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WHO International Collaborative Study of the proposed 1st International Standard for Human, Pituitary Luteinizing Hormone

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **4 October 2014** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Jongwon Kim** at email: kimjon@who.int.

Summary

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2011) the need for a replacement International Standard (IS) for human, pituitary luteinizing hormone (LH) for the calibration of immunoassays to measure LH in human serum and plasma. We report here, the calibration of a candidate standard for human pituitary LH by immunoassay in terms of the current, 2nd IS for human, pituitary LH, coded 80/552, by an International Collaborative Study carried out by eleven laboratories in seven countries. The unweighted geometric mean of the laboratory estimates of the LH immunoreactivity of the candidate standard, coded 81/535, is 33.2 IU per ampoule (95% confidence limits 32.1 – 34.3). The results of this study also indicate that the candidate standard appears sufficiently stable, on the basis of a thermally accelerated degradation study, to serve as an IS. The study included a partial assessment of the impact of the new standard on the routine measurement of LH in human serum samples. All laboratories contributed data during the Collaborative Study through the concomitant measurement by immunoassay of 12 human serum samples. The results suggest that the candidate standard is suitable for the continued calibration of immunoassay methods for the measurement of LH. Therefore, it is proposed that the candidate preparation in ampoules coded, 81/535 is established as the 3rd IS for human, pituitary LH for immunoassay with an assigned content of 33 IU LH per ampoule.

Introduction

The glycoprotein hormone, luteinizing hormone (LH), produced in the anterior pituitary gland, plays a major role in the regulation of reproductive processes. Determinations of the LH concentration in samples of serum and plasma by immunoassays have a number of clinical applications, contributing to clinical diagnoses regarding infertility, menstrual irregularities, precocious or delayed puberty and in distinguishing primary and secondary ovarian/testicular failure.

The 2nd International International Standard (IS) for human, pituitary LH, coded 80/552, was established by the WHO Expert Committee on Biological Standardization (ECBS) in 1988 [1, 2] and has been widely used for the calibration of immunoassays to measure LH in serum and plasma. This preparation contained highly purified LH from acetone-dried human pituitary glands and was selected following a comparative study of seven preparations offered to NIBSC [3]. Stocks of the 2nd IS are low and there is a requirement to replace the standard. Continuity of the IU is required for the on going calibration of LH immunoassays. In the absence of a source of human pituitary glands from which to purify LH, a batch of ampoules, coded 81/535, filled using the same bulk material as 80/552, has been evaluated in this collaborative study to determine its immunoreactivity and to assess its suitability to serve as an International Standard. Participants in the collaborative study were requested to also determine the LH concentration of 12 human serum samples in order to assess the impact of the candidate preparation on the routine measurement of LH by immunoassay.

The aims of this study were therefore:

1. To calibrate the candidate standard, 81/535, in terms of the 2nd IS, 80/552, by immunoassay.
2. To assess the suitability of the candidate standard, 81/535, to serve as the 3rd IS for the calibration of immunoassays of LH.

3. To determine the stability of the candidate standard, 81/535, by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study.

Participants

Eleven laboratories in seven countries took part in the study and are listed alphabetically, by country, in Table 1. Throughout the study, each participating laboratory is referred to by a code number. These code numbers were randomly assigned and do not reflect the order of listing.

Table 1: List of participants in order of country

BELGIUM	Michel Hars and Michel Anciaux Diasource S.A., Rue du Bosquet 2,1348 Louvain-la-Neuve.
BELGIUM	Dr Stefaan Marivoet, Tosoh Europe N.V., Transportstraat 4, 3980 Tessenderlo.
CHINA	Dr Yang Zhen National Institutes for Food and Drug Control, Institute for Medical Devices Control, No. 2 Tiantan XiLi, Dongcheng District, Beijing, 100050.
FRANCE	Dr Patrick Gradon and Dr Gerard Baudino BioMerieux, Chemin de L'Orme, 69280 Marcy L'Etoile.
GERMANY	Dr Andreas Gleixner Roche Diagnostics GmbH, Nonnenwald 2, 82372 Penzberg.
GERMANY	Dr Matthias Herkert and Dr Bernd Roeder DRG Instruments, Frauenbergstrasse 18, 35039 Marburg.
IRELAND	Dr Steven Dierks Abbott Diagnostics, Lisnamuck, Longford.
UK	Kevin Bradford SPD Development Company Ltd., Clearblue Innovation Centre, Priory Business Park, Bedford MK44 3UP.
UK	Dr Jackie Ferguson National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Herts, EN6 3QG.
USA	Douglas Clark Siemens Healthcare Division, 700 GBC Drive, Newark, DE 19702.
USA	Ryan Masica Beckman Coulter Inc., Mail Stop R-530-B, 1000 Lake Hazeltine Drive, Chaska, MN 55318-1084.

Bulk Materials and Processing

A bulk preparation of human pituitary LH was generously donated to the WHO by Drs J G Loeber and R M Lequin (formerly of Nijmegen, the Netherlands) and Dr G. Hennen (formerly of Liege, Belgium). The bulk preparation, coded NM15, contained highly–purified, human pituitary LH isolated from acetone-dried human pituitary glands [4] by the method of Closset et al., (1975)[5]. After comparison with other preparations [3], batch NM15 was selected as the bulk material for the batch of ampoules that comprises the current, 2nd IS, 80/552 [6]. The remainder of preparation NM15 was used to prepare a second batch of ampoules, coded 81/535. The batch

of ampoules coded 81/535 was filled on 11 June 1981, within the Standards Processing Division of NIBSC. As described in [6], 24.46 mg of LH from preparation NM15 was dissolved in a 30 ml volume of a solution containing 0.2% (w/v) purified human plasma albumin which was free of peptidase activity (batch AK3; Lister Institute, Elstree), 1% (w/v) lactose and 3 mM sodium chloride. The solution was centrifuged at 10 000 x *g* for 15 min at 4°C and the supernatant (29.214 g) diluted with diluent to a final concentration of approximately 11.6 µg LH/ml. The solutions were distributed into ampoules as 0.5 ml aliquots. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen. NIBSC does not routinely perform an automated 100% post sealing check on container integrity. Visual checks on seal quality and general container integrity are made at the time of sealing, whilst labeling and again at the time of picking material ready for dispatch to customers.

Characterization of the freeze dried product

A final total of 3518 ampoules of human pituitary LH, each coded 81/535, were obtained, with a mean fill weight of 0.501 g (n = 82; CV 0.20%), a mean dry weight of 4.7 mg (n = 5; range as a percentage of the mean, 18.08%) and a residual moisture content, determined by Karl Fischer titration, of 0.0773 % (n = 3; CV 34.86%). Each ampoule of 81/535 contains approximately 5.8 µg LH, 5 mg lactose, 1 mg human plasma albumin and 90µg sodium chloride. Using currently available tests, ampoules of the candidate standard, 81/535, were tested at NIBSC and found non-reactive for the viral markers, anti-HIV1/2, HBsAg and HCV RNA. A total of two thousand, nine hundred ampoules are offered to WHO. NIBSC will act as custodian of the preparation which is stored at -20°C under assured temperature controlled conditions in the Institute's Centre for Biological Reference Materials at Potters Bar, Hertfordshire, UK.

The bioactivity of the candidate standard, 81/535, was assessed at NIBSC by *in vivo* bioassays of LH measuring seminal vesicle weight gain (SVW) in immature male rats [7]. Due to limited stocks of the current IS, 80/552, the bioactivity of the candidate standard, 81/535, was measured in terms of the 5th International Standard for human, urinary follicle stimulating hormone and urinary luteinizing hormone, coded 10/286, which had been calibrated in terms of the 4th International Standard for human, urinary follicle stimulating hormone and urinary luteinizing hormone, coded 98/704 [8]. A previous study had demonstrated using SVW bioassays, that 0.8 IU of 98/704 was equivalent to 1 IU of 80/552 [9]. From two SVW bioassays, the bioactivity of the candidate standard, 81/535, in terms of 10/286, was determined as 26.5 IU/amp (95% confidence limits 21.8 – 32.1). Taking into account the relationship between the urinary and pituitary LH bioactivity, the potency determined for 81/535 in terms of 10/286 was in agreement with values determined previously for 81/535 during the calibration of 80/552 [6].

Collaborative Study for the calibration of 81/535

Study design and methods

The collaborative study, coded CS502, was organized by NIBSC. All participants were provided with ampoules of the current and candidate IS, 80/552 and 81/535, and a further selection of samples based on assay capacity and sample availability. Some thermally accelerated degradation samples were only available in limited numbers. A study protocol, shown in Appendix 2 and instructions for use were provided with the samples. Participants were requested to carry out the LH immunoassay(s) normally in use in their laboratory and, where possible, to perform at least two independent assays, using fresh ampoules, each assay to include all of the preparations allocated, measured at no less than five dilutions in the linear part of their dose-response curve. In instances where there was not a fresh ampoule for subsequent assays, it was

suggested that fresh dilutions be made from frozen stock solutions. Where dilutions of a stored stock solution were used, participants were asked to provide details of its storage and identification of the initial preparation. Participants were asked to provide details of the assay method used, including dilution steps, together with all raw assay data for central computation at NIBSC. Participants' own estimates of immunoreactivity as calculated by the method normally used in their laboratory were also requested**.** The materials for this study, which may be identified only by code letter, are listed in Table 2.

Table 2: Ampouled preparations provided to participants in collaborative study.

Code	**Preparation**	**Ampoule unitage and nominal content**
Not coded	2nd International Standard (80/552)	35 IU LH per ampoule
C	Candidate 3rd International Standard (81/535) stored at -20°C	Nominally 35 IU/amp
G	Coded duplicate of C	Nominally 35 IU/amp
A and D	Accelerated thermal degradation (ATD) samples of 81/535 stored at +37°C and +45°C for 2 years.	Content assumed identical to 81/535 stored at -20ºC
B, E and F	Accelerated thermal degradation (ATD) samples of 81/535 stored at +37°C, +20°C and +4°C for 26 years.	Content assumed identical to 81/535 stored at -20ºC

In addition to the ampouled preparations, participants were provided with a panel of 12 human serum samples. Serum samples coded EHuS004 to EHuS009 and EHuS011 to EHuS015 from individual human donors were obtained from First Link UK (Wolverhampton, UK). Samples were certified non-reactive for HIV 1/2, HIV p24, HBsAg, anti-HCV and Syphilis TP by the supplier. Serum samples EHuS011 to EHuS014 were filtered through sterile 30µm filter (Whatman 113V (GE Healthcare Life Sciences, Little Chalfont, UK)). Serum sample EHuS010 was a pool of eight individual donor sera obtained from Cerba Specimen Services (Saint Ouen l'Aumône, France). The pool was tested at NIBSC and found to be non-reactive for HCV RNA, anti-HIV 1/2 and HBsAg.

The concentration of human chorionic gonadotropin (hCG) in each serum sample was assessed by an hCG-specific ELISA (ALPCO, Salem, USA) and was shown to be <1.0 mIU/ml. Participants were requested to assay the serum samples in the same assays as the ampouled preparations and their house standards. Some serum samples were in limited supply and if necessary, participants were asked to include them all in one run of each method offered to NIBSC or, where possible, to perform both runs in one day and to sample from the same vial.

Samples of female human urine, collected at the time of LH surge or LH surge +1 day were provided and measured by Laboratory 3.

The assays provided by each laboratory are shown in Table 3

Table 3: Immunoassay methods contributed

Lab	Method	Details of calibration of kit/method	Number of assays with 80/552 and 81/535	Number of assays with serum samples	Number of serum samples provided [1]
1	Automated one-step immunoassay	Calibrated to 80/552	4	4	12
2	^{125}I-IRMA	Calibrated to 80/552	2	2	12
3a	Automated time-resolved fluorescent immunoassay	Calibrated to 80/552	3	3	12
3b	Automated 2-step immunoassay	Calibrated to the 3rd IS for human, urinary FSH and LH, 71/264	3	3[2]	12
3c	Automated 3-step immunoassay	Calibrated to 80/552	3	3	12
4a	^{125}I-RIA	Calibrated to 80/552	2	1[2]	11
4b	ELISA	Calibrated to 80/552	2	1[2]	11
4c	Time-resolved fluorescent immunoassay	Calibrated to 80/552	4	4	11
4d	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	11
4e	Automated chemiluminescent immunoassay	Calibrated to 80/552.	2	2	11
5a	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	12
5b	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	12
6	ELISA	Calibrated to 80/552	2	2	12
7a	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	1[3]	12
7b	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	1[3]	12
8	ELISA	Calibrated to 80/552	2	2	12
9	Automated fluorescent immunoassay	Calibrated to 80/552	2	2	12
10	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	12
11a	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	11
11b	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	11
11c	Automated chemiluminescent immunoassay	Calibrated to 80/552	2	2	11

[1] Some participants did not receive serum sample, EHuS010, due to limited stocks.
[2] Serum measurements were excluded from further analysis if measured independently of the current and candidate IS or if the method is not calibrated to the current IS, 80/552.
[3] Serum samples were measured once between Run 1 and Run 2 due to insufficient volume of serum.

Statistical analysis

An independent statistical analysis of all immunoassay data was performed at NIBSC. Estimates of the immunoreactivity for the candidate IS, 81/535, and the accelerated thermal degradation samples were calculated relative to the current IS, 80/552, by fitting a parallel-line model comparing log assay response to log concentration [10]. Linearity was assessed visually, and

parallelism was assessed by the ratio of slopes of the reference and test samples, with ratios outside of 0.80 to 1.25 considered as invalid.

Laboratory mean results were calculated as unweighted geometric mean values and overall mean results were calculated as the unweighted geometric mean of laboratory mean results. Variability between laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\} \times 100\%$ where s is the standard deviation of the $\log_{10}$-transformed immunoreactivity estimates).

The relative immunoreactivities of the accelerated thermal degradation samples were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [11], and hence predict the degradation rates when stored at a range of temperatures.

Analysis of the LH concentration in mIU/ml of the human serum samples used laboratory reported results calculated using in-house references or kit standards, and results calculated relative to the current and proposed International Standards using the method described above. For each serum sample, $\log_{10}$-transformed concentrations obtained using these three different standards were compared by Analysis of Variance to assess differences in mean concentrations and Levene's Test to compare between-laboratory variability.

Results

Data returned for analysis

Data were contributed by 11 laboratories. A total of 49 individual assays from 21 methods (19 different methods) were performed giving 49 sets of results for sample C. Mean immuno-reactivity estimates for 81/535 are summarized in Table 4 and Figure 1. Results from individual assays are given in Appendix Table A1.1.

Assay validity

All assays showed a significant dose-response and allowed statistically valid estimates of relative immunoreactivity to be calculated. Tables showing the slopes of the assayed samples relative to the current IS, 80/552, are included in Appendix Table A1.2 for information.

Immunoreactivity of 81/535 calculated relative to 80/552

Analysis gave a geometric mean immunoreactivity estimate of 33.2 IU per amp (n=21; 95% confidence limits 32.1 – 34.3; GCV 7.4%) for the candidate IS, 81/535.

Stability of 81/535

Depending on assay capacity and sample availability, participants were provided with ampoules of 81/535 that had been stored at elevated temperatures for a period of 2 years (+37°C and +45°C) or 26 years (+4°C, +20°C, +37°C). Participants were asked to measure LH immunoreactivity in the thermally accelerated degradation samples concurrently with measurements of the current and candidate standards. Estimates of the immunoreactivity of ampoules are summarized in Table 5. Analysis showed a predicted loss of immunoreactivity per year of 0.014% when stored at -20°C and 0.129% when stored at +4°C. The predicted loss of immunoreactivity per month is 0.038% when stored at +20°C. The nature of the non-intact LH in the thermally-accelerated degradation samples and its immunoreactivity in each of the 19 different assay methods is not known.

Assessment of the LH concentration of human serum samples

The human serum samples supplied to participants included two samples from premenopausal females, EHuS004 and EHuS005 (menstrual phase not known), one pooled sample from eight post-menopausal females, EHuS010, and nine samples from individual, postmenopausal female donors, EHuS006-009 and EHuS011-015. With the exception of serum sample, EHuS015, which gave serum LH concentrations of >100 mIU/ml using some methods, the values obtained were in accordance with the range of expected serum LH concentrations for pre- and post-menopausal females. For example, one manufacture cites a reference range of 1.9 – 12.5 mIU/ml for premenopausal females (follicular phase) and 0.5 – 16.9 mIU/ml (luteal phase) and 5.0 – 52.3 mIU/ml for postmenopausal females. However, each method and laboratory will have defined a reference range by routine measurement of clinical samples.

Data were not included in the analysis if the LH concentration of the serum samples was performed in a different run to the current and candidate IS (2 assays) or if the method was not calibrated to the current IS, 80/552, (1 assay) as shown in Table 3.

Analysis of Variance showed no significant difference in the mean LH concentrations obtained using different standards, as shown in Figure 2 and Table 6. Additionally, Levene's Test showed no significant difference in the levels of between-laboratory variability using different standards, also listed in Table 6. Appendix Tables A1.3, A1.4 and A1.5 show individual assay results. Pairwise linear assessment of commutability by fitting prediction intervals or by the assessment of relative residuals was not possible due to the inter-method variability between estimates.

Assessment of human urine samples

The LH content of individual human urine samples was measured by Laboratory 3 using three methods. Methods 3a and 3c were calibrated to 80/552. Method 3b was calibrated to the 3rd WHO IS for human, urinary, follicle stimulating hormone (FSH) and urinary LH, 71/264. Two urine samples, coded GNO00017C01D15 and GNO00017C01D16 were measured in each of three runs of each method (Appendix Table A1.6). Determinations of urinary LH were method-dependent, suggesting differences in the selectivity of the assays for urinary LH metabolites such as the stable, dimeric LH beta core fragment and also reflecting the use of different standard preparations.

Summary

Immunoassays of LH are widely used alongside determinations of serum concentrations of follicle stimulating hormone, estradiol and progesterone for the investigation of male and female infertility, precocious puberty and other conditions involving gonadal dysfunction. Assays for LH are also used to indicate ovulation and home-use kits are available for this purpose. Thus, multiple methods and platforms are available to determine LH concentration by immunoassay, and the current IS, 80/552, has been widely used to calibrate such assays. International reference materials for the calibration of LH by immunoassay have been available since the 1970s when it was recommended by WHO that standards for immunoassays should comprise highly purified hormone, as defined by high specific activity, which therefore would minimize discontinuity of unitage arising from differences in the purity of replacement standards [12]. Both the current IS, 80/552, and candidate IS, 81/535, were prepared using a batch of LH, coded NM15, that had been purified from human pituitary glands and which had been selected from a number of preparations offered to NIBSC [3]. As stocks of the current IS are exhausted, a proposal to

calibrate 81/535 as a replacement IS was endorsed by WHO in 2011. This report describes the collaborative study to calibrate 81/535 in terms of the current IS, 80/552.

The batch of ampoules coded 81/535 was filled in 1981 and stored at -20°C at NIBSC. LH bioactivity was confirmed prior to the launch of the collaborative study by rat seminal weight gain bioassay and the absence of viral markers was checked using current methods. Thermally-accelerated degradation samples incubated for 26 years were available for analysis during the study alongside samples incubated at a higher temperature for 2 years to provide a prediction of stability at -20°C. Eleven laboratories contributed data to the collaborative study using 19 different methods which provided 49 sets of results for the determination of LH immunoreactivity. Analysis of the fitted slopes for the dose-response of the candidate IS (samples coded C) and the current IS, 80/552, allowed statistically valid estimates of relative potency to be calculated from all laboratories, fulfilling the requirement of a replacement international standard in terms of parallelism of assay response with the existing IS. Analysis of log-transformed assay responses provided geometric mean potency determinations that were in agreement. The candidate IS, 81/535, was predicted to exhibit a loss of immunoreactivity per year of 0.014% when stored at -20°C indicating that it is sufficiently stable to be a WHO IS. Participants in the collaborative study were also asked to measure eleven individual, female serum samples and one pooled, female serum sample alongside their kit standards, and the dilutions of 80/552 and 81/535. Calculation of the LH content of each serum sample in terms of kit, current or candidate IS demonstrated that replacement of the current IS with 81/535 is unlikely to cause a negative effect on the variability that is currently observed between methods. In addition, future harmonization strategies to improve the between-method variability of LH immunoassays, perhaps utilizing panels of matrix-matched, human serum samples, will continue to require traceability to an International Standard and the establishment of 81/535 provides this continued traceability.

Proposal

It is recommended that the preparation in ampoules coded 81/535 is established as the 3rd IS for human, pituitary LH with an assigned potency of 33 IU LH per ampoule.

Acknowledgements

We gratefully acknowledge the important contributions of all the participants in the collaborative study, Dr J G Loeber, Dr R M Lequin and Dr G. Hennen who kindly donated the human, pituitary LH and the Centre for Biological Reference Materials, NIBSC for the preparation and despatch of the ampouled materials.

References

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Table 4: Laboratory mean estimates of LH immunoreactivity in IU/amp of 81/535, calculated relative to 80/552

Lab	Assay 1	Assay 2	Assay 3	Assay 4	GM	GCV
1	38.6	42.0	38.0	40.4	39.7	4.6%
2	32.9	33.7			33.3	
3a	34.1	32.1	33.2		33.1	3.1%
3b	33.6	32.1	33.3		33.3	2.5%
3c	33.4	31.7	34.0		33.5	3.8%
4a	31.9	31.6			31.8	
4b	36.0	36.9			36.4	
4c	36.1	35.1	34.6	33.7	34.8	2.9%
4d	37.8	34.1			33.6	
4e	30.2	32.8			33.6	
5a	34.4	34.2			34.2	
5b	33.3	35.0			34.2	
6	35.1	32.4			33.7	
7a	32.9	34.6			33.7	
7b	34.8	32.3			33.5	
8	33.3	32.9			33.1	
9	28.8	28.9			28.8	
10	33.8	32.5			33.2	
11a	31.2	27.0			29.0	
11b	30.8	31.6			31.2	
11c	31.5	31.2			31.3	
GM **95% CI** **GCV** **n**					33.2 32.1 – 34.3 7.4% 21	

Figure 1: LH immunoreactivity estimates in IU/amp for 81/535, calculated relative to 80/552

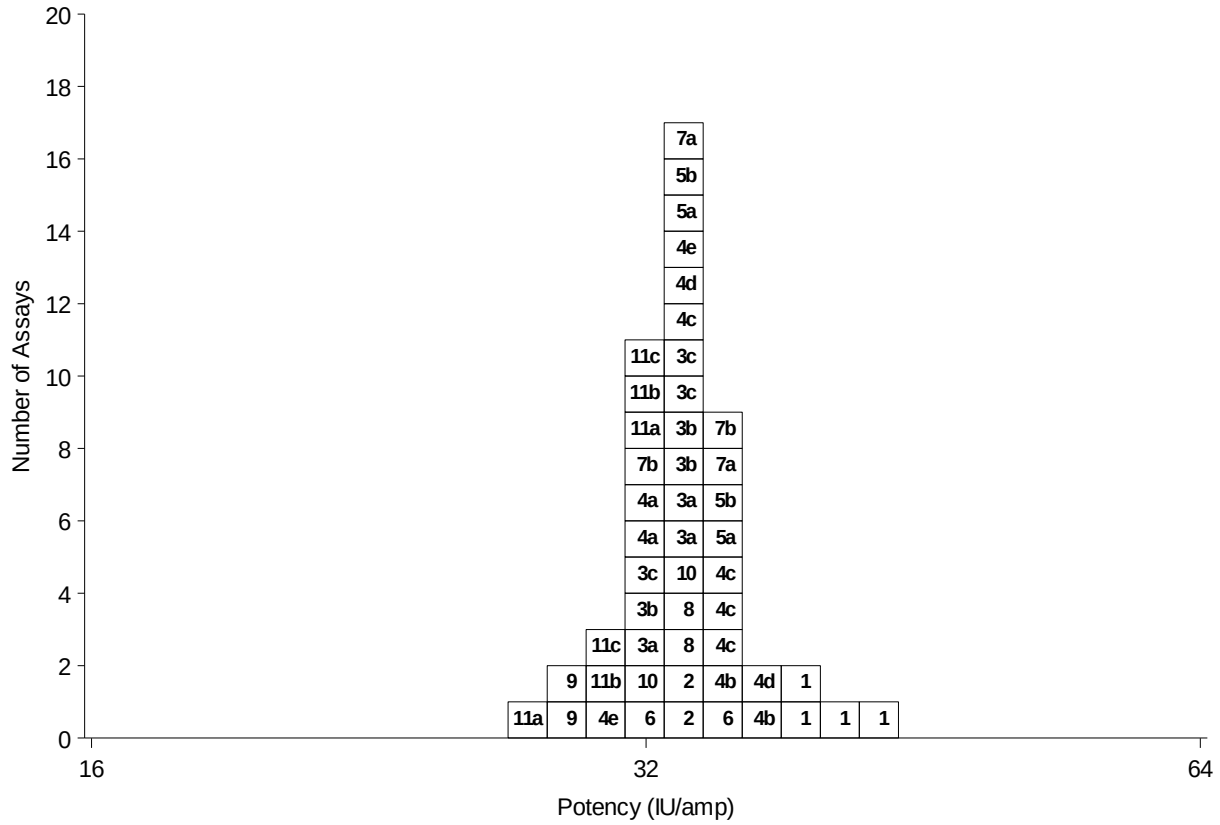
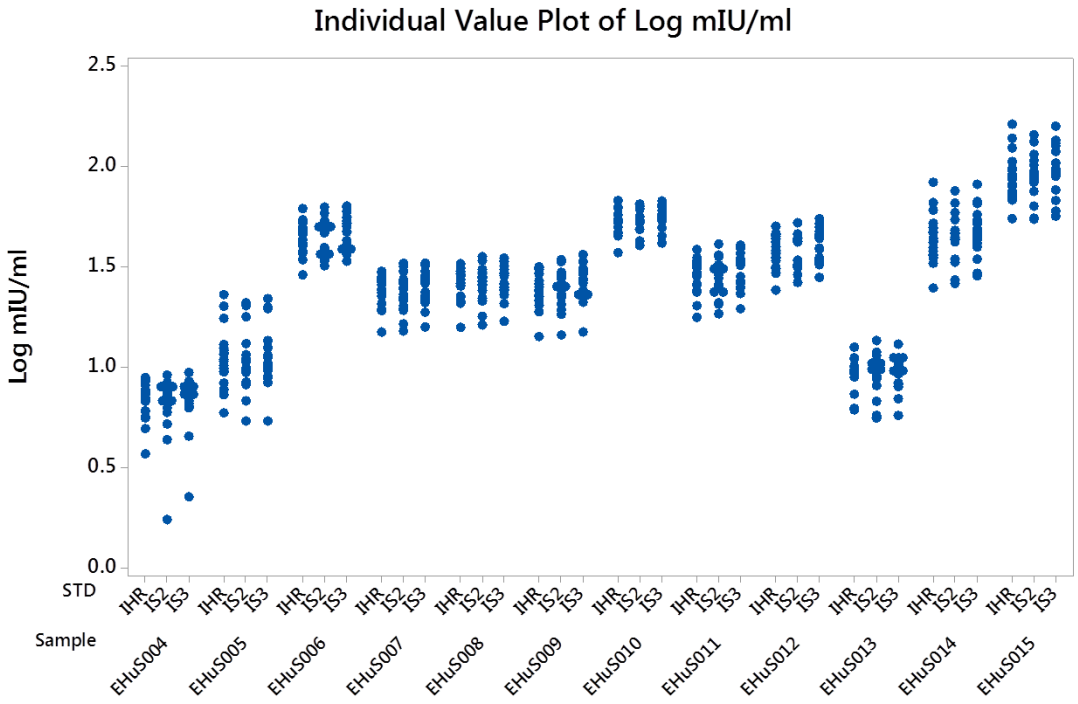


Table 5: LH immunoreactivity in IU/amp of accelerated degradation study samples of 81/535, calculated relative to 80/552

Lab	81/535 33 years, -20°C	A 2 years, +37°C	D 2 years, +45°C	F 26 years, +4°C	E 26 years, +20°C	B 26 years, +37°C
1	39.7	36.6	36.1			
2	33.3	29.4				
3a	33.1				32.2	29.0
3b	33.0				28.5	19.2
3c	33.0				28.2	18.2
4a	31.8			30.2		28.1
4b	36.4			34.0		29.7
4c	34.8			32.2		22.5
4d	35.9			35.0		25.8
4e	31.5			30.8		20.9
5a	34.3	33.4	30.0			
5b	34.1	33.5	29.9			
6	33.8					
7a	33.7				29.6	21.2
7b	33.5				29.2	21.3
8	33.1					
9	28.8	28.2	25.5			
10	33.2		24.9			
11a	29.0					
11b	31.2					
11c	31.3					
GM	33.2	32.1	29.0	32.4	29.5	23.2

Figure 2: Estimates of the LH concentration in Log mIU/ml of human serum samples, as determined relative to the participants' in-house reference/kit standards (IHR), 80/552 (IS2) and 81/535 (IS3) and shown by (i) individual value plot, and (ii) boxplot.

(i)



(ii)

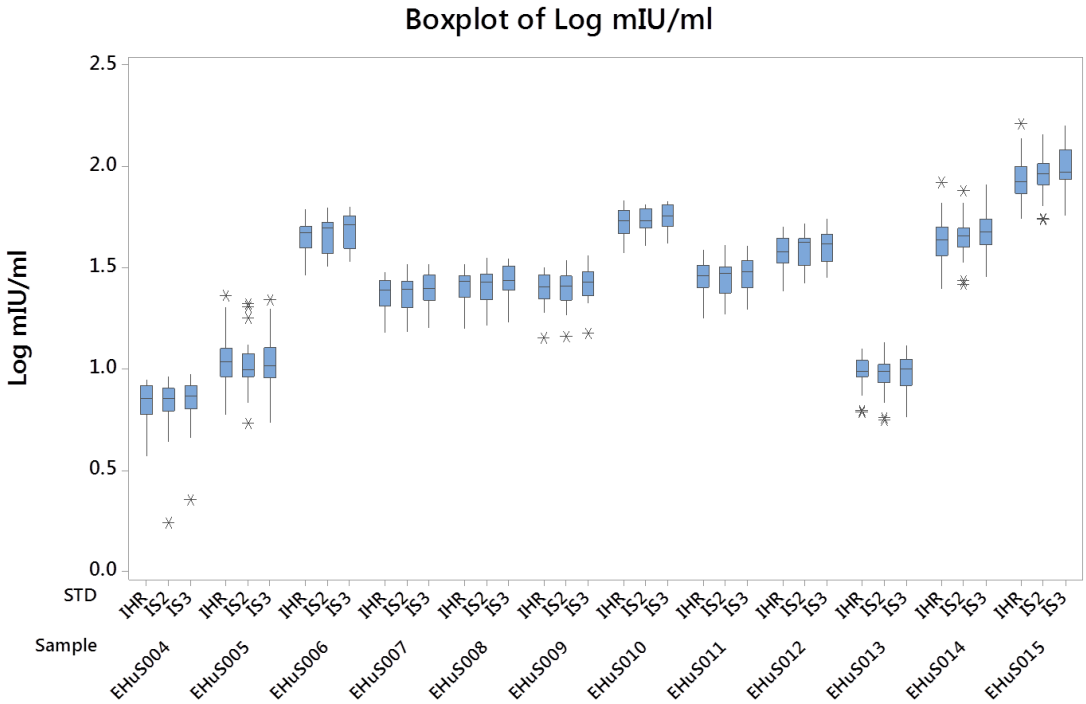


Table 6: Geometric mean and variance of the inter-laboratory estimates of the LH concentration of serum samples in mIU/ml, determined relative to participants' in-house reference/kit standards, 80/552 and 81/535.

Serum Sample	[LH] relative to in-house ref/kit standards (mIU/ml)		[LH] relative to 80/552 (mIU/ml)		[LH] relative to 81/535 (mIU/ml)		ANOVA p-value	Levene's Test p-value
	GM	GCV	GM	GCV	GM	GCV		
EHuS004	6.89	25.3%	6.51	45.1%	6.82	37.8%	0.845	0.685
EHuS005	11.13	40.9%	10.67	41.2%	11.18	40.6%	0.903	0.999
EHuS006	45.01	20.4%	45.75	22.9%	48.43	22.7%	0.515	0.818
EHuS007	23.79	20.1%	23.66	23.6%	24.93	22.0%	0.686	0.818
EHuS008	25.82	19.4%	25.78	22.8%	27.19	21.1%	0.641	0.863
EHuS009	24.82	22.7%	24.77	24.2%	26.05	22.9%	0.717	0.975
EHuS010	52.76	18.3%	54.03	16.3%	56.27	16.6%	0.606	0.909
EHuS011	28.13	21.5%	28.13	23.7%	29.72	22.0%	0.646	0.912
EHuS012	37.77	20.7%	38.20	21.2%	40.38	20.9%	0.532	0.993
EHuS013	9.59	22.1%	9.24	26.2%	9.66	23.0%	0.799	0.823
EHuS014	44.21	32.0%	44.62	31.1%	47.35	31.0%	0.717	0.992
EHuS015	88.53	30.4%	89.62	29.4%	95.57	31.6%	0.653	0.966

Appendix 1

Table A1.1 Estimates of LH immunoreactivity in IU/amp of 81/535 relative to 80/552 for the candidate standard (Sample C) and the coded duplicate (Sample G)

	Sample Code C				Sample Code G			
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**
1	38.6	42.0	38.0	40.4				
2	32.9	33.7						
3a	34.7	32.9	33.2		33.5	31.2	33.2	
3b	34.1	32.6	33.3		33.1	31.6	33.4	
3c	35.0	33.0	34.3		32.0	30.4	33.7	
4a	31.7	30.9			32.1	32.4		
4b	35.0	33.6			37.1	40.4		
4c	36.6	34.1	35.5	33.4	35.5	36.1	33.6	33.9
4d	37.5	33.4			38.1	34.7		
4e	30.9	33.5			29.5	32.1		
5a	34.4	34.2						
5b	33.3	35.0						
6	35.2	32.4						
7a	33.3	34.9			32.5	34.2		
7b	36.0	32.2			33.6	32.4		
8	32.5	32.5			34.0	33.3		
9	29.1	29.4			28.5	28.4		
10	33.8	32.5						
11a	31.2	27.0						
11b	30.8	31.6						
11c	31.5	31.2						

Table A1.2: Fitted slopes for 81/535 relative to 80/552 for the candidate standard (Sample C) and the coded duplicate (Sample G)

	Sample Code C				Sample Code G			
Lab	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**	**Assay 1**	**Assay 2**	**Assay 3**	**Assay 4**
1	1.000	0.998	0.987	1.010				
2	0.979	1.007						
3a	0.995	1.008	0.996		0.997	1.005	1.003	
3b	0.973	1.039	0.988		0.986	1.024	0.984	
3c	0.907	1.136	1.001		1.011	1.195	0.995	
4a	1.011	1.013			1.001	1.088		
4b	1.042	1.035			0.967	0.984		
4c	0.944	0.972	0.959	0.975	0.972	0.971	0.977	0.973
4d	0.968	0.964			0.989	0.962		
4e	0.998	1.000			1.015	0.984		
5a	1.014	1.010						
5b	1.018	1.017						
6	0.995	1.006						
7a	0.971	0.974			0.984	0.981		
7b	0.973	0.978			1.021	0.981		
8	1.038	1.042			1.038	1.086		
9	1.006	0.997			1.001	1.019		
10	0.974	1.006						
11a	1.059	0.981						
11b	0.849	0.937						
11c	1.037	1.062						

Table A1.3: Laboratory-reported LH concentration[1] in mIU/ml of the serum samples and the immunoreactivity of 80/552 and 81/535 in mIU/amp calculated relative to in-house references or kit standards

Lab	EHuS 004	EHuS 005	EHuS 006	EHuS 007	EHuS 008	EHuS 009	EHuS 010	EHuS 011	EHuS 012	EHuS 013	EHuS 014	EHuS 015	80/552	81/535
1	6.8	9.9	46.0	24.5	26.5	25.1	53.0	28.5	42.1	9.0	42.6	81.6	32598	36948
2	5.0	5.9	28.9	15.0	15.8	14.3	37.4	17.8	24.4	6.2	24.9	55.1	36135	35086
3a	7.5	10.3	54.4	26.5	28.7	29.4	62.6	33.0	44.0	10.1	44.0	86.5	35419	33981
3c	5.6	7.8	43.4	19.6	22.4	22.5	45.1	24.2	29.4	7.4	36.1	73.8	30190	29314
4c	7.1	10.7	61.7	30.2	32.8	31.4	nd	38.6	50.5	11.2	52.4	106.1	31776	35035
4d	6.9	9.5	48.0	25.9	27.5	24.1	nd	28.9	37.7	9.7	38.1	80.6	30288	35470
4e	6.9	17.6	34.4	19.1	21.3	20.4	nd	23.7	31.5	9.6	60.9	124.3	33591	32147
5a	8.8	11.7	49.9	28.1	29.3	29.2	62.1	32.3	43.6	9.5	47.3	91.3	35504	34683
5b	8.9	12.2	52.6	29.6	31.3	30.9	67.7	34.0	46.6	10.1	49.6	95.9	36383	35433
6	7.8	12.5	54.2	27.6	30.1	31.8	57.6	35.3	45.7	12.7	47.0	80.0	42463	40904
7a	8.5	23.1	40.3	23.7	26.2	24.3	57.6	26.1	35.4	11.1	83.6	162.6	37636	36773
7b	7.7	20.2	37.2	22.7	25.6	23.0	54.6	25.8	34.0	10.2	66.4	138.3	36077	35240
8	8.6	11.0	46.9	26.9	27.5	29.3	50.1	31.2	36.7	12.7	39.6	70.7	29114	27325
9	5.7	7.3	37.8	19.3	20.9	18.9	46.6	20.4	31.0	6.3	33.1	75.8	41006	34157
10	6.1	8.3	41.7	20.7	22.7	21.6	46.9	25.8	35.0	9.3	36.4	73.3	32046	30354
11a	8.2	11.8	48.9	27.3	28.6	26.0	nd	31.3	45.2	9.8	47.3	98.0	34340	29046
11b	7.3	9.6	49.3	24.3	28.8	26.9	nd	29.6	40.0	9.8	41.9	88.5	33361	30273
11c	3.7	13.0	47.3	23.6	25.6	26.1	nd	28.6	38.4	11.1	35.2	68.3	46015	42189

1. Geometric mean of reported LH measurements

nd Not determined, participant did not receive this serum sample

Immunoreactivity of 80/552 and 81/535 were calculated at NIBSC from raw data. (Other (unshaded) values were as reported by participants.)

Table A1.4: LH concentration in mIU/ml of the serum samples calculated relative to the current IS, 80/552

Lab	EHuS 004	EHuS 005	EHuS 006	EHuS 007	EHuS 008	EHuS 009	EHuS 010	EHuS 011	EHuS 012	EHuS 013	EHuS 014	EHuS 015
1	6.8	10.0	46.9	24.9	26.8	25.4	54.3	28.9	42.9	9.1	43.4	83.5
2	4.4	5.4	32.1	15.2	16.3	14.5	42.7	18.5	26.6	5.6	27.3	63.7
3a	7.2	9.9	53.8	26.0	28.2	29.1	61.9	32.6	43.4	9.9	43.5	86.1
3c	6.0	8.5	51.5	22.3	25.7	25.8	53.5	27.8	34.2	8.1	42.1	90.2
4c	9.2	13.1	62.9	33.1	35.7	34.4	nd	41.3	52.6	13.6	54.4	101.9
4d	8.4	11.6	58.8	31.9	34.0	29.8	nd	35.7	46.2	11.9	46.9	95.2
4e	7.4	17.9	34.0	19.3	21.4	20.7	nd	23.8	31.5	9.8	59.2	115.2
5a	8.1	10.7	49.0	27.0	28.2	28.0	61.5	31.1	42.7	8.8	46.4	91.4
5b	7.9	10.8	50.2	27.5	29.2	28.7	65.2	31.7	44.1	8.9	47.2	93.4
6	6.3	9.8	38.2	20.3	22.2	23.3	40.4	25.5	32.6	10.0	33.4	54.8
7a	8.1	21.0	36.8	21.7	24.2	22.4	52.5	23.8	32.2	10.5	75.6	144.6
7b	8.1	20.3	37.5	23.1	25.9	23.1	54.3	25.9	34.3	10.5	65.8	133.4
8	7.0	9.6	58.8	30.1	30.9	33.6	63.6	36.2	44.2	11.5	48.3	92.7
9	5.2	6.8	39.7	19.8	21.5	19.4	48.7	21.0	32.5	5.8	34.6	75.4
10	6.8	9.6	50.2	24.7	27.0	25.9	56.4	31.0	42.2	10.7	43.8	87.2
11a	7.5	11.0	50.2	26.6	28.0	25.4	nd	31.0	46.0	9.1	48.3	107.2
11b	7.2	9.5	52.7	25.0	29.9	27.8	nd	30.8	42.5	9.8	44.6	92.4
11c	1.8	8.2	36.8	16.5	18.0	18.4	nd	20.6	28.9	6.8	26.2	55.5

nd Not determined, participant did not receive this serum sample

Table A1.5: LH concentration in mIU/ml of the serum samples calculated relative to the candidate IS, 81/535

Lab	EHuS 004	EHuS 005	EHuS 006	EHuS 007	EHuS 008	EHuS 009	EHuS 010	EHuS 011	EHuS 012	EHuS 013	EHuS 014	EHuS 015
1	6.3	8.9	42.9	22.6	24.5	23.3	49.7	26.3	39.4	8.2	39.7	76.8
2	4.6	5.4	33.9	15.9	17.0	15.1	45.2	19.6	28.2	5.8	28.7	67.7
3a	7.6	10.4	56.1	27.0	29.5	30.2	64.7	33.9	45.2	10.3	45.4	89.7
3c	6.4	8.9	52.2	22.8	26.3	26.4	54.3	28.5	34.8	8.4	43.2	90.7
4c	8.6	12.5	63.1	32.5	35.0	33.7	nd	40.8	52.4	13.0	54.2	104.3
4d	7.4	10.5	56.0	30.1	31.9	27.7	nd	33.6	44.1	10.9	44.5	92.8
4e	8.1	20.0	38.9	21.7	24.2	23.1	nd	27.0	35.4	11.2	67.2	130.9
5a	8.4	11.4	50.6	28.0	29.2	29.2	63.1	32.4	43.9	9.3	47.8	93.5
5b	8.4	11.5	52.1	28.8	30.6	30.1	67.5	33.4	46.0	9.6	49.1	96.2
6	6.6	10.1	39.5	21.0	22.9	24.2	41.8	26.6	33.8	10.3	34.6	56.9
7a	8.1	22.1	38.9	22.8	25.2	23.1	56.0	24.9	34.0	10.5	81.9	159.6
7b	7.4	19.6	36.8	22.1	24.9	22.4	53.9	25.2	33.3	9.8	65.5	135.1
8	8.2	11.2	62.8	33.0	33.7	36.6	67.6	39.4	47.5	13.1	52.1	97.2
9	6.3	8.4	47.3	23.8	25.9	23.3	58.1	25.2	38.8	7.0	41.5	90.4
10	7.0	10.0	53.7	26.3	28.7	27.3	60.4	32.9	45.0	11.2	46.9	93.6
11a	9.5	13.7	59.8	32.3	33.8	30.5	nd	37.2	55.0	11.2	57.7	126.7
11b	6.8	9.1	63.6	27.5	33.6	31.0	nd	34.8	50.0	9.6	52.6	119.4
11c	2.3	9.6	40.6	18.9	20.8	21.2	nd	23.4	32.6	8.0	29.5	60.2

nd Not determined, participant did not receive this serum sample

Table A1.6: Laboratory-reported results for the LH concentration in mIU/ml of urine samples, relative to in-house reference/kit standard samples reported by Laboratory 3.

	Urine sample GNO0017C01D15 (LH surge, day 0) [LH] mIU/ml				Urine sample GNO0017C01D16 (LH surge, day +1) [LH] mIU/ml			
Method	**Assay 1**	**Assay 2**	**Assay 3**	**Mean**	**Assay 1**	**Assay 2**	**Assay 3**	**Mean**
3a	121	125	126	124	67.4	70.5	70	69.3
3b	36.5	34.2	31.6	34.1	9.62	9.43	10.4	9.82
3c	24.3	27.7	25.7	25.9	5.03	4.75	5.85	5.21

Appendix 2

International Collaborative Study to Establish the 3rd WHO International Standard for Human, Pituitary Luteinizing Hormone Study Protocol

Introduction

The 2nd International Standard (IS) for human, pituitary luteinizing hormone (LH), in ampoules coded 80/552, was established in 1988 and has been widely used for the calibration of immunoassays to measure LH in human serum and plasma. Determination of LH concentration is used to aid the diagnosis of hypothalamic, pituitary and gonadal dysfunctions and to monitor therapy. Confirmation of the menopause, identification of ovulation and investigations of disorders of puberty also require determination of LH concentration. Stocks of the 2nd IS, 80/552, are almost exhausted and there is an urgent requirement to replace this standard.

The candidate 3rd IS consists of a batch of ampoules, coded 81/535, containing purified human, pituitary LH. It is therefore intended to set up an international collaborative study with expert laboratories to aid in the value assignment of the candidate 3rd IS.

The aims of the study would be:

1. to calibrate 81/535 in terms of the current IS, 80/552.
2. to demonstrate the suitability of 81/535 to serve as the IS for the calibration of human, pituitary LH immunoassays by examining its behaviour in immunoassays and through the assessment of coded human serum samples.
3. to assess the stability of 81/535 after accelerated thermal degradation.

Materials

The materials for this study, which may be identified only by code letter, are listed in Table 1. Each participant will be allocated a set of core preparations and a further selection of samples based on assay capacity and sample availability (some thermally accelerated degradation samples and serum samples are only available in limited numbers).

Table 1: Preparations for inclusion in the collaborative study

Preparation	**Contents**
2nd IS for human, pituitary LH, 80/552	35 IU ampoule
Candidate 3rd IS for human pituitary LH, 81/535, coded	Nominally, 35 IU ampoule
Accelerated thermal degradation samples of 81/535 stored at +4°C, +20°C, +37°C, +45°C, coded	Nominally, 35 IU ampoule
Human serum samples, coded EHuS004 to EHuS015 * (11-12 samples with an LH concentration of 2 - 60 mIU/ml)	0.5 ml or 1.0 ml

***** Due to limited stocks, some participants may not receive human serum sample, EHuS010.

The core preparations are:

2nd IS for human, pituitary LH, 80/552
The 2nd IS contains the residue, after freeze-drying, of 0.5 ml of a solution which contained approximately 5.8 µg LH extract, 1 % (w/v) lactose, 0.2 % (w/v) human plasma albumin and 3mM sodium chloride. The 2nd IS has been tested and found to be negative for HBsAg, anti-HIV and HCV NAT.

Please note that due to extremely limited stocks of 80/552, in some instances we cannot provide fresh ampoules for each immuoassay, particularly to those laboratories offering multiple platforms. Please provide details of any stock solutions made and any freeze-thaw steps.

Candidate 3rd IS for human, pituitary LH, 81/535
The candidate 3rd IS contains the residue, after freeze-drying, of 0.5 ml of a solution which contained approximately 5.8 µg LH extract, 1 % (w/v) lactose, 0.2 % (w/v) human plasma albumin and 3mM sodium chloride. The candidate 3rd IS has been tested and found to be negative for HBsAg, anti-HIV and HCV NAT. Ampoules will be identified by code letter only.

Additional preparations which are available subject to assay capacity and sample availability are:

Accelerated thermal degradation (ATD) samples of the candidate 3rd IS, 81/535
Ampoules of the candidate 3rd IS which have been incubated at +4°C, +20°C, +37°C and +45°C will be included in the study to assess the stability of the candidate standard. The ampoule contents are as described above for the candidate 3rd IS. Ampoules will be identified by code letter only.

On receipt, all ampoules 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 ampoule are reconstituted in a suitable assay diluent, eg. PBS, and appropriate dilutions made from this stock solution. Since there may be extensive dilutions to achieve required assay doses, protein cover (typically 0.1 % (w/v) BSA or HSA) to prevent surface adsorption should be provided. If practical, our recommendation is to resuspend the contents of each ampoule in 3.5ml diluent to prepare a (nominally)10 IU/ml stock solution. Participants are requested to provide details of the reconstitution of the ampoules and all pre-dilutions.
In instances where there is not a fresh ampoule for subsequent assays, it is suggested that fresh dilutions are made from frozen stock solutions and where this is the case, participants are requested to provide details of freeze-thaw steps.

Serum samples
Individual, human serum samples of 0.5 ml or 1.0 ml volume will be provided to further assess the performance of the candidate standard. The serum samples have been tested and found negative for HBsAg, anti-HIV and HCV NAT. This material is only to be used in accordance with the Human Tissue Act or equivalent national legislation and is to be destroyed at the end of the collaborative study.

All material of human origin should be considered as potentially hazardous and handled with appropriate care. It should be used and discarded according to your own laboratory's safety procedures.

Serum samples should be thawed at room temperature prior to each assay and mixed thoroughly by inversion. As the stability of each serum sample is not known, participants are requested to assay immediately and perform both runs in one day. Duplicate serum samples may be available if this is not feasible.

Tests Requested
Participants will receive a selection of coded, ampouled preparations (core preparations and ATDs), distributed on the basis of assay capacity, sample availability and study design. Participants are requested to carry out the assay(s) normally in use in their laboratory and to perform at least two independent runs, using fresh ampoules, each run to include all of the preparations allocated at no less than five dose levels in the linear part of the dose-response curve.

In the same runs as the ampouled preparations, participants are requested to assay all the coded serum samples. Participants are requested to provide details of any dilutions made to serum samples.

Participants are also asked to ensure that all assays include their local standard and to provide details of the assay method used, together with all raw assay data in electronic format in the form of clearly annotated optical densities, counts, etc. for central computation at NIBSC. An example of a reporting table is detailed in Appendix 1.
Participants' own estimates of LH concentration, as calculated by the method normally used in their laboratory, are also requested.

Report
A preliminary report will be prepared and circulated to all participants for comment before submission to the Expert Committee on Biological Standardization of WHO. In the report, participating laboratories will be identified by a laboratory number only and any requests to treat information in confidence will be respected.

For further information, please contact Dr Jackie Ferguson (e-mail: jackie.ferguson@nibsc.org)

National Institute for Biological Standards and Control (http://www.nibsc.org)
Tel: 44 (0) 1707 641135; Fax: 44 (0) 1707 641057

Study Protocol Appendix 1: <u>Human, pituitary LH collaborative study sample data reporting tables</u>

Laboratory Name: ……
Immunoassay Platform/Kit: ……
Assay Number: ……
Date: ……

Sample	**Dilution (or kit standard concentration)**	**Read* 1**	**Read 2**	**Read 3**
Blanks/baselines (please provide details)				
Kit standard 1				
Kit standard 2				
Kit standard 3				
etc…				
Current Standard 80/552				
Current Standard 80/552				
Current Standard 80/552				
etc….				
Candidate ampoule Code (eg. A)				
Candidate ampoule Code (eg. A)				
Candidate ampoule Code (eg. A)				
etc…				
Serum Sample Code (eg. EHuS004)				
etc…				

* **'Read'** refers to the raw data obtained from each individual replicate (eg. absorbance units or counts).

Appendix 3: Draft Instructions for use

WHO International Standard

3rd International Standard for Luteinizing Hormone, human, pituitary
NIBSC Code: 81/535
Instructions for use
(Version 1.0, Dated 01/11/2014)

1. INTENDED USE

The 2nd International Standard (IS) for Luteinizing Hormone (LH), human, pituitary, in ampoules coded 80/552, has been widely used for the calibration of immunoassays for LH. Stocks of the 2nd IS are exhausted and the WHO Expert Committee on Biological Standardization (ECBS) has recognized (2011) the need for a replacement IS. The 3rd IS for Luteinizing Hormone, human, pituitary, in ampoules coded 81/535 containing highly purified Luteinizing Hormone (LH) of human, pituitary origin, was established at the 65th Meeting of WHO ECBS (2014). This material replaces the 2nd IS for Pituitary LH for 80/552, which is discontinued.

2. CAUTION

This preparation is not for administration to humans. The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. However, as with all materials of human origin, the preparation cannot be assumed to be free from infectious agents. The container and its contents should be used and discarded according to your own laboratory procedures. Such procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening the container to avoid cuts.

3. UNITAGE

33 INTERNATIONAL UNITS per ampoule

4. CONTENTS

Country of origin of biological material: Netherlands
Each ampoule contains the residue, after freeze-drying, of 0.5ml of a solution which contained:

LH extract	approx 5.8 μg
Lactose	5 mg
Human plasma albumin	1 mg
Sodium chloride	90 μg

Nitrogen gas at slightly less than atmospheric pressure.

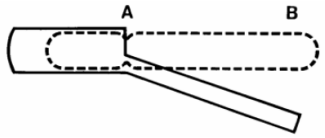
5. STORAGE

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

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass

fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

For practical purposes each ampoule contains the same quantity of LH. Depending on the intended use, dissolve the total contents of the ampoule in a known amount of a suitable diluent. If extensive dilutions are prepared, a carrier protein (0.05 - 0.1% w/v BSA or HSA) should be added. The ampoules do not contain bacteriostat and a solution of the reagent should not be assumed to be sterile.

8. PREPARATION OF AMPOULES

Some 50mg of highly purified pituitary LH, batch no. NM15, were generously donated to WHO by Drs R.M. Lequin and J.G. Loeber (Nijmegen, the Netherlands) and Dr G. Hennen (Liége, Belgium). This material was isolated by Drs Loeber and Lequin from acetone-dried human pituitary glands (Loeber, 1977) as described by Closset et al (1975). The batch of ampoules, coded 81/535, was prepared as described in Storring and Gaines Das, 1993 and evaluated in a collaborative study in which eleven laboratories in seven countries participated, with the aims being:

i. to calibrate 81/535 in terms of the current IS, 80/552.
ii. to demonstrate the suitability of 81/535 to serve as the IS for the calibration of human, pituitary LH immunoassays by examining its behaviour in immunoassays and through the assessment of coded human serum samples.
iii. to assess the stability of 81/535 after accelerated thermal degradation.

From this study, the geometric mean estimate of immunoreactivity of 81/535 in terms of 80/552 was 33.2 IU per amp (n=21; 95% confidence limits 32.1 – 34.3; GCV 7.4%). Preparation 81/535 is sufficiently stable to serve as an IS. Analysis of thermally-accelerated degradation samples gave a predicted loss of immunoreactivity per year of 0.014% when stored at -20°C.

9. STABILITY

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

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended. Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Unopened ampoules should be stored on receipt as indicated on the label. In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

10. REFERENCES

Closset K, Vandalem J-L, Hennen G & Lequin RM (1975). European Journal of Biochemistry 57, 325-333.

Loeber JG (1977). Acta Endocrinologica 85 (Suppl 210), 1-130.

Storring PL & Gaines Das, RE (1993). Journal of Endocrinology 138, 345-359

11. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants in the collaborative study and Dr J G Loeber, Dr R M Lequin and Dr G.Hennen who kindly donated the human, pituitary LH.

12. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards: http://www.who.int/biologicals/en/
JCTLM Higher order reference materials:
http://www.bipm.org/en/committees/jc/jctlm/
Derivation of International Units:
http://www.nibsc.org/products/biological_reference_materials/frequently_asked_questions/how_are_international_units.aspx
Ordering standards from NIBSC:
http://www.nibsc.org/products/ordering_information/frequently_asked_ questions.aspx NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

13. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

14. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

15. MATERIAL SAFETY SHEET

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

16. LIABILITY AND LOSS

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

17. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 6mg
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable. Attached: No

18. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards
http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol
efstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.