83.6 |
| 34 | 91.9 | 82.8 | 106.0 | 90.1 | 100.0 | 92.2 | 108.4 | 100.2 | 101.4 | 108.2 | 65.1 | 107.8 | 74.9 |
| 35 | 106.6 | 97.3 | 117.0 | 96.7 | 112.8 | 92.7 | 112.8 | 100.0 | 99.5 | 118.2 | 81.3 | 115.5 | 88.0 |
| 36 | 100.7 | 95.4 | 100.2 | 91.7 | 107.3 | 93.9 | 107.3 | 95.8 | 99.8 | 115.3 | 73.8 | 109.3 | x |
| 37 | 104.0 | 94.9 | 98.6 | 98.0 | 107.0 | 93.1 | 108.8 | 101.4 | 97.9 | 115.6 | x | 113.1 | 78.0 |
| 38 | x | 100.0 | x | 91.9 | 114.0 | 87.8 | x | x | 101.9 | 113.4 | x | x | 86.3 |
| 39 | 106.8 | 99.5 | 98.9 | 100.0 | 106.0 | 94.8 | 109.6 | 101.5 | 99.5 | 122.8 | 83.0 | 108.1 | 85.3 |
| 40 | 106.4 | 101.3 | 93.1 | 100.0 | 94.7 | 97.2 | 114.6 | 89.9 | 102.9 | 119.3 | 84.8 | 112.8 | 89.5 |
| 41 | 107.1 | 91.6 | 116.1 | 96.4 | 100.0 | 85.4 | 102.3 | 101.7 | 92.0 | 112.0 | 83.0 | 114.8 | 74.7 |
| 42 | 118.1 | 90.8 | 116.8 | 93.8 | 98.9 | 93.3 | 107.9 | 103.6 | 100.0 | 115.0 | 83.4 | 122.0 | 82.5 |
| 43 | 112.0 | 100.0 | 123.4 | 90.2 | 97.5 | 90.0 | 106.0 | 105.1 | 96.0 | 127.1 | 82.2 | 120.5 | 86.0 |
| 44 | 114.4 | 97.9 | 113.7 | 99.9 | 92.1 | 88.8 | 109.1 | 100.1 | 93.5 | 114.1 | x | 113.9 | 61.1 |
| 45 | 120.6 | 97.9 | x | 100.9 | 100.0 | 93.8 | 108.8 | 96.8 | 100.0 | 127.7 | 82.8 | 125.6 | 83.2 |
| 46 | 116.3 | 99.5 | 123.5 | 95.2 | 90.8 | 96.4 | 103.3 | 103.8 | 100.0 | 114.2 | 87.0 | 124.9 | 89.7 |
| 47 | 129.2 | 102.9 | 115.1 | x | 85.2 | 89.4 | 105.7 | 96.0 | 97.1 | 118.7 | 83.1 | 112.8 | 78.6 |
| 48 | 133.7 | 101.3 | 118.1 | 93.3 | 83.3 | 91.9 | 109.0 | 85.2 | 100.0 | 127.1 | 77.8 | 115.6 | 81.3 |
| 49 | 131.2 | x | 92.8 | 111.3 | 86.7 | 101.8 | x | x | 100.0 | 125.6 | 61.5 | 99.0 | x |
| 50 | 130.7 | 101.9 | 112.1 | 100.4 | 83.8 | 94.0 | 100.0 | 97.0 | 99.2 | 112.9 | 83.5 | 115.2 | 66.5 |
| | | | | | | | | | | | | | |
| Median | 91.1 | 94.9 | 112.0 | 93.8 | 126.4 | 93.3 | 108.3 | 105.1 | 100.0 | 119.3 | 78.3 | 115.0 | 77.5 |
| GM | 95.4 | 94.0 | 109.5 | 94.9 | 121.0 | 93.7 | 108.6 | 105.8 | 99.6 | 119.1 | 77.3 | 115.5 | 78.2 |
| 95% LCL | 91.1 | 92.5 | 106.5 | 93.1 | 114.8 | 92.1 | 107.3 | 103.1 | 98.7 | 117.2 | 75.2 | 113.2 | 76.4 |
| 95% UCL | 99.8 | 95.5 | 112.5 | 96.7 | 127.4 | 95.3 | 109.9 | 108.5 | 100.6 | 121.0 | 79.4 | 117.8 | 79.9 |
| Slope | 0.121 | 0.021 | 0.000 | 0.017 | -0.155 | -0.005 | -0.009 | -0.050 | -0.008 | -0.003 | 0.007 | -0.004 | 0.012 |

**Figure 2:** Bias in estimates for serum samples relative to the candidate IS, 81/615 (lab GM estimate as % of study median value for sample). Each box displays the results in Table 4 from a single laboratory method (Lab A, B, etc), with % values along the y-axis, and sample ID 1-50 plotted from left to right along the x-axis.

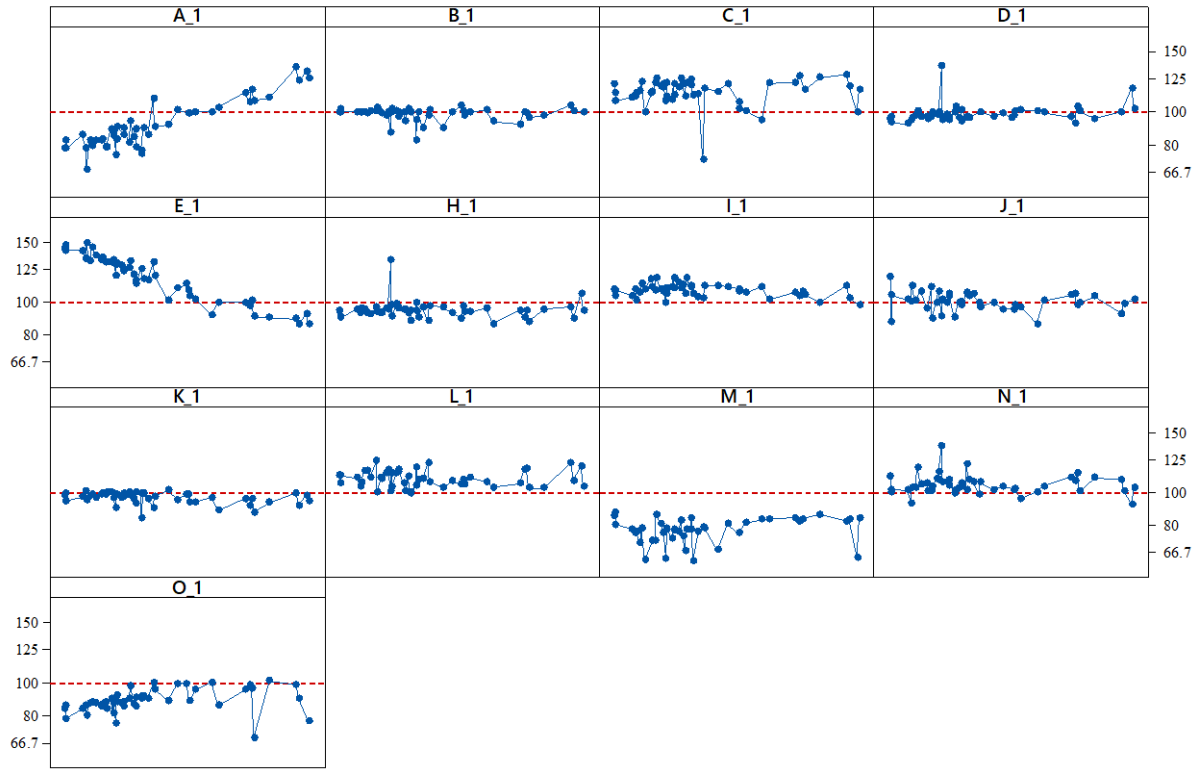


**Figure 3:** Bias in estimates for serum samples relative to the current IS, 81/565 (lab GM estimate as % of study median value for sample). Each box displays the results in Table 5 from a single laboratory method (Lab A, B, etc), with % values along the y-axis, and sample ID 1-50 plotted from left to right along the x-axis.

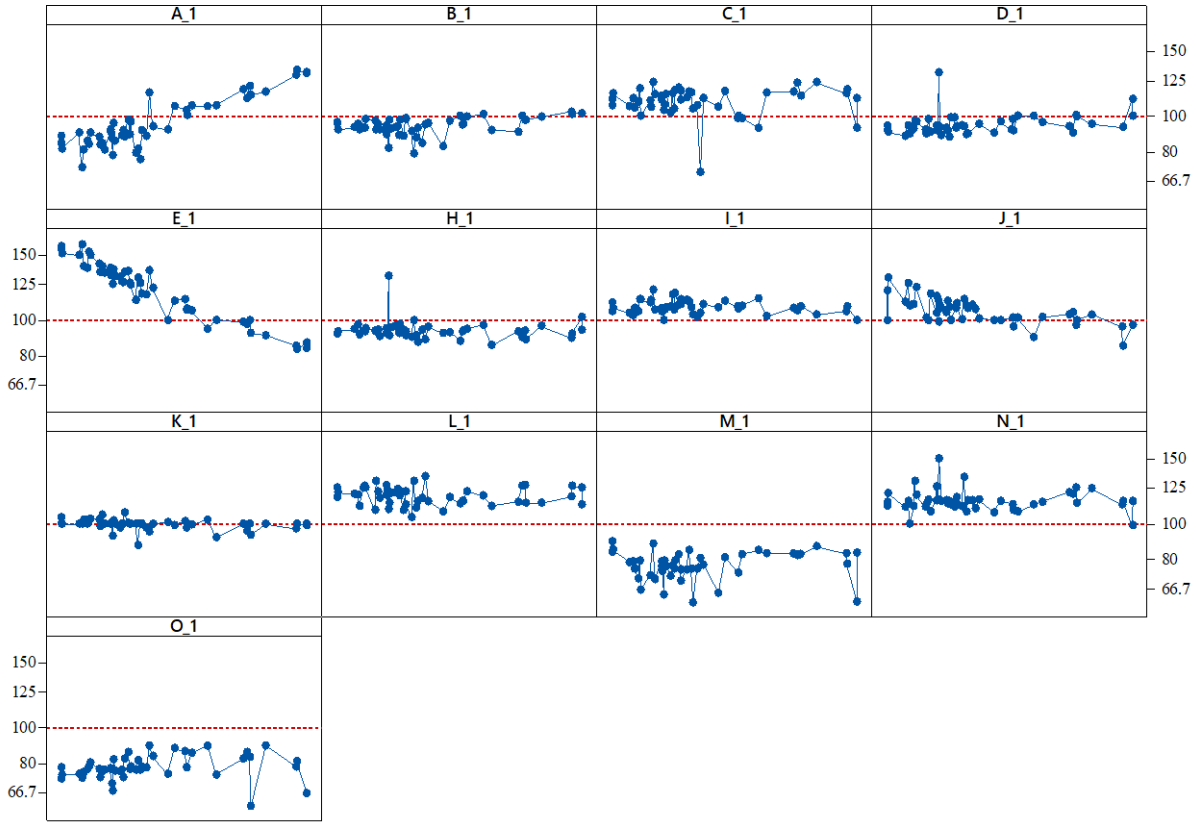
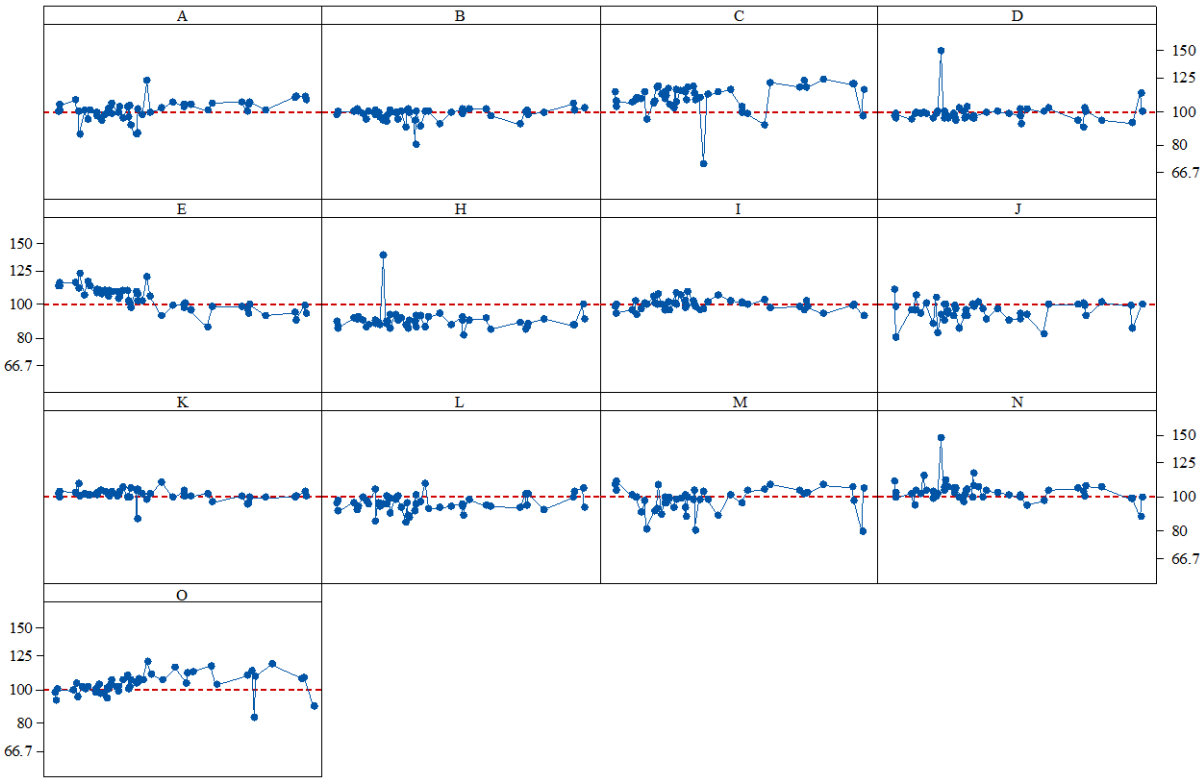


**Table 6:** Bias in estimates for serum samples reported relative to assay calibrators/standards. Samples are identified 1-50 by increasing median TSH concentration. “x” indicates that a result was not reported.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 100.0 | 98.1 | 113.6 | 97.0 | 112.5 | 89.3 | 98.8 | 110.0 | 102.4 | 96.6 | 108.9 | 111.5 | 98.8 |
| 2 | 100.9 | 99.5 | 102.7 | 95.9 | 112.7 | 86.3 | 100.1 | 98.5 | 100.0 | 97.6 | 104.7 | 103.0 | 93.7 |
| 3 | 104.5 | 99.9 | 106.8 | 98.2 | 115.1 | 85.6 | 94.3 | 80.6 | 104.0 | 91.1 | 111.5 | 100.0 | 100.6 |
| 4 | 107.5 | 99.8 | 106.0 | 95.1 | 115.8 | 91.3 | 96.4 | 95.9 | 103.2 | 96.6 | 101.4 | 102.6 | 100.0 |
| 5 | 99.8 | 101.4 | 108.1 | 98.9 | 111.5 | 90.9 | 102.6 | 96.3 | 109.3 | 92.3 | 100.0 | 95.0 | 104.3 |
| 6 | 86.0 | 100.0 | 109.5 | 99.1 | 123.0 | 92.1 | 93.3 | 106.2 | 101.1 | 94.2 | 100.0 | 104.5 | 95.4 |
| 7 | 100.6 | 98.3 | 108.7 | 98.4 | 106.5 | 89.7 | 96.8 | 93.9 | 102.2 | 100.0 | 90.6 | 102.1 | 102.2 |
| 8 | 95.2 | 95.1 | 113.5 | 99.5 | 116.0 | 85.9 | 100.9 | x | 101.5 | 97.8 | 97.7 | 115.2 | 100.5 |
| 9 | 100.7 | 99.9 | 94.8 | 98.5 | 112.8 | 87.3 | 100.0 | 101.0 | 101.4 | 95.8 | 81.1 | 105.0 | 102.5 |
| 10 | 97.3 | 97.8 | 105.7 | 95.9 | 107.5 | 90.0 | 105.1 | 87.7 | 101.7 | 105.4 | x | 103.6 | 98.3 |
| 11 | 99.4 | 100.8 | 107.4 | 95.6 | 110.5 | 88.1 | 100.5 | x | 103.3 | 85.4 | 91.2 | 99.5 | 100.8 |
| 12 | 95.5 | 99.4 | 116.8 | 98.2 | 109.2 | 88.7 | 100.0 | 104.6 | 104.6 | 96.2 | 92.5 | 100.6 | 104.2 |
| 13 | 93.8 | 96.6 | 118.4 | 100.0 | 107.3 | 87.1 | 107.1 | 82.8 | 104.3 | 94.4 | 108.5 | 102.1 | 97.5 |
| 14 | 97.4 | 94.0 | 112.3 | 150.0 | 109.3 | 138.2 | 100.0 | 93.3 | 103.6 | 95.5 | 89.4 | 148.4 | 97.5 |
| 15 | 101.6 | 93.5 | 109.1 | 95.7 | 105.5 | 87.8 | 96.4 | 90.2 | 101.5 | 95.4 | 100.0 | 104.7 | 101.6 |
| 16 | 99.5 | 96.9 | 113.8 | 100.0 | 107.9 | 89.1 | 98.0 | 100.2 | 103.4 | 100.4 | 96.2 | 107.1 | 94.9 |
| 17 | 102.0 | 97.6 | 110.9 | 97.7 | 109.9 | 89.6 | 97.0 | 99.8 | 100.4 | 100.0 | 96.1 | 112.4 | 101.1 |
| 18 | 98.5 | 99.3 | 116.4 | 95.5 | 108.6 | 93.2 | 101.3 | 95.6 | 104.2 | 99.2 | 100.0 | 106.7 | 103.7 |
| 19 | 105.2 | 100.7 | 104.8 | 96.6 | 108.9 | 85.5 | 96.1 | 94.0 | 102.7 | 89.7 | 99.3 | x | 107.0 |
| 20 | x | 99.1 | 102.7 | 98.7 | 108.4 | 93.4 | 101.0 | 92.9 | 100.9 | 98.8 | 93.3 | 105.9 | 102.1 |
| 21 | 99.2 | 99.0 | 115.1 | 96.5 | 104.0 | 91.7 | 108.3 | 99.6 | 102.5 | 100.0 | 98.5 | 103.1 | 102.6 |
| 22 | 103.4 | 95.1 | 106.1 | 94.4 | 105.7 | 89.9 | 100.0 | 97.0 | 104.1 | 100.6 | 98.4 | 106.5 | 99.3 |
| 23 | 95.2 | 100.0 | 114.4 | 101.9 | 109.3 | 91.1 | 106.3 | 85.5 | 107.2 | 93.5 | 99.0 | 99.7 | 106.9 |
| 24 | 103.3 | 89.7 | 115.3 | 100.4 | 109.6 | 87.1 | 97.7 | x | 99.6 | 84.6 | 101.8 | 97.0 | 110.3 |
| 25 | 96.3 | 100.8 | 114.0 | 95.6 | 101.9 | 87.5 | 102.2 | 93.0 | 100.0 | 96.1 | 93.7 | 101.3 | 100.8 |
| 26 | 103.6 | 101.4 | 108.1 | 96.4 | 101.0 | 85.1 | 99.6 | 96.2 | 100.0 | 88.8 | 87.6 | 104.4 | 102.0 |
| 27 | 91.2 | 99.5 | 117.1 | 102.9 | 97.8 | 89.8 | 108.8 | 92.6 | 106.6 | 87.1 | 100.0 | 105.4 | 106.9 |
| 28 | 85.9 | 94.0 | 118.5 | 95.9 | 108.9 | 89.4 | 102.2 | 100.2 | 104.7 | 91.2 | 98.6 | 100.0 | 105.0 |
| 29 | 86.8 | 80.5 | 112.5 | 95.3 | 106.9 | 93.0 | 100.0 | 98.4 | 86.7 | 101.8 | 104.8 | 117.0 | 105.7 |
| 30 | 101.8 | 100.0 | 107.5 | 96.7 | 102.2 | 86.2 | 98.2 | 99.0 | 105.2 | 95.2 | 80.4 | 107.7 | 107.5 |
| 31 | 97.4 | 90.7 | 109.8 | x | 102.5 | 92.6 | 96.4 | 101.3 | 102.7 | 97.2 | 98.7 | 107.3 | 106.7 |
| 32 | 122.5 | 100.0 | 70.8 | x | 119.8 | 85.7 | 97.2 | 97.1 | 98.1 | 109.8 | 103.8 | 100.0 | 120.7 |
| 33 | 99.4 | 100.0 | 111.8 | 99.5 | 105.3 | 91.9 | 101.9 | 90.8 | 102.1 | 93.1 | 98.8 | 104.4 | 111.6 |
| 34 | 101.9 | 92.1 | 113.4 | 100.0 | 92.6 | 94.4 | 106.6 | 97.4 | 110.6 | 93.3 | 88.5 | 103.1 | 106.9 |
| 35 | 106.6 | 99.5 | 115.7 | 98.6 | 99.1 | 87.6 | 102.4 | 90.1 | 100.0 | 94.1 | 101.9 | 101.7 | 116.0 |
| 36 | 103.4 | 101.3 | 103.2 | 97.1 | 100.1 | 92.4 | 101.4 | 90.8 | 104.5 | 95.9 | 96.3 | 99.9 | x |
| 37 | 104.6 | 98.7 | 99.5 | 101.6 | 97.6 | 89.8 | 100.8 | 94.4 | 100.5 | 94.2 | x | 101.3 | 105.0 |
| 38 | x | 100.0 | x | 91.7 | 100.4 | 81.4 | x | x | 100.5 | 88.8 | x | x | 111.7 |
| 39 | 104.6 | 101.7 | 98.4 | 101.9 | 96.0 | 90.0 | 100.0 | 93.4 | 100.5 | 98.7 | 104.5 | 95.2 | 113.1 |
| 40 | 100.4 | 101.7 | 91.5 | 100.0 | 86.2 | 91.0 | 103.2 | 82.2 | 102.4 | 94.7 | 105.2 | 97.6 | 117.3 |
| 41 | 105.7 | 97.3 | 121.3 | 101.9 | 98.7 | 84.7 | 97.6 | 100.0 | 96.9 | 94.2 | 109.0 | 104.9 | 103.7 |
| 42 | 106.6 | 91.9 | 117.4 | 94.3 | 98.4 | 88.7 | 98.8 | 100.0 | 100.9 | 93.2 | 104.8 | 106.0 | 110.0 |
| 43 | 100.0 | 100.0 | 123.1 | 89.8 | 96.7 | 84.7 | 96.1 | 100.8 | 95.9 | 102.2 | 102.3 | 103.6 | 113.7 |
| 44 | 104.5 | 100.9 | 116.9 | 102.3 | 94.2 | 86.2 | 102.0 | 99.1 | 96.3 | 94.6 | x | 100.9 | 83.1 |
| 45 | 106.4 | 97.8 | x | 100.1 | 99.9 | 88.2 | 98.5 | 92.7 | 99.8 | 102.6 | 102.9 | 107.7 | 109.9 |
| 46 | 100.8 | 99.3 | 123.7 | 94.3 | 92.8 | 90.9 | 93.8 | 101.4 | 100.0 | 92.1 | 108.5 | 107.0 | 119.0 |
| 47 | 109.8 | 105.3 | 119.7 | x | 94.8 | 87.2 | 99.5 | 99.2 | 100.4 | 99.6 | 107.0 | 99.2 | 108.0 |
| 48 | 110.4 | 101.1 | 119.8 | 92.4 | 90.2 | 87.4 | 100.0 | 85.3 | 100.7 | 104.2 | 97.6 | 99.1 | 109.1 |
| 49 | 110.4 | x | 97.0 | 113.2 | 99.4 | 100.0 | x | x | 104.0 | 106.4 | 79.7 | 87.6 | x |
| 50 | 107.9 | 102.7 | 115.2 | 100.3 | 93.9 | 90.5 | 92.9 | 100.0 | 101.1 | 93.7 | 106.1 | 99.7 | 90.2 |
| | | | | | | | | | | | | | |
| Median | 100.9 | 99.5 | 112.1 | 98.2 | 105.6 | 89.4 | 100.0 | 96.3 | 101.6 | 95.7 | 99.6 | 103.1 | 104.0 |
| GM | 101.0 | 98.1 | 109.5 | 98.9 | 104.2 | 89.8 | 99.9 | 95.4 | 101.7 | 95.9 | 98.4 | 103.8 | 104.1 |
| 95% LCL | 99.1 | 96.8 | 106.6 | 96.8 | 102.0 | 88.0 | 98.8 | 93.5 | 100.7 | 94.5 | 96.1 | 101.7 | 102.0 |
| 95% UCL | 102.9 | 99.3 | 112.5 | 101.0 | 106.5 | 91.7 | 101.0 | 97.3 | 102.8 | 97.4 | 100.8 | 106.0 | 106.3 |
| Slope | 0.025 | 0.007 | 0.013 | 0.000 | -0.053 | -0.003 | -0.005 | 0.000 | -0.008 | 0.009 | 0.007 | -0.018 | 0.019 |

**Figure 4:** Bias in estimates for serum samples reported relative to assay calibrators/standards. Each box displays the results in Table 6 from a single laboratory method (Lab A, B, etc), with % values along the y-axis, and sample ID 1-50 plotted from left to right along the x-axis.



## Stability of the candidate IS, 81/615

The accelerated thermal degradation study included two further laboratories, Lab Q and Lab R, each performing a different immunoassay method (using LIAISON® XL and cobas® e411 analyzers). The results of this analysis are summarised in Table 6. Results are expressed as relative potencies, where the TSH concentration in each sample stored at elevated temperature is expressed relative to that of the reference sample (stored at -20ºC).

**Table 6:** Potencies (i.e. TSH contents by immunoassay) of ATD samples relative to the reference sample (stored at -20ºC) after storage at elevated temperatures for 2.6 years.

| Storage temperature | Potency (as % of -20ºC sample) | | | | | |
|---|---|---|---|---|---|---|
| | Lab Q | | | Lab R | | |
| | 95% LCL | Estimate | 95% UCL | 95% LCL | Estimate | 95% UCL |
| 4ºC | 95.93 | 104.87 | 114.68 | 94.13 | 96.64 | 99.21 |
| 20ºC | 86.44 | 94.49 | 103.31 | 88.36 | 90.71 | 93.13 |
| 37ºC | 81.60 | 89.19 | 97.51 | 71.62 | 73.54 | 75.50 |
| 45ºC | 80.15 | 87.61 | 95.78 | 66.37 | 68.14 | 69.96 |

No consistent stability profile was observed across the two laboratories, with Lab Q showing no significant loss in potency at 4ºC or 20ºC, and approximately 12% loss at 45ºC, while Lab R showed significant losses at all elevated temperatures, up to approximately 32% loss at 45ºC. Consequently, it was difficult to obtain a robust prediction of long-term stability using the Arrhenius model when incorporating all reported data. When data from all temperatures were included, Lab Q yielded a predicted loss of 0.12% per year at -20ºC, whilst Lab R showed a significantly poor model fit from which a prediction could not be derived. By excluding data for samples stored at 45ºC, the model fit improved somewhat for Lab R, enabling prediction of a loss of 0.18% per year at -20oC, whilst the predicted loss from Lab Q was reduced to 0.03%.

# Discussion

Stocks of the 3rd International Standard for thyroid-stimulating hormone, human, for immunoassay, coded 81/565, are now exhausted, necessitating the establishment of a replacement IS. A candidate IS, coded 81/615, prepared at around the same time (in 1981) and using the same batch of TSH material as the current IS, has been evaluated in an international multi-laboratory study, aiming to assess its overall suitability to serve as an IS, including value-assignment of TSH content and assessments of commutability and stability. This study was carried out in collaboration with the International Federation of Clinical Chemistry & Laboratory Medicine (IFCC) Committee for Standardisation of Thyroid Function Tests (C-STFT), in parallel with its efforts to replace its serum sample panel designed to improve harmonisation of TSH immunoassays, and the US Centres for Disease Control and Prevention (CDC).

The current IS, 81/565, the candidate IS, 81/615, and the C-STFT panel, were shipped together to 15 laboratories across 8 countries for immunoassay analysis. Each laboratory performed a different method, resulting in performance of 15 different commercial immunoassays across the study. All assay data returned was valid, however two laboratories (Lab G and P) reported significantly reduced estimates of TSH contents in the candidate IS, 81/615, compared to the current IS, 81/565 (Table A1.1). Considering the previously established equivalence, in terms of both production and contents [1,5], of the two materials, this data was excluded from subsequent analysis. The remaining data was analysed to value-assign the contents of the candidate IS, 81/615, relative to the current IS, 81/565. Across 13 laboratories, TSH contents for the candidate IS, 81/615, ranging from 11.0-12.7 mIU per ampoule were determined, with a geometric mean of 11.7 mIU per ampoule and a low inter-laboratory geometric coefficient of variance (4.5%, n=13). This is in good agreement with previous analyses of the candidate IS [1,5].

The two laboratories excluded from value-assignment (Lab G and P) were each provided additional ampoules of the candidate IS, 81/615, for further analysis, to confirm measurement of the approximately expected immunoreactivity (Table A1.5). This data is not for incorporation into the value-assignment calculations but to provide assurances over the suitability the candidate IS in these assays. Upon repeat analysis, Lab P reported TSH contents in the candidate IS, 81/615, which were more closely comparable to the contents reported for the current IS, 81/565, in the initial analysis. Lab G used an alternative for diluent for repeat analysis of the candidate IS, 81/615, which yielded results closer to the expected value for each dilution. Both sets of results provide assurances that the candidate IS, 81/615, is suitable for use in the respective assays.

The analysis of the current and candidate IS alongside the IFCC C-STFT serum sample panel provided an opportunity to assess the commutability of the WHO preparations. It is widely believed that WHO reference standards for TSH have historically had poor commutability with patient samples. Since the establishment of the 2$^{nd}$ International Reference Preparation, 80/558, in the early 1980s, WHO standards for TSH have contained the same source of TSH, derived from deceased human pituitary, which may comprise different glycosylation forms to circulating serum TSH [3]. This has likely contributed to the lack of harmonisation of TSH measurements across WHO-traceable immunoassays which developed over time. The recent efforts of the IFCC C-STFT, including the provision of the serum sample panel, have led to a significant improvement in TSH measurement harmonisation [3,4].

The assessment of commutability performed here for the both the candidate IS, 81/615, and the current IS, 81/565, confirms that these materials have poor overall commutability with patient samples. Although good commutability was observed for some methods (i.e. bias values close to 100% when serum sample measurements were expressed relative to either the current or candidate IS), in the majority of assays poor commutability was observed, with bias values ranging from <80% to >120% (Tables 4-5, Figures 1-2). However, the recent availability of the IFCC C-STFT sample panel has led to recalibration of TSH immunoassay and a resulting improvement in harmonisation of TSH measurement. This is reflected by the

fact that improved bias values are observed when IFCC C-STFT panel serum samples are reported relative to kit standards/calibrator (Table 6, Figure 3).

Despite its poor commutability with patient samples, and the recent availability of the IFCC C-STFT panel for assay recalibration, the WHO IS for TSH has continued to sell in relatively high numbers (approximately 200 units per year). This indicates that there is a continued high demand for the WHO standard for TSH and it is therefore appropriate to replace the 3rd IS, 81/565, now that stocks are exhausted. Importantly, the WHO IS defines the (milli-) International Unit (mIU) of activity to which TSH measurements, including those of the C-STFT panel components, are traceable. It is therefore desirable to maintain availability of a WHO standard for TSH alongside the C-STFT itself. Due to the availability of the C-STFT panel, enabling better harmonisation of TSH measurements, the like-for-like replacement of a standard with known poor commutability is also considered to be acceptable at this time, as opposed to the development of a better commutable WHO preparation, for which the sourcing of material would likely prove to be extremely challenging.

An accelerated thermal degradation (ATD) study of the candidate IS, 81/615, has also been performed, although it has not been possible to derive a straightforward prediction of loss of potency per year during storage at -20ºC, due to differences in the patterns of potency loss at elevated temperatures observed in both laboratories. Using various combinations of data, annual losses of potency of 0.03-0.18% at -20ºC have been predicted. It is worth noting that the ATD study of the current IS, 81/565, yielded a predicted annual loss of 0.04% at -20ºC [1]. This material was available for approximately 20 years, during which time no concerns over loss of potency were received from end users, indicating that the product did indeed have good real-time stability at -20ºC. The same can also thus be expected of the candidate IS, 81/615, considering that it is effectively identical to 81/565.

Overall, this study has demonstrated that the candidate IS, coded 81/615, is suitable to serve as a replacement International Standard for TSH. The material has been robustly value-assigned in good agreement with previous evaluations, is expected to have good long-term stability, and will provide good continuity from the current IS, 81/565, as a WHO IS for TSH. Although the preparation has poor commutability, the impact of this can be offset by use in parallel of the IFCC C-STFT serum panel to ensure harmonisation of the TSH measurements between laboratories.

# Proposal

It is proposed that the candidate preparation in ampoules coded 19/166 is established as the **4th WHO International Standard for thyroid-stimulating hormone, human, for immunoassay** with an assigned content of **11.7 mIU per ampoule**.

# Acknowledgements

We gratefully acknowledge the important contributions of all participants of the collaborative study, as well as IFCC C-STFT, for overall co-ordination of the study, and CDC, for dispatch of the WHO study material alongside the C-STFT panels.

## Comments from participants

A draft copy of this report was circulated to study participants ahead of submission and comments were invited. A number of comments were received, many of which related to corrections of typing errors, or clarifications of statistical methods.

One participant queried why data from current calibrations was used rather than data following recalibration to the C-STFT panel. The participant was informed that at the time of statistical analysis, recalibrated results were not available for all participants, hence current calibrations were used to ensure consistency across all participants. This was considered to be appropriate, since the current calibration represented the assay calibration at the time that the analysis was performed. The participant was also reminded that the purpose of the WHO study was to evaluate and demonstrate suitability of the candidate IS, 81/615. The outcomes of value assignment of 81/615, and commutability assessments of both WHO preparations, would not be affected by recalibration, as this involves adjustment of results by applying a uniform correction factor. Thus, the potency of 81/615 relative to 81/565, and the % / bias values when results are expressed relative to each WHO preparation, would remain the same after recalibration, therefore the conclusions of this report are not affected. Nonetheless, it is noted that recalibration to the C-STFT panel should lead to further improvement in harmonisation between assays, however this is considered to be beyond the scope of the WHO study. Instead, it is expected that recalibration and harmonisation will be presented and discussed in future publication(s) by IFCC C-STFT describing the ongoing curation of a harmonisation panel.

# References

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## APPENDIX 1

**Table A1.1:** geometric mean reported estimates for dilutions of the candidate IS, 81/615, and current IS, 81/565, relative to assay calibrators/standards

| Sample ID | nominal TSH conc. (mIU/L) | A | B | C | D | E | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 81/565 | 50 | 54.2475 | 57.9320 | 61.9252 | 55.6975 | 55.8163 | 72.8500 | 54.6530 | 10.7400 |
| | 12.5 | 13.8290 | 14.4860 | 14.4531 | 14.8210 | 13.3054 | 18.7500 | 13.6985 | 2.5850 |
| | 3.125 | 3.4860 | 3.7300 | 3.3742 | 3.7870 | 3.2303 | 4.3000 | 3.4485 | 0.6550 |
| | 0.781 | 0.8610 | 0.9370 | 0.8320 | 0.9520 | 0.8391 | 0.8775 | 0.8590 | 0.1650 |
| | 0.195 | 0.2725 | 0.2410 | 0.2115 | 0.2340 | 0.1696 | 0.2300 | 0.2095 | 0.0400 |
| | 0.049 | 0.1055 | 0.0610 | 0.0562 | 0.0625 | 0.0189 | 0.0450 | 0.0525 | 0.0100 |
| 81/615 | 50 | 56.8580 | 58.5625 | 59.4501 | 55.8035 | 54.4084 | 39.8000 | 55.4230 | 11.0100 |
| | 12.5 | 14.3050 | 14.6510 | 13.9149 | 14.5090 | 13.3611 | 8.8550 | 13.5315 | 2.7000 |
| | 3.125 | 3.6355 | 3.6405 | 3.3430 | 3.6375 | 3.4427 | 2.1500 | 3.4220 | 0.6550 |
| | 0.781 | 0.9165 | 0.8770 | 0.8001 | 0.9010 | 0.8931 | 0.5420 | 0.8510 | 0.1600 |
| | 0.195 | 0.2895 | 0.2150 | 0.2051 | 0.2260 | 0.1861 | 0.0925 | 0.2070 | 0.0400 |
| | 0.049 | 0.1115 | 0.0535 | 0.0540 | 0.0595 | 0.0227 | 0.0270 | 0.0530 | 0.0100 |

| Sample ID | nominal TSH conc. (mIU/L) | J | K | L | M | N | O | P |
|---|---|---|---|---|---|---|---|---|
| 81/565 | 50 | 60.0800 | 57.1630 | 47.5745 | 72.2420 | 51.2525 | NR | 36.6037 |
| | 12.5 | 13.8300 | 14.2415 | 11.5441 | 18.4345 | 12.5110 | 19.7200 | 9.2029 |
| | 3.125 | 3.1055 | 3.8155 | 2.8774 | 4.4620 | 3.0735 | 4.8350 | 2.3297 |
| | 0.781 | 0.7450 | 0.9240 | 0.7053 | 1.1072 | 0.8070 | 1.1000 | 0.5828 |
| | 0.195 | 0.1650 | 0.2280 | 0.1705 | 0.2996 | 0.2090 | 0.2850 | 0.1507 |
| | 0.049 | 0.0405 | 0.0550 | 0.0425 | 0.0689 | 0.0540 | 0.0750 | 0.0387 |
| 81/615 | 50 | 62.1650 | 60.7025 | 51.4036 | 71.8430 | 56.5100 | 58.1700 | 19.7474 |
| | 12.5 | 14.1050 | 15.0750 | 12.8115 | 18.5515 | 13.9465 | 18.1450 | 5.4366 |
| | 3.125 | 3.2420 | 3.9560 | 3.1368 | 4.4737 | 3.4485 | 4.6200 | 1.3238 |
| | 0.781 | 0.7405 | 0.9380 | 0.7664 | 1.0887 | 0.8670 | 1.1000 | 0.3412 |
| | 0.195 | 0.1689 | 0.2315 | 0.1873 | 0.3093 | 0.2280 | 0.2700 | 0.0870 |
| | 0.049 | 0.0688 | 0.0565 | 0.0463 | 0.0708 | 0.0580 | 0.0600 | 0.0243 |

**Table A1.2:** geometric mean reported estimates for serum samples relative to the candidate IS, 81/615. "x" indicates that a result was not reported.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.58 | 0.74 | 0.89 | 0.71 | 1.07 | 0.70 | 0.81 | 0.88 | 0.73 | 0.84 | 0.63 | 0.83 | 0.63 | 0.74 |
| 2 | 0.59 | 0.76 | 0.82 | 0.71 | 1.08 | 0.69 | 0.83 | 0.80 | 0.72 | 0.85 | 0.61 | 0.78 | 0.60 | 0.76 |
| 3 | 0.62 | 0.77 | 0.86 | 0.73 | 1.11 | 0.69 | 0.79 | 0.66 | 0.76 | 0.81 | 0.66 | 0.76 | 0.65 | 0.76 |
| 4 | 0.85 | 0.99 | 1.09 | 0.91 | 1.41 | 0.94 | 1.03 | 1.01 | 0.96 | 1.10 | 0.77 | 1.01 | 0.83 | 0.99 |
| 5 | 0.83 | 1.06 | 1.17 | 1.00 | 1.43 | 0.99 | 1.16 | 1.06 | 1.07 | 1.10 | 0.80 | 0.98 | 0.92 | 1.06 |
| 6 | 0.72 | 1.07 | 1.21 | 1.03 | 1.59 | 1.02 | 1.08 | 1.20 | 1.02 | 1.15 | 0.82 | 1.11 | 0.86 | 1.07 |
| 7 | 0.93 | 1.13 | 1.30 | 1.10 | 1.50 | 1.08 | 1.21 | 1.15 | 1.11 | 1.32 | 0.81 | 1.17 | 0.99 | 1.13 |
| 8 | 0.93 | 1.16 | 1.44 | 1.18 | 1.71 | 1.09 | 1.33 | x | 1.16 | 1.36 | 0.92 | 1.40 | 1.03 | 1.17 |
| 9 | 1.02 | 1.25 | 1.24 | 1.20 | 1.71 | 1.14 | 1.36 | 1.34 | 1.20 | 1.38 | 0.79 | 1.31 | 1.08 | 1.24 |
| 10 | 1.12 | 1.38 | 1.55 | 1.32 | 1.83 | 1.33 | 1.61 | 1.31 | 1.36 | 1.70 | x | 1.46 | 1.17 | 1.37 |
| 11 | 1.16 | 1.43 | 1.59 | 1.33 | 1.88 | 1.31 | 1.54 | x | 1.38 | 1.39 | 1.00 | 1.41 | 1.21 | 1.39 |
| 12 | 1.16 | 1.47 | 1.80 | 1.42 | 1.94 | 1.38 | 1.61 | 1.64 | 1.46 | 1.64 | 1.06 | 1.49 | 1.31 | 1.47 |
| 13 | 1.18 | 1.47 | 1.88 | 1.49 | 1.96 | 1.39 | 1.77 | 1.34 | 1.50 | 1.65 | 1.28 | 1.56 | 1.26 | 1.49 |
| 14 | 1.30 | 1.51 | 1.88 | 2.37 | 2.09 | 2.32 | 1.74 | 1.59 | 1.57 | 1.76 | 1.12 | 2.40 | 1.33 | 1.74 |
| 15 | 1.44 | 1.58 | 1.92 | 1.59 | 2.12 | 1.55 | 1.77 | 1.62 | 1.62 | 1.85 | 1.32 | 1.78 | 1.46 | 1.62 |
| 16 | 1.41 | 1.64 | 2.01 | 1.66 | 2.17 | 1.58 | 1.80 | 1.80 | 1.66 | 1.95 | 1.27 | 1.82 | 1.36 | 1.66 |
| 17 | 1.46 | 1.67 | 1.98 | 1.64 | 2.23 | 1.61 | 1.80 | 1.81 | 1.62 | 1.96 | 1.28 | 1.93 | 1.47 | 1.67 |
| 18 | 1.48 | 1.77 | 2.16 | 1.67 | 2.29 | 1.74 | 1.95 | 1.80 | 1.76 | 2.03 | 1.39 | 1.91 | 1.57 | 1.77 |
| 19 | 1.61 | 1.82 | 1.97 | 1.71 | 2.32 | 1.62 | 1.88 | 1.80 | 1.75 | 1.86 | 1.40 | x | 1.64 | 1.77 |
| 20 | x | 1.93 | 2.08 | 1.89 | 2.48 | 1.91 | 2.13 | 1.92 | 1.86 | 2.20 | 1.42 | 2.07 | 1.69 | 1.92 |
| 21 | 1.70 | 2.00 | 2.42 | 1.92 | 2.47 | 1.94 | 2.36 | 2.12 | 1.95 | 2.31 | 1.55 | 2.09 | 1.76 | 2.00 |
| 22 | 1.80 | 1.94 | 2.25 | 1.89 | 2.52 | 1.92 | 2.20 | 2.09 | 2.00 | 2.35 | 1.56 | 2.18 | 1.72 | 2.00 |
| 23 | 1.77 | 2.17 | 2.58 | 2.18 | 2.76 | 2.07 | 2.49 | 1.96 | 2.20 | 2.32 | 1.68 | 2.18 | 1.97 | 2.18 |
| 24 | 2.09 | 2.09 | 2.80 | 2.31 | 2.96 | 2.13 | 2.47 | x | 2.20 | 2.26 | 1.86 | 2.28 | 2.18 | 2.23 |
| 25 | 1.97 | 2.38 | 2.81 | 2.23 | 2.81 | 2.17 | 2.62 | 2.33 | 2.24 | 2.61 | 1.73 | 2.42 | 2.03 | 2.33 |
| 26 | 2.15 | 2.42 | 2.69 | 2.27 | 2.80 | 2.13 | 2.57 | 2.43 | 2.26 | 2.43 | 1.63 | 2.52 | 2.07 | 2.42 |
| 27 | 1.93 | 2.43 | 2.98 | 2.49 | 2.79 | 2.31 | 2.88 | 2.40 | 2.47 | 2.45 | 1.92 | 2.61 | 2.23 | 2.45 |
| 28 | 2.00 | 2.52 | 3.30 | 2.54 | 3.35 | 2.52 | 2.97 | 2.84 | 2.66 | 2.80 | 2.07 | 2.71 | 2.40 | 2.66 |
| 29 | 2.06 | 2.20 | 3.20 | 2.58 | 3.34 | 2.67 | 2.96 | 2.84 | 2.24 | 3.18 | 2.24 | 3.24 | 2.46 | 2.67 |
| 30 | 2.48 | 2.75 | 3.08 | 2.64 | 3.24 | 2.50 | 2.93 | 2.88 | 2.74 | 3.01 | 1.74 | 3.01 | 2.52 | 2.75 |
| 31 | 2.55 | 2.69 | 3.38 | x | 3.46 | 2.88 | 3.09 | 3.16 | 2.88 | 3.29 | 2.29 | 3.22 | 2.69 | 2.98 |
| 32 | 3.56 | 3.18 | 2.35 | x | 4.27 | 2.87 | 3.35 | 3.26 | 2.95 | 3.99 | 2.59 | 3.23 | 3.27 | 3.24 |
| 33 | 3.03 | 3.38 | 3.93 | 3.34 | 4.02 | 3.27 | 3.73 | 3.24 | 3.27 | 3.60 | 2.63 | 3.59 | 3.22 | 3.34 |
| 34 | 3.79 | 3.72 | 4.76 | 4.03 | 4.21 | 4.02 | 4.66 | 4.15 | 4.23 | 4.31 | 2.81 | 4.25 | 3.69 | 4.15 |
| 35 | 4.93 | 4.87 | 5.88 | 4.83 | 5.35 | 4.52 | 5.42 | 4.65 | 4.64 | 5.27 | 3.94 | 5.10 | 4.87 | 4.87 |
| 36 | 5.81 | 5.91 | 6.27 | 5.70 | 6.37 | 5.70 | 6.42 | 5.59 | 5.80 | 6.41 | 4.46 | 6.00 | x | 5.86 |
| 37 | 5.91 | 5.79 | 6.08 | 6.00 | 6.25 | 5.57 | 6.41 | 5.84 | 5.60 | 6.33 | x | 6.11 | 5.30 | 5.95 |
| 38 | x | 5.93 | x | 5.47 | 6.47 | 5.10 | x | x | 5.66 | 6.03 | x | x | 5.69 | 5.69 |
| 39 | 6.60 | 6.59 | 6.64 | 6.66 | 6.75 | 6.18 | 7.02 | 6.38 | 6.19 | 7.32 | 5.38 | 6.36 | 6.31 | 6.59 |
| 40 | 8.60 | 8.71 | 8.17 | 8.68 | 7.91 | 8.27 | 9.57 | 7.43 | 8.34 | 9.28 | 7.20 | 8.66 | 8.68 | 8.60 |
| 41 | 10.00 | 9.07 | 11.75 | 9.65 | 9.68 | 8.39 | 9.86 | 9.81 | 8.61 | 10.06 | 8.13 | 10.16 | 8.35 | 9.68 |
| 42 | 17.18 | 13.86 | 18.39 | 14.57 | 15.05 | 14.25 | 16.14 | 15.83 | 14.51 | 16.08 | 12.73 | 16.74 | 14.41 | 15.05 |
| 43 | 17.39 | 16.24 | 20.75 | 14.97 | 15.87 | 14.67 | 16.92 | 17.20 | 14.87 | 18.97 | 13.41 | 17.66 | 16.07 | 16.24 |
| 44 | 18.98 | 16.96 | 20.42 | 17.68 | 16.00 | 15.46 | 18.58 | 17.50 | 15.47 | 18.19 | x | 17.82 | 12.16 | 17.59 |
| 45 | 19.70 | 16.71 | x | 17.58 | 17.14 | 16.07 | 18.24 | 16.64 | 16.27 | 20.02 | 14.20 | 19.33 | 16.34 | 16.92 |
| 46 | 24.82 | 22.02 | 28.48 | 21.60 | 20.41 | 21.55 | 22.59 | 23.59 | 21.21 | 23.37 | 19.51 | 25.07 | 23.05 | 22.59 |
| 47 | 45.70 | 37.19 | 43.93 | x | 32.02 | 33.07 | 38.14 | 36.73 | 33.98 | 40.21 | 30.90 | 37.38 | 33.53 | 36.96 |
| 48 | 47.63 | 36.91 | 45.41 | 35.19 | 31.52 | 34.24 | 39.59 | 32.68 | 35.23 | 43.39 | 29.12 | 38.58 | 34.97 | 35.23 |
| 49 | 55.21 | x | 42.10 | 49.47 | 38.93 | 44.79 | x | x | 41.55 | 50.61 | 27.19 | 39.02 | x | 42.10 |
| 50 | 55.20 | 43.79 | 51.04 | 44.81 | 37.73 | 41.48 | 43.01 | 44.65 | 41.35 | 45.67 | 37.08 | 45.50 | 33.86 | 43.79 |

**Table A1.3:** geometric mean reported estimates for serum samples relative to the current IS, 81/565. "x" indicates that a result was not reported.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.61 | 0.69 | 0.86 | 0.68 | 1.14 | 0.70 | 0.81 | 0.98 | 0.75 | 0.91 | 0.64 | 0.91 | 0.56 | 0.75 |
| 2 | 0.63 | 0.71 | 0.79 | 0.68 | 1.15 | 0.68 | 0.83 | 0.89 | 0.74 | 0.93 | 0.62 | 0.85 | 0.54 | 0.74 |
| 3 | 0.66 | 0.72 | 0.83 | 0.70 | 1.19 | 0.68 | 0.79 | 0.75 | 0.78 | 0.88 | 0.67 | 0.83 | 0.58 | 0.75 |
| 4 | 0.90 | 0.93 | 1.06 | 0.88 | 1.49 | 0.94 | 1.04 | 1.12 | 0.99 | 1.20 | 0.78 | 1.11 | 0.75 | 0.99 |
| 5 | 0.87 | 0.99 | 1.14 | 0.96 | 1.51 | 0.99 | 1.16 | 1.18 | 1.11 | 1.21 | 0.81 | 1.08 | 0.82 | 1.08 |
| 6 | 0.76 | 1.00 | 1.18 | 0.99 | 1.68 | 1.02 | 1.08 | 1.32 | 1.05 | 1.26 | 0.83 | 1.21 | 0.77 | 1.05 |
| 7 | 0.99 | 1.07 | 1.26 | 1.06 | 1.59 | 1.08 | 1.21 | 1.27 | 1.15 | 1.44 | 0.82 | 1.28 | 0.89 | 1.15 |
| 8 | 0.98 | 1.09 | 1.39 | 1.14 | 1.80 | 1.09 | 1.33 | x | 1.20 | 1.49 | 0.93 | 1.53 | 0.92 | 1.17 |
| 9 | 1.08 | 1.18 | 1.20 | 1.16 | 1.80 | 1.14 | 1.36 | 1.47 | 1.24 | 1.50 | 0.79 | 1.44 | 0.97 | 1.20 |
| 10 | 1.19 | 1.31 | 1.50 | 1.28 | 1.93 | 1.32 | 1.62 | 1.44 | 1.40 | 1.86 | x | 1.60 | 1.05 | 1.42 |
| 11 | 1.22 | 1.35 | 1.54 | 1.28 | 1.98 | 1.30 | 1.55 | x | 1.43 | 1.52 | 1.01 | 1.55 | 1.08 | 1.39 |
| 12 | 1.23 | 1.40 | 1.74 | 1.38 | 2.04 | 1.37 | 1.62 | 1.79 | 1.52 | 1.78 | 1.07 | 1.64 | 1.16 | 1.52 |
| 13 | 1.24 | 1.40 | 1.82 | 1.44 | 2.06 | 1.39 | 1.78 | 1.47 | 1.55 | 1.80 | 1.30 | 1.71 | 1.12 | 1.47 |
| 14 | 1.38 | 1.43 | 1.82 | 2.30 | 2.20 | 2.32 | 1.75 | 1.73 | 1.63 | 1.92 | 1.13 | 2.64 | 1.18 | 1.75 |
| 15 | 1.52 | 1.50 | 1.86 | 1.54 | 2.23 | 1.55 | 1.78 | 1.76 | 1.68 | 2.02 | 1.33 | 1.95 | 1.29 | 1.68 |
| 16 | 1.49 | 1.56 | 1.94 | 1.61 | 2.28 | 1.58 | 1.81 | 1.95 | 1.72 | 2.13 | 1.28 | 2.00 | 1.21 | 1.72 |
| 17 | 1.55 | 1.59 | 1.91 | 1.59 | 2.34 | 1.60 | 1.81 | 1.96 | 1.68 | 2.14 | 1.29 | 2.12 | 1.30 | 1.68 |
| 18 | 1.56 | 1.68 | 2.09 | 1.62 | 2.40 | 1.73 | 1.97 | 1.96 | 1.82 | 2.21 | 1.40 | 2.10 | 1.39 | 1.82 |
| 19 | 1.70 | 1.73 | 1.91 | 1.66 | 2.43 | 1.61 | 1.89 | 1.95 | 1.82 | 2.03 | 1.41 | x | 1.46 | 1.77 |
| 20 | x | 1.84 | 2.02 | 1.83 | 2.59 | 1.90 | 2.15 | 2.07 | 1.93 | 2.40 | 1.43 | 2.28 | 1.50 | 1.97 |
| 21 | 1.80 | 1.90 | 2.34 | 1.85 | 2.58 | 1.93 | 2.38 | 2.29 | 2.03 | 2.52 | 1.56 | 2.30 | 1.56 | 2.03 |
| 22 | 1.90 | 1.85 | 2.18 | 1.83 | 2.64 | 1.91 | 2.22 | 2.25 | 2.08 | 2.56 | 1.57 | 2.40 | 1.52 | 2.08 |
| 23 | 1.86 | 2.07 | 2.50 | 2.11 | 2.88 | 2.07 | 2.52 | 2.12 | 2.28 | 2.53 | 1.69 | 2.40 | 1.75 | 2.12 |
| 24 | 2.21 | 2.00 | 2.71 | 2.24 | 3.08 | 2.13 | 2.49 | x | 2.28 | 2.47 | 1.87 | 2.51 | 1.94 | 2.26 |
| 25 | 2.08 | 2.28 | 2.72 | 2.17 | 2.93 | 2.17 | 2.64 | 2.50 | 2.33 | 2.84 | 1.75 | 2.66 | 1.80 | 2.33 |
| 26 | 2.27 | 2.32 | 2.60 | 2.20 | 2.93 | 2.13 | 2.60 | 2.60 | 2.34 | 2.65 | 1.65 | 2.77 | 1.84 | 2.34 |
| 27 | 2.04 | 2.33 | 2.88 | 2.41 | 2.91 | 2.30 | 2.91 | 2.57 | 2.56 | 2.67 | 1.93 | 2.87 | 1.97 | 2.56 |
| 28 | 2.11 | 2.42 | 3.20 | 2.47 | 3.47 | 2.51 | 3.00 | 3.03 | 2.76 | 3.06 | 2.08 | 2.99 | 2.12 | 2.76 |
| 29 | 2.17 | 2.10 | 3.09 | 2.50 | 3.47 | 2.66 | 2.98 | 3.03 | 2.33 | 3.47 | 2.26 | 3.57 | 2.18 | 2.66 |
| 30 | 2.61 | 2.65 | 2.98 | 2.56 | 3.36 | 2.49 | 2.96 | 3.07 | 2.85 | 3.28 | 1.75 | 3.31 | 2.23 | 2.85 |
| 31 | 2.69 | 2.58 | 3.27 | x | 3.60 | 2.87 | 3.12 | 3.36 | 2.99 | 3.59 | 2.31 | 3.55 | 2.38 | 3.05 |
| 32 | 3.75 | 3.06 | 2.28 | x | 4.41 | 2.86 | 3.38 | 3.45 | 3.07 | 4.35 | 2.61 | 3.55 | 2.89 | 3.23 |
| 33 | 3.19 | 3.26 | 3.81 | 3.25 | 4.15 | 3.26 | 3.77 | 3.44 | 3.40 | 3.93 | 2.64 | 3.96 | 2.84 | 3.40 |
| 34 | 3.99 | 3.60 | 4.61 | 3.92 | 4.35 | 4.01 | 4.71 | 4.36 | 4.41 | 4.71 | 2.83 | 4.69 | 3.26 | 4.35 |
| 35 | 5.19 | 4.74 | 5.70 | 4.71 | 5.49 | 4.51 | 5.49 | 4.87 | 4.84 | 5.75 | 3.96 | 5.63 | 4.29 | 4.87 |
| 36 | 6.11 | 5.79 | 6.07 | 5.56 | 6.51 | 5.69 | 6.51 | 5.81 | 6.05 | 6.99 | 4.48 | 6.63 | x | 6.06 |
| 37 | 6.21 | 5.67 | 5.89 | 5.85 | 6.39 | 5.56 | 6.50 | 6.06 | 5.85 | 6.91 | x | 6.75 | 4.66 | 5.97 |
| 38 | x | 5.80 | x | 5.33 | 6.61 | 5.09 | x | x | 5.91 | 6.58 | x | x | 5.00 | 5.80 |
| 39 | 6.94 | 6.47 | 6.43 | 6.50 | 6.89 | 6.16 | 7.13 | 6.60 | 6.47 | 7.98 | 5.40 | 7.02 | 5.55 | 6.50 |
| 40 | 9.03 | 8.60 | 7.91 | 8.49 | 8.04 | 8.25 | 9.73 | 7.63 | 8.73 | 10.13 | 7.20 | 9.58 | 7.60 | 8.49 |
| 41 | 10.50 | 8.97 | 11.38 | 9.44 | 9.80 | 8.37 | 10.02 | 9.97 | 9.01 | 10.97 | 8.13 | 11.25 | 7.32 | 9.80 |
| 42 | 18.00 | 13.84 | 17.80 | 14.30 | 15.07 | 14.22 | 16.45 | 15.79 | 15.24 | 17.54 | 12.71 | 18.59 | 12.57 | 15.24 |
| 43 | 18.23 | 16.28 | 20.09 | 14.69 | 15.87 | 14.65 | 17.25 | 17.10 | 15.62 | 20.69 | 13.38 | 19.61 | 14.00 | 16.28 |
| 44 | 19.88 | 17.01 | 19.77 | 17.37 | 16.00 | 15.43 | 18.95 | 17.39 | 16.26 | 19.84 | x | 19.80 | 10.63 | 17.38 |
| 45 | 20.64 | 16.75 | x | 17.27 | 17.11 | 16.05 | 18.61 | 16.57 | 17.11 | 21.84 | 14.16 | 21.48 | 14.24 | 17.11 |
| 46 | 25.98 | 22.23 | 27.58 | 21.25 | 20.29 | 21.52 | 23.07 | 23.17 | 22.33 | 25.49 | 19.44 | 27.90 | 20.02 | 22.33 |
| 47 | 47.75 | 38.01 | 42.54 | x | 31.47 | 33.02 | 39.07 | 35.47 | 35.90 | 43.85 | 30.71 | 41.68 | 29.04 | 36.95 |
| 48 | 49.76 | 37.71 | 43.97 | 34.72 | 31.00 | 34.19 | 40.57 | 31.70 | 37.22 | 47.32 | 28.95 | 43.02 | 30.27 | 37.22 |
| 49 | 57.65 | x | 40.77 | 48.92 | 38.09 | 44.73 | x | x | 43.95 | 55.18 | 27.04 | 43.51 | x | 43.95 |
| 50 | 57.64 | 44.92 | 49.42 | 44.29 | 36.95 | 41.43 | 44.09 | 42.79 | 43.74 | 49.80 | 36.82 | 50.78 | 29.32 | 44.09 |

**Table A1.4:** geometric mean reported estimates for serum samples relative to assay calibrators/standards. “x” indicates that a result was not reported.

| Sample ID | A | B | C | D | E | H | I | J | K | L | M | N | O | Median |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.85 | 0.83 | 0.97 | 0.82 | 0.96 | 0.76 | 0.84 | 0.94 | 0.87 | 0.82 | 0.93 | 0.95 | 0.84 | 0.85 |
| 2 | 0.87 | 0.85 | 0.88 | 0.82 | 0.97 | 0.74 | 0.86 | 0.85 | 0.86 | 0.84 | 0.90 | 0.89 | 0.80 | 0.86 |
| 3 | 0.91 | 0.87 | 0.93 | 0.85 | 1.00 | 0.74 | 0.82 | 0.70 | 0.90 | 0.79 | 0.97 | 0.87 | 0.87 | 0.87 |
| 4 | 1.20 | 1.12 | 1.19 | 1.07 | 1.30 | 1.02 | 1.08 | 1.07 | 1.16 | 1.08 | 1.14 | 1.15 | 1.12 | 1.12 |
| 5 | 1.18 | 1.20 | 1.27 | 1.17 | 1.32 | 1.07 | 1.21 | 1.14 | 1.29 | 1.09 | 1.18 | 1.12 | 1.23 | 1.18 |
| 6 | 1.04 | 1.21 | 1.32 | 1.19 | 1.48 | 1.11 | 1.12 | 1.28 | 1.22 | 1.14 | 1.21 | 1.26 | 1.15 | 1.21 |
| 7 | 1.31 | 1.28 | 1.42 | 1.28 | 1.39 | 1.17 | 1.26 | 1.23 | 1.33 | 1.31 | 1.18 | 1.33 | 1.33 | 1.31 |
| 8 | 1.31 | 1.31 | 1.56 | 1.37 | 1.60 | 1.18 | 1.39 | x | 1.40 | 1.35 | 1.35 | 1.59 | 1.38 | 1.38 |
| 9 | 1.43 | 1.42 | 1.35 | 1.40 | 1.60 | 1.24 | 1.42 | 1.43 | 1.44 | 1.36 | 1.15 | 1.49 | 1.45 | 1.42 |
| 10 | 1.56 | 1.57 | 1.70 | 1.54 | 1.72 | 1.44 | 1.68 | 1.41 | 1.63 | 1.69 | x | 1.66 | 1.57 | 1.60 |
| 11 | 1.60 | 1.62 | 1.73 | 1.54 | 1.78 | 1.42 | 1.62 | x | 1.67 | 1.38 | 1.47 | 1.60 | 1.62 | 1.61 |
| 12 | 1.61 | 1.67 | 1.97 | 1.65 | 1.84 | 1.49 | 1.68 | 1.76 | 1.76 | 1.62 | 1.56 | 1.70 | 1.75 | 1.68 |
| 13 | 1.63 | 1.67 | 2.05 | 1.73 | 1.86 | 1.51 | 1.85 | 1.43 | 1.81 | 1.64 | 1.88 | 1.77 | 1.69 | 1.73 |
| 14 | 1.78 | 1.72 | 2.06 | 2.74 | 2.00 | 2.53 | 1.83 | 1.71 | 1.89 | 1.75 | 1.64 | 2.71 | 1.78 | 1.83 |
| 15 | 1.96 | 1.80 | 2.10 | 1.84 | 2.03 | 1.69 | 1.85 | 1.73 | 1.95 | 1.84 | 1.92 | 2.01 | 1.95 | 1.92 |
| 16 | 1.92 | 1.87 | 2.20 | 1.93 | 2.08 | 1.72 | 1.89 | 1.93 | 1.99 | 1.94 | 1.86 | 2.07 | 1.83 | 1.93 |
| 17 | 1.99 | 1.90 | 2.16 | 1.90 | 2.14 | 1.74 | 1.89 | 1.94 | 1.96 | 1.95 | 1.87 | 2.19 | 1.97 | 1.95 |
| 18 | 2.00 | 2.02 | 2.36 | 1.94 | 2.20 | 1.89 | 2.05 | 1.94 | 2.11 | 2.01 | 2.03 | 2.17 | 2.10 | 2.03 |
| 19 | 2.16 | 2.07 | 2.15 | 1.99 | 2.24 | 1.76 | 1.97 | 1.93 | 2.11 | 1.84 | 2.04 | x | 2.20 | 2.06 |
| 20 | x | 2.20 | 2.28 | 2.19 | 2.40 | 2.07 | 2.24 | 2.06 | 2.24 | 2.19 | 2.07 | 2.35 | 2.26 | 2.22 |
| 21 | 2.28 | 2.28 | 2.65 | 2.22 | 2.39 | 2.11 | 2.49 | 2.29 | 2.36 | 2.30 | 2.26 | 2.37 | 2.36 | 2.30 |
| 22 | 2.40 | 2.21 | 2.46 | 2.19 | 2.45 | 2.09 | 2.32 | 2.25 | 2.41 | 2.34 | 2.28 | 2.47 | 2.30 | 2.32 |
| 23 | 2.36 | 2.47 | 2.83 | 2.52 | 2.70 | 2.25 | 2.63 | 2.11 | 2.65 | 2.31 | 2.45 | 2.47 | 2.64 | 2.47 |
| 24 | 2.75 | 2.39 | 3.07 | 2.67 | 2.92 | 2.32 | 2.60 | x | 2.65 | 2.25 | 2.71 | 2.58 | 2.93 | 2.66 |
| 25 | 2.60 | 2.72 | 3.08 | 2.59 | 2.76 | 2.37 | 2.76 | 2.51 | 2.70 | 2.60 | 2.53 | 2.74 | 2.72 | 2.70 |
| 26 | 2.82 | 2.76 | 2.95 | 2.63 | 2.75 | 2.32 | 2.71 | 2.62 | 2.73 | 2.42 | 2.39 | 2.85 | 2.78 | 2.73 |
| 27 | 2.55 | 2.78 | 3.27 | 2.88 | 2.74 | 2.51 | 3.04 | 2.59 | 2.98 | 2.44 | 2.80 | 2.95 | 2.99 | 2.80 |
| 28 | 2.63 | 2.88 | 3.63 | 2.94 | 3.34 | 2.74 | 3.13 | 3.07 | 3.21 | 2.80 | 3.02 | 3.07 | 3.22 | 3.07 |
| 29 | 2.71 | 2.51 | 3.51 | 2.98 | 3.34 | 2.90 | 3.12 | 3.07 | 2.71 | 3.18 | 3.27 | 3.65 | 3.30 | 3.12 |
| 30 | 3.21 | 3.15 | 3.39 | 3.05 | 3.22 | 2.72 | 3.09 | 3.12 | 3.32 | 3.00 | 2.54 | 3.40 | 3.39 | 3.15 |
| 31 | 3.30 | 3.07 | 3.72 | x | 3.47 | 3.13 | 3.26 | 3.43 | 3.48 | 3.29 | 3.34 | 3.63 | 3.61 | 3.39 |
| 32 | 4.46 | 3.64 | 2.58 | x | 4.36 | 3.12 | 3.54 | 3.53 | 3.57 | 4.00 | 3.78 | 3.64 | 4.39 | 3.64 |
| 33 | 3.85 | 3.88 | 4.34 | 3.86 | 4.08 | 3.56 | 3.95 | 3.52 | 3.96 | 3.61 | 3.83 | 4.05 | 4.32 | 3.88 |
| 34 | 4.73 | 4.27 | 5.26 | 4.64 | 4.30 | 4.38 | 4.94 | 4.52 | 5.13 | 4.33 | 4.10 | 4.79 | 4.96 | 4.64 |
| 35 | 6.01 | 5.61 | 6.52 | 5.56 | 5.58 | 4.94 | 5.77 | 5.08 | 5.64 | 5.31 | 5.74 | 5.73 | 6.54 | 5.64 |
| 36 | 6.98 | 6.83 | 6.96 | 6.55 | 6.75 | 6.23 | 6.84 | 6.12 | 7.05 | 6.46 | 6.49 | 6.73 | x | 6.74 |
| 37 | 7.09 | 6.69 | 6.74 | 6.89 | 6.62 | 6.08 | 6.83 | 6.40 | 6.81 | 6.38 | x | 6.86 | 7.11 | 6.78 |
| 38 | x | 6.85 | x | 6.28 | 6.88 | 5.57 | x | x | 6.88 | 6.08 | x | x | 7.64 | 6.85 |
| 39 | 7.84 | 7.62 | 7.37 | 7.64 | 7.20 | 6.74 | 7.49 | 7.00 | 7.53 | 7.39 | 7.83 | 7.13 | 8.48 | 7.49 |
| 40 | 9.98 | 10.10 | 9.09 | 9.93 | 8.56 | 9.04 | 10.25 | 8.16 | 10.17 | 9.41 | 10.45 | 9.69 | 11.65 | 9.93 |
| 41 | 11.45 | 10.53 | 13.13 | 11.03 | 10.68 | 9.17 | 10.56 | 10.83 | 10.49 | 10.20 | 11.81 | 11.36 | 11.22 | 10.83 |
| 42 | 18.76 | 16.17 | 20.67 | 16.60 | 17.32 | 15.61 | 17.38 | 17.60 | 17.76 | 16.41 | 18.45 | 18.65 | 19.36 | 17.60 |
| 43 | 18.98 | 18.98 | 23.36 | 17.05 | 18.35 | 16.08 | 18.23 | 19.14 | 18.20 | 19.41 | 19.42 | 19.66 | 21.58 | 18.98 |
| 44 | 20.54 | 19.83 | 22.98 | 20.11 | 18.52 | 16.94 | 20.04 | 19.49 | 18.94 | 18.60 | x | 19.84 | 16.34 | 19.66 |
| 45 | 21.26 | 19.53 | x | 19.99 | 19.96 | 17.62 | 19.67 | 18.51 | 19.93 | 20.50 | 20.56 | 21.51 | 21.95 | 19.98 |
| 46 | 26.24 | 25.83 | 32.18 | 24.53 | 24.16 | 23.65 | 24.42 | 26.39 | 26.03 | 23.98 | 28.23 | 27.84 | 30.97 | 26.03 |
| 47 | 45.80 | 43.90 | 49.90 | x | 39.54 | 36.34 | 41.48 | 41.37 | 41.85 | 41.55 | 44.60 | 41.38 | 45.06 | 41.70 |
| 48 | 47.56 | 43.56 | 51.60 | 39.79 | 38.87 | 37.64 | 43.08 | 36.74 | 43.40 | 44.88 | 42.05 | 42.70 | 46.99 | 43.08 |
| 49 | 54.43 | x | 47.80 | 55.78 | 48.98 | 49.29 | x | x | 51.25 | 52.44 | 39.27 | 43.18 | x | 49.29 |
| 50 | 54.42 | 51.78 | 58.08 | 50.57 | 47.33 | 45.64 | 46.85 | 50.43 | 51.00 | 47.26 | 53.49 | 50.29 | 45.50 | 50.43 |

**Table A1.5:** geometric mean reported estimates for dilutions of the candidate IS, 81/615, and current IS, 81/565, in initial and repeat analyses performed by Lab G and Lab P. The current IS, 81/565, was not provided for repeat analysis.

| Sample ID | nominal TSH conc. (mIU/L) | Lab G | | Lab P | |
|---|---|---|---|---|---|
| | | 1st run | 2nd run | 1st run | 2nd run |
| 81/565 | 50 | 72.8500 | - | 36.6037 | - |
| | 12.5 | 18.7500 | - | 9.2029 | - |
| | 3.125 | 4.3000 | - | 2.3297 | - |
| | 0.781 | 0.8775 | - | 0.5828 | - |
| | 0.195 | 0.2300 | - | 0.1507 | - |
| | 0.049 | 0.0450 | - | 0.0387 | - |
| 81/615 | 50 | 39.8000 | 57.0500 | 19.7474 | 34.329 |
| | 12.5 | 8.8550 | 12.300 | 5.4366 | 8.8061 |
| | 3.125 | 2.1500 | 3.1750 | 1.3238 | 2.2132 |
| | 0.781 | 0.5420 | 0.7325 | 0.3412 | 0.5463 |
| | 0.195 | 0.0925 | 0.1205 | 0.0870 | 0.1387 |
| | 0.049 | 0.0270 | 0.0105 | 0.0243 | 0.0404 |

**APPENDIX 2**

## INSTRUCTIONS FOR MEASUREMENT – WHO STANDARDS

### 1. INTRODUCTION

To enable evaluation and value-assignment of the candidate 4th WHO IS for human TSH, study participants are requested to perform TSH measurements of the current and candidate WHO IS (coded 81/565 and 81/615 respectively), alongside the TSH follow-up panel.

### 2. MATERIALS

The WHO materials provided for TSH measurement are listed in Table 1.

| **TSH preparation** | **Ampoule/vial content** |
|---|---|
| Candidate IS (81/615), stored at -20 °C | Nominally 11.5 mIU TSH<br>1 mg human serum albumin<br>5 mg lactose |
| 3rd WHO IS (81/565) | 11.5 mIU TSH<br>1 mg human serum albumin<br>5 mg lactose |

***Table 1.*** *Materials provided to collaborative study participants.*

On receipt all ampoules of 81/565 and 81/615 should be stored at -20°C until use. Before opening, ampoules should be brought to room temperature to minimise moisture uptake. It is recommended that the contents of each lyophilised ampoule are reconstituted as described in the protocol in Section 3 below.

All samples have been tested negative for HIV-1/2, HbsAg and HCV NAT. Nonetheless, as with all materials of biological origin, these preparations should be regarded as potentially hazardous to health. They should be used and discarded according to your own laboratory's safety procedures.

### 3. TESTS REQUESTED

Participants are requested to analyse the candidate IS, 81/615, the 3rd IS, 81/565, using the assay method normally used in their laboratory. These samples must be analysed in the same test run as the TSH follow-up panel.

The test run must include measurement of a set of six dilutions of each material, 81/565 and 81/615, containing **50, 12.5, 3.125, 0.781, 0.195 and 0.049** mIU/L TSH. Dilutions should be prepared and measured in duplicate. This amounts to a total of 24 samples (12 for each standard, 6 dilutions, each in duplicate).

Instructions for the preparation of dilutions are as follows:

1. Before opening, ampoules should be brought to room temperature to minimize moisture uptake.

2. Open ampoules using the metal ampoule breaker provided, following guidance provided in the Instructions for Use for each material.
3. Reconstitute each ampoule in 1150 µL PBS plus 0.1% BSA, or an alternative buffer appropriate for your chosen assay (giving a nominal TSH concentration of 10,000 mIU/L).
4. Add 1150 µL reconstituted material to 10.35 mL of PBS plus 0.1% BSA, or appropriate alternative buffer, giving a total volume of 11.5 mL (a 1:10 dilution, giving a nominal TSH concentration 1,000 mIU/L).
5. Perform a further 1:20 dilution into PBS plus 0.1% BSA, or appropriate alternative buffer (nominal TSH concentration 50 mIU/L). This will form a stock solution ("Dilution 1") from which serial dilutions should be made:

    - Perform a 1:4 dilution of "Dilution 1" into PBS plus 0.1% BSA or appropriate alternative buffer, forming "Dilution 2" (12.5 mIU/L).
    - Perform a 1:4 dilution of "Dilution 2" into PBS plus 0.1% BSA or appropriate alternative buffer, forming "Dilution 3" (3.125 mIU/L).
    - Perform three further 1:4 serial dilutions as described above. Table 1 below provides the full details of the six dilutions and their expected TSH concentrations.

| Step / dilution | Nominal TSH concentration (mIU/L) |
|---|---|
| Step 3 – ampoule reconstitution | 10,000 |
| Step 4 – dilution (1:10) | 1,000 |
| Step 5 – **dilution 1 (1:20)** | **50** |
| **dilution 2 (1:4)** | **12.5** |
| **dilution 3 (1:4)** | **3.125** |
| **dilution 4 (1:4)** | **0.781** |
| **dilution 5 (1:4)** | **0.195** |
| **dilution 6 (1:4)** | **0.049** |

***Table 1:*** *nominal TSH concentrations at each dilution step. The six core concentrations to be included in all assays are highlighted in bold.*

Measurements of the TSH contents of duplicate preparations of Dilution 1-6 of 81/565 and 81/615 should be calculated in comparison with the assay kit standard/calibrator.

Participants are requested to provide details of the assay method used, including dilution steps. A template for data reporting of WHO and follow-up panel sample results is provided.

## 4. REPORT

For the candidate 4th WHO IS, 81/615, a preliminary report will be prepared and circulated to all participants for comment before submission to the Expert Committee on Biological Standardization of WHO. Study participants will be provided with a copy of this report and asked for comments prior to submission. In the report, participating laboratories will be identified by a laboratory number only and any requests to treat information in confidence will be respected.

## APPENDIX 3

## INSTRUCTIONS FOR USE / MATERIAL SAFETY DATA SHEET

**4th WHO International Standard for Thyroid Stimulating Hormone, Human, Pituitary**
**NIBSC code: 81/615**

**"This material is not for *in vitro* diagnostic use"**

### 1. INTRODUCTION / CONTENTS

This material contains the lyophilized residue of 0.5 mL of a solution which contained:

| | |
|---|---|
| Purified human pituitary TSH extract | nominally 2 μ TSH |
| Peptidase-free human serum albumin | 1 mg |
| Lactose | 5 mg |

Each ampoule contains approximately 11.7 mIU TSH activity.

On receipt, the material should be stored at -20°C or below.

### 2. CAUTION

**THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS OR ANIMALS IN THE HUMAN FOOD CHAIN**.

The preparation contains 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 safety procedures 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. 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.

### 4. LIABILITY AND LOSS

4.1 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.

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

4.3 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.

4.4 Subject to clause 4.3:

4.4.1 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

4.4.2 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.

4.5 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.

## 5. MATERIAL SAFETY SHEET

| **Physical properties (at room temperature)** | | | |
|---|---|---|---|
| Physical appearance | White freeze dried powder | | |
| Fire hazard | None | | |
| **Chemical properties** | | | |
| Stable | Yes | Corrosive: | No |
| Hygroscopic | Yes | 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. | |