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WHO International Collaborative Study of the Proposed 2nd International Standard for Parathyroid Hormone 1-34, Recombinant, Human

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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 **18 September 2017** 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 M. Nübling** at email: nueblingc@who.int.

Summary

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized the need for a replacement International Standard (IS) for parathyroid hormone 1-34, recombinant, human (rhPTH 1-34) in terms of which the content of therapeutic products can be expressed. We report here the calibration of a candidate standard for rhPTH 1-34 by HPLC assay in terms of a primary calibrant, by an international collaborative study carried out by twelve laboratories in eight countries. The mean of the laboratory estimates of the rhPTH 1-34 content of the candidate standard, coded 15/304, is 0.914 milligrams per ampoule (CV = 2.16%, n=28, expanded uncertainty of 0.902, 0.926 mg/amp) in terms of the primary calibrant. The study also included an assessment of the purity, bioactivity and stability of the candidate standard. The mean of the laboratory estimates of the rhPTH 1-34 purity of the candidate standard is 99.08% (CV = 0.59%, n=11). Bioassay data shows good agreement between the potencies of the candidate standard and the 1st WHO IS, 04/200. The results of this study also indicate that the candidate standard is sufficiently stable to serve as an IS.

Therefore, it is proposed that the candidate preparation in ampoules, coded **15/304**, is established as the **2nd WHO IS for parathyroid hormone 1-34, recombinant, human** with an assigned content of 0.914 mg per ampoule.

Introduction

Human PTH 1-34 is the N-terminal, biologically active fragment of parathyroid hormone. The recombinant form of this peptide, expressed in *E. coli* cells, is commonly known as teriparatide. Teriparatide is currently prescribed in the USA and Europe as a treatment for osteoporosis, under the brand names Forteo and Forsteo respectively (Eli Lilly & Co.). The product is under patent protection until 2019, after which it is anticipated that teriparatide biosimilars will arrive on the market.

The 1st IS for parathyroid hormone 1-34, recombinant, human, coded 04/200, was established by the WHO Expert Committee on Biological Standardization (ECBS) in 2007 for the calibration of therapeutic teriparatide (hereafter termed rhPTH 1-34) preparations [1]. This preparation contained rhPTH 1-34 donated by Eli Lilly & Co. Stocks of the 1st IS are low and there is a requirement to replace the standard.

The same manufacturer has once again provided a donation of rhPTH 1-34 with which to produce a replacement WHO IS. This material has been formulated and distributed into ampoules, coded 15/304, which have been evaluated in this collaborative study. Participants have been requested to determine the mass content of the candidate standard using a primary calibrant approach. The primary calibrant, coded PRS0404, was previously donated to WHO by Eli Lilly & Co. in 2004, for the purpose of calibrating the 1st IS by international collaborative study. Only half of the donated vials were used, however the purity and content of the remaining vials were recently verified by HPLC analysis, demonstrating that the material is also fit for use as a primary calibrant in this collaborative study.

The aims of this study were therefore:

1. To calibrate the candidate standard, 15/304, in terms of a primary calibrant, PRS0404, by HPLC assay.

2. To determine the purity of the candidate standard, 15/304.

3. To confirm the bioactivity of the candidate standard, 15/304, and its continuity with the 1st WHO IS, 04/200.

4. To determine the stability of the candidate standard, 15/304, by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study.

Participants

Twelve laboratories in eight 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. The code numbers were randomly assigned and do not reflect the order of listing.

Table 1: List of participants in order of country

AUSTRALIA	Dr Kevin Grant, Dr Tursun Kerim, Dr Sadiqur Talukder, Samuel Ling & Margeret Butt Therapeutic Goods Administration, Biochemistry Section LB, 136 Narrabundah Lane, Symonston ACT 2609
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Bulk materials and processing

A bulk preparation of highly purified lyophilized rhPTH 1-34 was generously donated to the WHO by Eli Lily & Co. Approximately 8 g of rhPTH 1-34 was dissolved in 8 L of a solution containing 10 mg/ml trehalose and 1 mM acetic acid to give a solution containing approximately 1 mg/ml rhPTH 1-34. The solution was distributed into ampoules as 1.0 ml aliquots. The ampoule contents were freeze-dried and sealed under nitrogen. Ampoules were stored at -20°C.

Characterization of the freeze dried product

A total of 7,527 ampoules were produced. Check-weights measured during filling demonstrated a mean fill weight of 1.0066 g (CV = 0.248%, n = 277). The mean residual moisture was estimated to be 0.515% (n = 12) and the mean oxygen head space was estimated to be 0.4% (n =12). No microbial contamination was detected.

Collaborative study for the calibration of 15/304

The collaborative study was organised by NIBSC. The materials provided to participants are summarized in Table 2. A study protocol, shown in Appendix 1, and instructions for use were provided with the samples.

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

rhPTH 1-34 preparation	Vial content
Primary calibrant (PRS0404)	1 mg rhPTH 1-34
Candidate 2nd IS for rhPTH 1-34, recombinant, human (15/304) stored at -20°C	Nominally 1 mg rhPTH 1-34, plus 10 mg trehalose
1st IS for rhPTH 1-34, recombinant, human (04/200) stored at -20°C	0.89 mg rhPTH 1-34, plus 10 mg trehalose
Accelerated thermal degradation (ATD) samples of 15/304, stored at +4°C, +20°C, +37°C and +45°C for 179 days (randomly coded A-D)	Contents assumed to be identical to 15/304

Participants were asked to provide estimates of the rhPTH 1-34 content of the provided preparations by comparison with primary calibrant via HPLC assay. It was requested that three independent assays be performed, using fresh ampoules/vials for each assay. Due to limited supplies, the inclusion of the 1st IS and ATD samples in only one assay was permitted. Participants were also requested to provide estimates of the rhPTH 1-34 purity of the candidate standard via HPLC.

Additionally, in order to provide supporting information for the use of the candidate standard in a bioassay, participants were requested to carry out the bioassay normally in use in their laboratory. It was requested that three independent assays be performed, using fresh ampoules/vials for each assay, and that each assay include candidate standard, 15/304, and 1st IS, 04/200, preparations 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.

The assays provided by each laboratory are shown in Table 3.

Table 3: Methods contributed by each laboratory

Lab	Method	Comments
1	HPLC (content and purity)	As protocol.
2	HPLC (content and purity)	As protocol. Mobile phase composition adjusted to obtain desired retention times in both tests. 100 Å column pore size.
3	HPLC (content and purity)	As protocol.
4	*In vitro* Bioassay	cAMP measurement in UMR106 cells, stably transfected with a cAMP Responsive Element (CRE)-Luciferase gene construct. Detection via luminescence.

5	HPLC (content and purity)	As protocol.
6	HPLC (content)	As protocol.
7	*In vitro* Bioassay	cAMP measurement in UMR106.01 cells. Detection via radioimmunoassay.
8	HPLC (content and purity)	As protocol. Mobile phase composition adjusted to obtain desired retention times in purity test.
9	HPLC (content and purity)	As protocol. 100 Å column pore size.
10	HPLC (content and purity)	As protocol. Mobile phase composition adjusted to obtain desired retention times in both tests.
11	HPLC (content and purity)	As protocol. Mobile phase composition adjusted to obtain desired retention times in both tests.
12	HPLC (content and purity)	As protocol. Mobile phase composition adjusted to obtain desired retention times in both tests.

Results

Data returned for analysis

Data were contributed by 12 laboratories who performed in total 30 individual HPLC content assays, 11 HPLC purity assays and 9 *In vitro* bioassays.

Assay validity

The majority of HPLC content and purity assays satisfied the system suitability criteria, thus allowing valid estimates of rhPTH 1-34 content and purity to be calculated. However Assay 2 for content from Laboratory 6 did not satisfy the system suitability criteria for rhPTH 1-34 peak RSD% or peak tailing, and was therefore excluded in the analysis. Assay 3 for content from Laboratory 6 was also excluded as an outlier.

Estimated content of the candidate standard, 15/304, in terms of the primary calibrant

Estimates of rhPTH 1-34 content (mg/amp) reported by each laboratory were checked and verified. In the results sheet originally provided to participants, rhPTH 1-34 content (mg/ampoule) was calculated by multiplying the estimated rhPTH 1-34 concentration (mg/ml) by 4 (the ampoule reconstitution volume, in ml). Participants were also requested to report the accurately weighed mass of solvent added to ampoules during reconstitution. Following data receipt, rhPTH 1-34 content estimates were re-calculated, by instead multiplying the estimated rhPTH 1-34 concentration by the mass of solvent added to the ampoule.

During the collaborative study of the 1st WHO IS, 04/200, the primary calibrant, PRS0404, was estimated to contain 0.997 mg/vial rhPTH 1-34. PRS0404 was then provided to participants with an assigned content of 1.0 mg/vial and a correction was later applied to the mean of all estimates

of rhPTH 1-34 content. The same approach has been taken here for the candidate standard, 15/304.

Estimates for the rhPTH 1-34 content of the candidate standard, 15/304, in terms of the primary calibrant, PRS0404, are summarized in Table 4. The estimated mean content across nine laboratories is 0.917 mg/amp, which corrected for the estimated content of the primary calibrant (0.997 mg/vial) is 0.914 mg/amp. The relative standard uncertainty was determined as shown in Table 5 with an overall estimate of 0.65%. A final estimate of content for the candidate standard, 15/304, is 0.914 (0.902, 0.926) mg/amp (expanded uncertainty with coverage factor of k=2 taken to correspond to a 95% level of confidence). The rhPTH 1-34 content of the candidate standard, 15/304, was also estimated in terms of the 1st IS, using its assigned content of 0.89 mg/amp, and found to be 0.936 mg/amp (95% confidence interval of 0.931, 0.942), as summarized in Table 6.

Estimated content of the 1st WHO IS, 04/200, in terms of the primary calibrant

Estimates of the rhPTH 1-34 content of the 1st WHO IS, 04/200, in terms of the primary calibrant, PRS0404, are summarized in Table 6. The estimated mean content across nine laboratories is 0.871 (0.860, 0.883) mg/amp (expanded uncertainty calculated the same way as for the candidate standard), or 0.869 (0.857, 0.880) mg/amp when correcting for primary calibrant content. The collaborative study of the 1st IS, carried out in 2007, assigned an estimated content of 0.89 mg/amp. Although an uncertainty was not assigned to the 1st IS at the time, this has been retrospectively determined to be 0.868, 0.912 mg/amp.

Assessment of 15/304 purity

Estimates reported by each laboratory were checked and verified prior to value assignment. Where no peak threshold had been employed, a relative peak area (%RPA) threshold of ≥0.03% was applied. Estimates for the rhPTH 1-34 purity of candidate standard, 15/304, are summarized in Table 7. The mean estimated purity is 99.08% (95% confidence interval of 98.69, 99.47).

Assessment of stability

The relative contents of the accelerated thermal degradation samples were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [2] and hence predict the degradation rates when stored at -20°C.

Estimates of the content of candidate standard, 15/304, ampoules stored at elevated temperatures for a period of 179 days, in terms of the primary calibrant, PRS0404, are summarized in Table 8. Estimates of the relative potencies of these ampoules, in terms of 15/304 ampoules stored at -20°C, are summarized in Table 9. Laboratory 10's reported data was excluded from the analysis due to having outlying results. Analysis showed a predicted loss of content per year of 0.042% when stored at -20°C.

Bioassay data

Two laboratories each returned data from bioassays including the current standard, 04/200, and the candidate standard, 15/304. Bioassay data was analysed in CombiStats v5.0 [3] using a sigmoid curves model and checked for significant non-parallelism between samples using

analysis of variance. All assays showed no deviation from parallelism when considered significant at the 1% level ($p<0.01$). Relative potency estimates are shown in Table 10.

Discussion & conclusions

The aim of this collaborative study was to assign a value to the candidate 2nd WHO IS for rhPTH 1-34, 15/304, using a HPLC assay. Estimates for the content of 15/304 were in good agreement and the content in terms of the primary calibrant, PRS0404, was determined as 0.914 mg per ampoule (CV 2.16%; n=28) (Table 4). The value includes a correction for the estimated content of the primary calibrant, PRS0404, which was provided to participants with an assigned value of 1.0 mg/vial for HPLC analysis, but was accurately estimated to contain 0.997 mg/vial during the collaborative study of the 1st WHO IS for rhPTH 1-34, 04/200. The relative uncertainty of the candidate standard has been estimated as 0.65% (Table 5) giving rise to an expanded uncertainty of 0.902, 0.926 mg/amp.

The rhPTH 1-34 content of the candidate standard, 15/304, has also been estimated in terms of the 1st IS, 04/200, using its assigned content of 0.89 mg (Table 6). The resulting estimate of 0.936 mg/amp differs by 0.022 mg (2.4%) from the estimate of 0.914 mg/amp derived in terms of the primary calibrant, which if not accounted for could cause a shift in potency assignments of rhPTH 1-34 therapeutic products upon uptake of the replacement IS. The difference is likely to be caused by inaccurate content assignment of the 1st IS. Repeat analysis of the 1st IS in this study estimates an rhPTH 1-34 content of 0.869 mg/amp in terms of the primary calibrant, which is lower (by 2.36%) than its assigned content of 0.89 mg/amp. It should be noted that the collaborative study of the 1st WHO IS reported significant deviations in estimates between laboratories performing HPLC assays (CV 5.05%, n=40), and the expanded uncertainty has been retrospectively determined here to be 0.868, 0.912 mg/amp. In this study the estimates for the 1st IS are in much closer agreement (CV 1.46%, n=10) with a reduced uncertainty of 0.857, 0.880 mg/amp. Therefore it is believed that the value of 0.869 mg/amp reported here is a more accurate estimation of rhPTH 1-34 content in the 1st IS. The estimated rhPTH 1-34 content of the candidate standard, 15/304, relative to the 1st IS, 04/200, using a corrected content of 0.869 mg, is 0.914 mg/amp; equal to the estimate relative to the primary calibrant.

The study also aimed to evaluate the purity of the candidate standard via HPLC, giving rise to an estimated mean rhPTH 1-34 purity of 99.08% (95% confidence interval of 98.69, 99.47) (Table 7). The candidate standard is therefore of very high purity, of a similar order to the current IS, and is therefore suitable to serve as a replacement IS.

The bioactivity of the candidate standard has also been demonstrated through bioassays performed in two laboratories. Data from laboratory 4 demonstrates very close agreement between the potencies of the candidate standard and the 1st WHO IS (Table 9). Whilst data from laboratory 7 showed more variability in relative potency, taken together the data demonstrates that use of the candidate standard would ensure continuity of rhPTH 1-34 measurements in bioassays.

Finally, the stability of the candidate standard, 15/304, was assessed by the measurement of accelerated thermal degradation (ATD) samples via HPLC assay (Table 8). The candidate standard

was predicted to exhibit a loss of content of 0.042% per year when stored at -20°C. This value is higher than expected, especially when compared with data from the collaborative study of 1st WHO IS for rhPTH 1-34, which predicted an annual loss of 0.001% based on HPLC assays. The candidate standard, 15/304, contains the same active ingredient from the same source, formulated in the same manner as the 1st IS. Mean moisture levels are lower in the candidate standard (0.5%) than the 1st WHO IS (1.31%), and oxygen levels, although not recorded for the 1st IS, are low in the candidate standard (0.4%). Therefore the candidate standard would be expected to be at least as stable as the 1st IS, for which HPLC chromatograms have been acquired in this study and exhibit little evidence of degradation. Furthermore closer inspection of the relative potencies of ATD samples reveals that laboratory 2 observed a larger decrease in relative potency at +4°C than all other laboratories (Table 9). Exclusion of laboratory 2 from the analysis reduces the predicted annual loss to 0.005% per year at -20°C.

Considering all of the above discussion, it is anticipated that the candidate standard, 15/304, is sufficiently stable to serve as a WHO IS stored at -20°C. However ATD samples will be continually monitored at NIBSC over the next 1-2 years, and if further analysis leads to predictions of significant losses then the material will be transferred to -70°C storage.

Prior to the establishment of the 1st WHO IS for rhPTH 1-34, 04/200, a NIBSC Research Reagent for PTH 1-34 (82/508) was in use, which was assigned units of activity (355U) relative to a previous in-house preparation (75/596) thought to contain 0.03 mg (i.e. using a conversion factor of 10000 units/mg). Although rhPTH 1-34 therapeutic preparations are routinely administered in mass units, activity units are sometimes quoted in addition, which may originally have been based on the NIBSC Reference Reagent. Consequently the 1st WHO IS was assigned a unitage in addition to its mass content (0.89 mg; 8900IU; 10000 U/mg). Since rhPTH 1-34 International Standards are suitable for use in bioassays and immunoassays, an assigned unitage may continue to be useful, and therefore the assigned mass content of the candidate standard, 15/304, should also be converted to units (0.914 mg; 9140IU; 10000 U/mg).

Proposal

It is recommended that the preparation in ampoules coded 15/304 is established as the 2nd IS for parathyroid hormone 1-34, recombinant, human, with an assigned content of 0.914 mg/amp. To maintain continuity with the assigned unitage of the 1st WHO IS for rhPTH 1-34, it is also proposed that this material be assigned an ampoule content of 9140 International Units (1 IU approximately equivalent to 100 ng).

Acknowledgements

We gratefully acknowledge the important contributions of all the participants in the collaborative study, Eli Lilly & Co., who donated the bulk material, and the Centre for Biological Reference Materials, NIBSC for preparation and despatch of the ampouled materials.

References

[1] WHO International Collaborative Study of the Proposed 1st International Standard for Parathyroid Hormone 1-34, Recombinant, Human. Geneva. World Health Organisation, Expert Committee on Biological Standardisation. WHO/BS07.2063
[2] Kirkwood, T. B. (1977). Predicting the stability of biological standards and products. Biometrics 33(4): 736-742.
[3] Daas A. Combistats. 5.0 edn: EDQM - Council of Europe, 2013.

Table 4: Laboratory estimates of rhPTH 1-34 content in the candidate standard, 15/304, calculated relative to the primary calibrant, PRS0404, with an assumed content of 1 mg/vial

Lab	Assay 1	Assay 2	Assay 3	Mean
01	0.915	0.911	0.920	0.915
02	0.930	0.927	0.928	0.928
03	0.911	0.916	0.906	0.911
05	0.924	0.922	0.936	0.927
06	0.884	0.979*	1.374[#]	0.884
08	0.923	0.924	0.915	0.921
09	0.958	0.896	0.914	0.923
10	0.900	0.850	0.934	0.895
11	0.939	0.936	0.931	0.935
12	0.919	0.911	0.905	0.912
			Mean	**0.917**
			Standard Uncertainty	**0.65%**
			Expanded Uncertainty (k = 2)	**(0.905, 0.929)**
			Corrected Mean†	**0.914**
			Corrected Expanded Uncertainty (k = 2)†	**(0.902, 0.926)**

See Table 5

** excluded as assay did not meet system suitability criteria*
[#]excluded as an outlying result
† corrected for estimated content of primary calibrant (0.997 mg/vial)

Table 5. Uncertainty of measurement determination for 15/304 and 04/200

Variability Source	Estimation Method	Relative Standard Uncertainty
Between Laboratory	Using a random effects model treating 'lab' as a random effect	0.04%
Between Assay		0.40%
Homogeneity of Fill	%CV of fill weights for 277 ampoules	0.25%
Primary Calibrant	From previous international collaborative study data	0.45%
Combined relative standard uncertainty		0.65%

Table 6: Laboratory estimates of rhPTH 1-34 content in the candidate standard, 15/304, calculated relative to the 1st IS, 04/200, with its assigned content of 0.89 mg and corrected content of 0.869 mg/amp, and in both 15/304 and 04/200 calculated relative to the primary calibrant, PRS0404, with an assumed content of 1 mg/vial

Lab	15/304 vs PRS0404	04/200 vs PRS0404	15/304 vs 04/200 (0.89 mg/amp)	15/304 vs 04/200 (0.869 mg/amp)
01	0.915	0.866	0.936	0.914
02	0.928	0.875	0.948	0.926
03	0.911	0.875	0.931	0.909
05	0.927	0.877	0.938	0.916
06	0.884	0.853	0.921	0.899
08	0.921	0.875	0.938	0.915
09	0.923	0.894	0.932	0.910
10	0.895	0.862	0.933	0.911
11	0.927	0.882	0.942	0.920
12	0.912	0.854	0.944	0.922
Mean	0.917 (0.914†)	0.871 (0.869†)	0.936	0.914
95% LCL	0.905 (0.902†)*	0.862 (0.857†)*	0.931	0.903
95% UCL	0.929 (0.926†)*	0.880 (0.880†)*	0.942	0.925

**expanded uncertainty with coverage factor k=2 taken to correspond to a 95% level of confidence*

† corrected for estimated content of primary calibrant (0.997 mg/vial)

Table 7: Laboratory estimates of rhPTH 1-34 purity in the candidate standard, 15/304

Lab	Injection 1	Injection 2	Injection 3	Mean
01	99.61	99.62	99.61	99.61
02	98.81	98.78	98.76	98.78
03	99.09	99.14	99.09	99.10
05	99.01	98.95	98.90	98.95
08	99.90	99.90	99.90	99.90
09	99.85	99.86	99.84	99.85
10	98.42	98.34	98.39	98.39
	98.25	98.30	98.27	98.27
	98.30	98.36	98.46	98.37
11	99.29	99.38	99.42	99.36
12	99.24	99.24	99.23	99.24
			Mean	99.08
			95% LCL	98.69
			95% UCL	99.47

** excluded as data did not meet system suitability criteria*

Table 8: Laboratory estimates of rhPTH 1-34 content of accelerated degradation study samples of 15/304 (179 days incubation), calculated relative to the primary calibrant, PRS0404, with an assumed content of 1 mg/vial

Lab	15/304 (-20°C)	B (+4°C)	A (+20°C)	C (+37°C)	D (+45°C)
01	0.911	0.913	0.913	0.899	0.888
02	0.930	0.910	0.910	0.896	0.892
03	0.911	0.912	0.912	0.897	0.889
05	0.924	0.915	0.927	0.912	0.901
06	0.884	0.886	0.888	0.909	0.886
08	0.923	0.922	0.917	0.907	0.897
09	0.914	0.914	0.905	0.892	0.892
10*	0.900*	0.804*	0.844*	1.093*	0.879*
11	0.936	0.932	0.931	0.918	0.901
12	0.919	0.897	0.900	0.884	0.871
Mean	0.920	0.914	0.917	0.901	0.892
95% LCL	0.911	0.906	0.907	0.892	0.884
95% UCL	0.928	0.921	0.926	0.910	0.900

** excluded as outlying results*

Table 9: Laboratory estimates of rhPTH 1-34 relative potencies of accelerated degradation study samples of 15/304 (179 days incubation), calculated relative to samples stored -20°C.

Lab	B (+4°C)	A (+20°C)	C (+37°C)	D (+45°C)
01	1.00	1.00	0.99	0.97
02	0.98	0.98	0.96	0.96
03	1.00	1.00	0.98	0.98
05	0.99	1.00	0.99	0.98
06	1.00	1.00	1.03	1.00
08	1.00	0.99	0.98	0.97
09	1.00	0.99	0.98	0.98
10	0.89*	0.94*	1.21*	0.98*
11	1.00	0.99	0.98	0.96
12	0.99	0.99	0.98	0.96

** excluded as outlying results*

Table 10: Estimated potencies of candidate standard, 15/304, relative to the current standard, 04/200, in bioassays

Lab	Assay	95% LCL	Estimate	95% UCL
04	1	0.91	0.99	1.07
	2	0.88	0.98	1.10
	3	0.84	0.94	1.05
	4	0.85	0.98	1.14
	5	0.73	0.84	0.97
	6	0.88	1.01	1.17
07	1	0.59	0.79	1.05
	2	0.64	0.71	0.79
	3	0.90	1.11	1.37

Appendix 1

COLLABORATIVE STUDY PROTOCOL - ESTABLISHMENT OF THE 2nd WHO INTERNATIONAL STANDARD FOR PARATHYROID HORMONE 1-34, RECOMBINANT, HUMAN (15/304)

INTRODUCTION

PTH 1-34 is the N-terminal, biologically active fragment of human parathyroid hormone. It is prescribed, under the brand name Forteo, in the USA and Europe as a treatment for osteoporosis. In 2004 the World Health Organisation (WHO) Expert Committee on Biological Standardisation (ECBS) recognised the need for an International Standard (IS) for recombinant, human, parathyroid hormone 1-34 (rhPTH 1-34, also known as teriparatide) in terms of which the content of therapeutic products can be expressed. This resulted in the establishment of the 1st WHO IS for rhPTH 1-34 at NIBSC (Code No. 04/200) in 2007. Stocks of this standard are expected to be exhausted within the next two years, necessitating the development of a replacement 2nd IS.

A preparation of rhPTH 1-34 has been received at NIBSC and distributed into ampoules (Code No. 15/304) as a candidate 2nd WHO IS, following procedures recommended by WHO (1). It is proposed to carry out an international collaborative study to establish this replacement standard, by calibration in terms of a primary calibrant of rhPTH 1-34 using HPLC, and confirmation of bioactivity via bioassay. The primary calibrant (Code No. PRS0404) was previously donated for the purpose of calibrating the 1st IS by international collaborative study, and assigned a mass value of 1.0 mg per vial by amino-acid analysis and UV spectroscopy. Only half of the donated vials were used, and the remainder were stored at -80°C. The purity and content of these vials has recently been verified by HPLC analysis at NIBSC, demonstrating that the material is fit for use as a primary calibrant in this collaborative study.

Therefore, the aim of the study is to calibrate the candidate standard (15/304) in terms of the primary calibrant (PRS0404) by HPLC, and to establish continuity with the existing International Standard for rhPTH 1-34 (04/200).

MATERIALS

Preparations supplied to participants in collaborative study.
The materials for this study are listed in Table 1. The study includes accelerated thermal degradation (ATD) samples of the candidate standard, which are coded by letter (A-D) in random order. Triplicate primary calibrant (PRS0404) vials and multiple candidate standard (15/304) ampoules are provided for analysis. Due to limited availability, restricted numbers of 1st WHO IS (04/200) and 15/304 ATD samples (A-D) are provided.

rhPTH 1-34 preparation	Vial content
Primary calibrant (PRS0404)	1 mg rhPTH 1-34
Candidate standard (15/304), stored at -20 °C	Nominally 1 mg rhPTH 1-34, plus 10 mg trehalose
1st WHO IS (04/200)	0.89 mg rhPTH 1-34, plus 10 mg trehalose
Accelerated thermal degradation (ATD) samples of the candidate standard, stored at +4°C, +20°C, +37°C and +45°C (randomly coded A-D)	Content assumed to be identical 15/304

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

Handling of material
On receipt all vials and ampoules should be stored at -20°C until use. Before opening, vials and ampoules should be brought to room temperature to minimise moisture uptake.

Bulk material and preparation of rhPTH 1-34 ampoules
Highly purified rhPTH 1-34, expressed in *E. coli* cells, was kindly donated by Eli Lilly & Co. (Indianapolis, IN, USA). The preparation was received as a lyophilised white powder, which was formulated at 1 mg/mL with pre-filtered trehalose (10 mg/mL) in 1 mM acetic acid and dispensed into 5 mL ampoules at 1 mL per ampoule. Ampoules were then lyophilised and sealed. A total of 7,527 ampoules were produced, with a mean fill mass of 1.0066 g, a mean dry weight of 0.0106 g, and a mean residual moisture content of 0.5154%. No microbial contamination was detected.

TESTS REQUESTED

HPLC for content assay and purity evaluation
Participants are asked to provide estimates of the rhPTH 1-34 content of provided preparations, following the enclosed HPLC protocol (Appendix A) as closely as is practicable. It is requested that participants perform three independent assays, and that the rhPTH 1-34 content of the candidate standard (15/304), its ATD samples (A-D), and the 1st WHO IS (04/200) is estimated by comparison with the primary calibrant (PRS0404) using its assigned mass value of 1.0 mg per vial. Because restricted numbers of 1st WHO IS (04/200) and ATD samples (A-D) have been provided, these may be included in only one HPLC assay.

Participants are also asked to provide estimates of the purity of the candidate standard (15/304) following the enclosed HPLC protocol (Appendix B) as closely as is practicable.

Bioassay
In order to provide supporting information for the use of the candidate standard (15/304) in a bioassay participants are requested to carry out the bioassay normally in use in their laboratory. Where possible it is requested that at least two independent assays be performed, using fresh ampoules, and that each assay includes candidate standard (15/304) and 1st WHO IS (04/200) preparations at preferably no less than five dose levels in the linear part of the dose-response

curve. In instances where there is not a fresh ampoule for subsequent assays, it is suggested that fresh dilutions are made from frozen stock solutions.

It is recommended that the contents of each ampoule are reconstituted in a suitable assay diluent e.g. PBS or saline (not water) and appropriate dilutions made from this stock solution. Since there are likely to be extensive dilutions to achieve required assay doses, protein cover (typically 0.1% BSA or HSA) to prevent surface adsorption should be provided. There is some evidence that if the initial dilution is carried out in a relatively large volume, variation resulting from the numerous dilution steps is minimized. If practicable, an initial dilution volume of 5 ml for all preparations is therefore suggested.

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.

REFERENCES

1. WHO Tech Rep Ser No 800, 1990, 181-214

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APPENDIX A

HPLC ASSAY OF THE CANDIDATE STANDARD (15/304) USING THE PRIMARY CALIBRANT (PRS0404) AS A REFERENCE STANDARD

1. KEY EQUIPMENT & MATERIALS

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

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

- **0.2 M sulphate buffer, pH 2.3**
  E.g. Dissolve 28.4 g of sodium sulphate (e.g. ACS reagent, ≥99%, Sigma-Aldrich Cat. No. 239313) in 900 ml water. Adjust the pH to 2.3 with 85% phosphoric acid (85-90%, for HPLC, Sigma-Aldrich Cat. no. 79606) and make up to 1 L with water.

- **Mobile phase A**
  90% sulphate buffer, 10% acetonitrile. Mix 1 volume of acetonitrile (e.g. HiPerSolv CHROMANORM® for HPLC, VWR Cat. No. 83639.320) with 9 volumes of sulphate buffer.

- **Mobile phase B**
  50% sulphate buffer, 50% acetonitrile. Mix equal volumes of acetonitrile and sulphate buffer. If necessary, incubate at 37°C for 10-15 minutes to ensure complete dissolution of reagents.

NB. All aqueous buffers should be prepared in double distilled, or HPLC grade water (e.g. Chromasolv® water for HPLC , Sigma-Aldrich, Cat. No. 270733), filtered and degassed.

2. STANDARD AND SAMPLE PREPARATION

- **Perform <u>three independent HPLC assays</u>, using fresh ampoules/vials for each assay.**
  Due to limited ampoule availability, the 1st WHO IS (04/200), and coded ATD samples (A-D) may be included in just one assay.

- **Preparation of the standard curve of the primary calibrant (PRS0404).**
  A standard curve of the mean peak area resulting from a minimum of five quantities of rhPTH 1-34 should be prepared. Dilutions should be prepared <u>in duplicate</u>, using mobile phase A as the diluent. The precise mass of solvent added to the primary calibrant vial during the initial reconstitution step should be measured using a weighing balance, <u>and reported in the provided results sheet</u>, alongside details of serial dilutions.

  For example, reconstitute the primary calibrant vial with 2 mL mobile phase A (and record the weight of solvent added to the vial), to obtain a rhPTH 1-34 concentration of 0.5 mg/mL. Use this

stock solution to accurately prepare dilutions (in duplicate) of 0.35, 0.2, 0.1 and 0.05 mg/mL. During HPLC analysis 20 µl injections of each preparation will therefore provide a standard curve with determinations of 10, 7, 4, 2 and 1 µg rhPTH 1-34.

- **Preparation of ampoules; 04/200, 15/304, A, B, C, D**
  Reconstitute ampoule contents in 4 ml mobile phase A to obtain a rhPTH 1-34 concentration of 0.25 mg/mL.

  The precise mass of solvent added to each ampoule during reconstitution should be measured using a weighing balance, and reported in the provided results sheet.

NB. Samples are stable for 48 hours when stored at refrigerated temperature in a properly sealed container.

3. SAMPLE ANALYSIS

- **HPLC operating conditions:**
  - Flow rate — 1.0 mL/min
  - Injection volume — 20 µL
  - Column temperature — 40 °C
  - Autosampler — 5 °C
  - Run time — 20 mins
  - Detection — UV at 214 nm
  - Mobile phase — 63% mobile phase A/37% mobile phase B (isocratic)
    If precipitation occurs then apply mild heading (20-25 ^{o}C).
    Stir continuously during analysis.

NB. The approximate retention time of rhPTH 1-34 is 7-11 mins. The mobile phase composition may be adjusted in order to obtain the desired retention time. Please report any deviations from the stated protocol.

- **System suitability**
  - Perform three injections of primary calibrant (PRS0404) containing 0.5 mg/mL rhPTH 1-34. See Figure 1 for a typical chromatogram.
  - The relative standard deviation (RSD) of the rhPTH 1-34 peak area must be ≤1.25%.
  - The mean for peak tailing (asymmetry) must be ≤1.5 for the rhPTH 1-34 peak, according to the USP definition:

$$T = \frac{w}{2f}$$

where w is the peak width measured at 5% of the peak height and f is the distance from peak start to the peak maximum at 5% of the peak height.

- **Sample analysis**
  - Perform one injection per individual primary calibrant (PRS0404) preparation (dilutions are prepared in duplicate, therefore a total of two injections per rhPTH 1-34 concentration will be performed).
  - Perform three injections per ampouled preparation.
  - Repeat the system suitability steps outlined above after every 15 injections, and additionally following the final injection. If the system suitability criteria are not met then the samples in the affected bracket must be rejected.

4. DATA ANALYSIS

For each independent assay perform the following steps:

- Using the mean rhPTH 1-34 peak area measured in the duplicate chromatograms of primary calibrant (PRS0404) dilutions, produce a standard curve of rhPTH 1-34 peak area vs rhPTH 1-34 concentration.

- Using the mean rhPTH 1-34 peak areas measured in the triplicate chromatograms of each ampouled preparation, calculate the rhPTH 1-34 content of each preparation using the standard curve of the primary calibrant, by linear regression analysis.

NB. Please record all details of system suitability samples, primary calibrant dilutions, and rhPTH 1-34 peak areas, concentrations and contents in the provided results sheet. Please include all corresponding chromatograms when returning data.

5. FIGURES

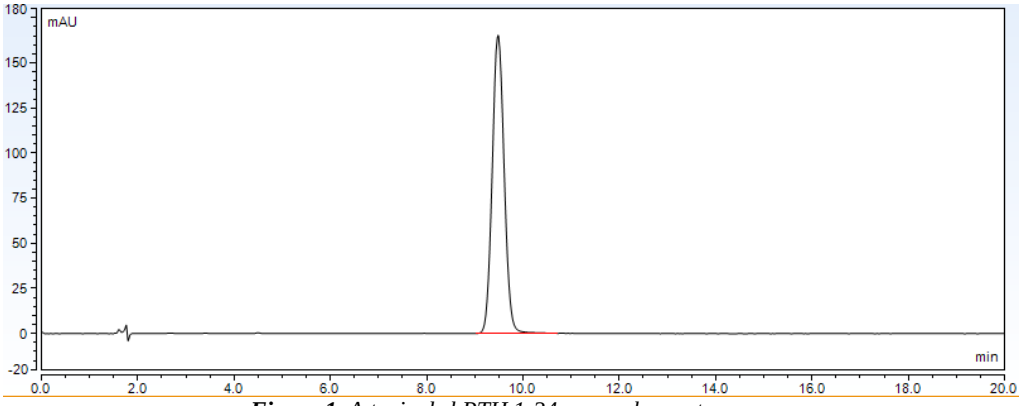

Figure 1. *A typical rhPTH 1-34 assay chromatogram.*

APPENDIX B

HPLC DETERMINATION OF PURITY OF THE CANDIDATE STANDARD (15/304)

1. KEY EQUIPMENT & MATERIALS

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

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

- **0.2 M sulphate buffer, pH 2.3**
  E.g. Dissolve 28.4 g of sodium sulphate (e.g. ACS reagent, ≥99%, Sigma-Aldrich Cat. No. 239313) in 900 ml water. Adjust the pH to 2.3 with 85% phosphoric acid (85-90%, for HPLC, Sigma-Aldrich Cat. no. 79606) and make up to 1 L with water.

- **Mobile phase A**
  90% sulphate buffer, 10% acetonitrile. Mix 1 volume of acetonitrile (e.g. HiPerSolv CHROMANORM® for HPLC, VWR Cat. No. 83639.320) with 9 volumes of sulphate buffer.

- **Mobile phase B**
  50% sulphate buffer, 50% acetonitrile. Mix equal volumes of acetonitrile and sulphate buffer. If necessary, incubate at 37^{o}C for 10-15 minutes to ensure complete dissolution of reagents.

NB. All aqueous buffers should be prepared in double distilled, or HPLC grade water (e.g. Chromasolv® water for HPLC , Sigma-Aldrich, Cat. No. 270733), filtered and degassed.

2. SAMPLE PREPARATION

- **Preparation of the system suitability sample.**
  A degraded sample is recommended for system suitability. Prepare this by reconstituting an ampoule of candidate standard (15/304) in 2 mL mobile phase A to give a rhPTH 1-34 concentration of 0.5 mg/ml. Adjust the pH to 3.0 using 1 M NaOH, and incubate the sample at 50 ^{o}C for 9 days.

NB. If precipitation is observed following sample incubation then add guanidine hydrochloride to a final concentration of 3 M.

- **Preparation of the candidate standard (15/304).**
  Reconstitute an ampoule of candidate standard (15/304) in 2 ml mobile phase A, to obtain a rhPTH 1-34 concentration of 0.5 mg/mL.

NB. Samples are stable for 48 hours when stored at refrigerated temperature in a properly sealed container.

3. SAMPLE ANALYSIS

- **HPLC operating conditions:**
  - Flow rate 1.0 mL/min
  - Injection volume 20 µL
  - Column temperature 40 °C
  - Autosampler 5 °C
  - Run time 45 mins
  - Detection UV at 214 nm
  - Mobile phase Gradient elution, as detailed in table 2.
    If precipitation occurs then apply mild heading (20-25 °C).
    Stir continuously during analysis.

Time (mins)	A (%)	B (%)
0	100	0
5	65	35
35	60	40
45	0	100
46	100	0
55	100	0

Table 2

NB. The approximate retention time of rhPTH 1-34 is 23 mins. If necessary the mobile phase B (%) at 5 and 35 minutes may be adjusted in order to obtain the desired retention time. Please report any deviations from the stated protocol.

- **System suitability**
  - Inject the system suitability sample. See Figures 2 & 3 for a typical chromatogram and integration.
  - The Peak-to-Valley ratio of the first post-main peak must be ≥1.5. It is defined by the formula:

$$R = \frac{h}{v}$$

    where h is the height of the first post-main peak and v is the height of the valley between the main peak and the first post-main peak.
  - Peak tailing (asymmetry) must be ≤2.0 for the rhPTH 1-34 peak, according to the USP definition:

$$T = \frac{w}{2f}$$

where w is the peak width measured at 5% of the peak height and f is the distance from peak start to the peak maximum at 5% of the peak height.

- **Sample analysis**
  - Inject a mobile phase A blank.
  - Perform <u>three</u> injections of the candidate standard (15/304) preparation. Integrate all peaks in the chromatograms except for those representing the sample solvent (mobile phase A).
  - Following sample injections, repeat the injection of a system suitability sample. If the system suitability criteria are not met then the test samples must be rejected.

4. DATA ANALYSIS

- Determine the mean relative peak areas (RPA%) of integrated peaks for the candidate standard (15/304) preparation. The mean RPA% for the main rhPTH 1-34 peak represents rhPTH 1-34 purity.

NB. Please record details of system suitability and test samples in the provided results sheet. Please include all corresponding chromatograms when returning data.

5. FIGURES

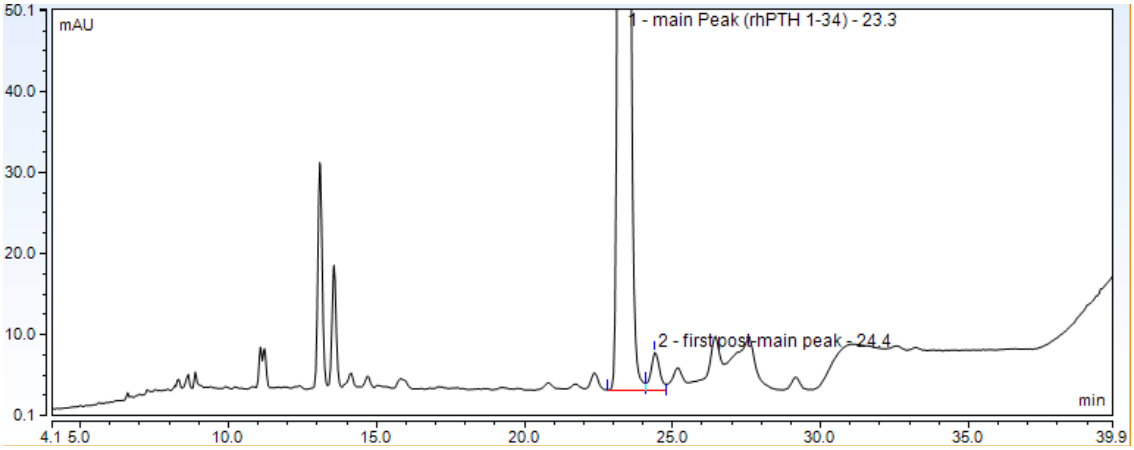

Figure 2. *Example of system suitability sample displaying adequate peak-to-valley ratio around the main peak.*

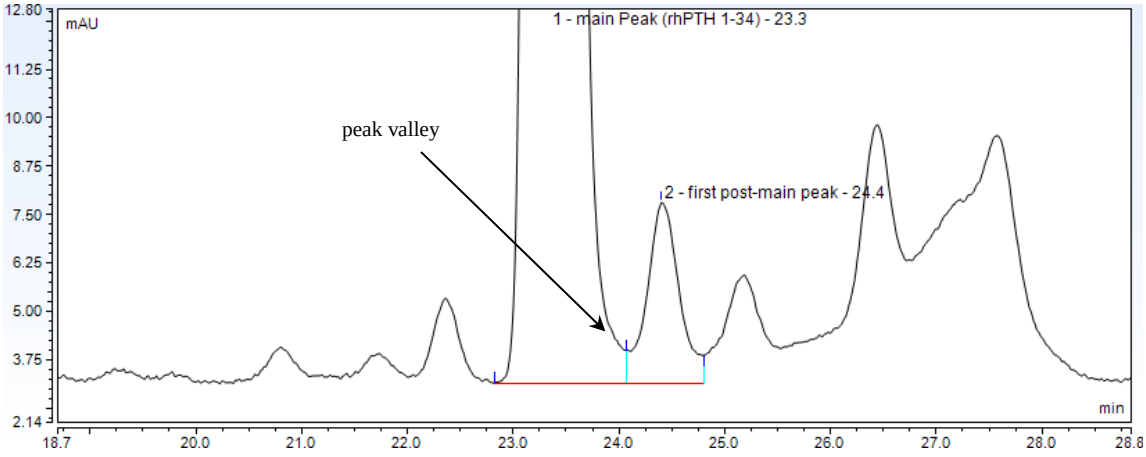

Figure 3. *Expanded view of the system suitability sample and the height and valley regions of the chromatogram*

Appendix 2: Draft Instructions for use

2nd WHO International Standard
Parathyroid Hormone 1-34, Recombinant, Human
NIBSC Code: 04/200

Instructions for use
(Version 1.0, Dated 22/05/2017)

1. INTENDED USE

The 1st WHO IS for rhPTH 1-34, coded 04/200, has been widely used for the calibration of HPLC assays, immunoassays and bioassays for rhPTH 1-34. Stocks of the 1st IS are almost exhausted and the WHO Expert Committee on Biological Standardization (ECBS) has recognized (2016) the need for a replacement IS. A preparation of rhPTH 1-34, coded 15/304, was ampouled and evaluated for its suitability to serve as a WHO International Standard by international collaborative study. This material was established at the 68th Meeting of WHO ECBS (2017) and replaces the 1st WHO IS for rhPTH 1-34, 04/200.

2. CAUTION

This preparation is not for administration to humans.

This material is not of human or bovine oririn. As with all materials of biological origin, the preparation should be regards as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials to avoid cuts.

3. UNITAGE

Each ampoule of 15/304 contains 0.91 mg rhPTH 1-34 per ampoule.
The assigned content has an estimated uncertainty of 0.65%, derived from between assay variability, the homogeneity of the fill and the uncertainty of primary calibrant content.
For bioassay and immunoassay purposes, it is also proposed that this material be assigned an ampoule content of 9100 IU.

4. CONTENTS

Country of origin of biological material: United States
Each ampoule contains the residue, after freeze-drying, of 1.0 ml of a solution which contained:

rhPTH 1-34	approximately 1 mg
Trehalose	10 mg in 1 mM acetic acid

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

DIN ampoules have an "easy-open" coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.
Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

For all practical purposes each ampoule contains the same quantity of the substances listed above. Dissolve the total contents of the ampoule in a known volume of a suitable solvent, with carrier protein (0.05 - 0.1% w/v BSA or HSA) if necessary. Rinse the ampoule several times using the known volume of solvent to ensure recovery of ampoule contents returning the rinses to the known volume. No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. For economy of use, it is recommended that the solution be sub-divided into several small containers and stored at -40°C, or below. The ampoules do not contain bacteriostat and a solutions of the material should not be assumed to be sterile.

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

9. ACKNOWLEDGEMENTS

We gratefully acknowledge the important contributions of all the participants in the collaborative study and Eli Lilly & Co. Ltd, who kindly donated the rhPTH 1-34 material.

10. 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

11. 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

12. 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.

13. MATERIAL SAFETY SHEET

Physical and Chemical properties (at room temperature)	
Physical appearance : Freeze dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify)	
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: 10 mg
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.

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