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PROPOSED 1ST WHO INTERNATIONAL STANDARD FOR ANTI-THYROID PEROXIDASE ANTIBODIES

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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 **17 September 2021** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technical Standards and Specifications (TSS). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

Summary

Stocks of the NIBSC Reference Reagent for anti-thyroid microsome serum, 66/387, are exhausted, and the World Health Organisation (WHO) Expert Committee on Biological Standardization (ECBS) has recognised (2018) the urgent need for a replacement standard to be prepared for the calibration of assays used in the diagnosis of thyroid autoimmunity and disease.

We report here the evaluation of a candidate preparation of anti-thyroid peroxidase autoantibodies (anti-TPO), filled into ampoules coded 19/260, in an international collaborative study carried out by 7 laboratories in 6 countries. The evaluation of the candidate preparation was performed by immunoassay in comparison with the existing NIBSC Reference Reagent, 66/387, and a panel of human serum and plasma samples containing a range of anti-TPO antibody concentrations. From the data provided by the 7 laboratories, a total of 13 different immunoassay methods were analysed. The two standards behaved in a similar manner in the immunoassays included in this study, with the relative potencies of the NRR, 66/387, and candidate standard 19/260 in reasonable agreement with an overall geometric mean of 871 IU/ml (95% CI 800 – 948 IU/ml), a median of 872 IU/ml and a robust mean of 879 IU/ml for the NRR 66/387, and a geometric mean of 623 IU/ml (95% CI 521 – 745 IU/ml) , a median of 637 IU/ml and a robust mean of 628 IU/ml for the candidate standard 19/260. Taking the robust mean for 66/387, this is equivalent to 1099 IU/amp, which is in good agreement with the assigned potency of 1000 IU/amp. Calculated relative to the NRR, the overall geometric mean potency for 19/260 was 571 IU/ml (95% CI 493 – 662 IU/ml), with a median of 533 IU/ml and a robust mean of 555 IU/ml. Taking the robust mean, this is equivalent to an overall geometric mean of 555 IU/amp. Potency estimates for 19/260 in terms of the NRR 66/387 were in reasonable agreement, with an inter-lab GCV of 32%. The data demonstrated the candidate IS, 19/260, with an assigned potency of 555 IU/amp, to be immunoreactive and behave in a similar manner to the NRR 66/387 in the immunoassays included in this study, indicating that continuity of anti-TPO measurements would be achieved with the introduction of 19/260 as the 1st International Standard to replace the NIBSC Reference Reagent, 66/387.

The inclusion of a panel of human serum and plasma samples in the study enabled an assessment of the impact of the new standard on the routine measurement of anti-TPO autoantibodies in human samples. These serum and plasma samples were measured in parallel with the candidate standard 19/260 and NRR 66/387 by all laboratories. The assessment of commutability was performed using a difference in bias approach. This assessment found that the candidate standard 19/260 and current NRR, 66/387 were both commutable with all laboratory methods, with the majority of bias values for individual sample potency estimates lying within the set commutability limits.

Analysis of the stability of the candidate standard was performed by immunoassay of accelerated thermal degradation (ATD) study samples stored at elevated temperatures for 7 months. Using the Arrhenius model to predict the degradation of the candidate over time, the estimated yearly loss of activity at storage temperatures of -20ºC was approximately 0.013%, indicating that the candidate standard is sufficiently stable to serve as an International Standard.

Taken together, these results indicate the candidate standard 19/260 is suitable to serve as an International Standard for the continued calibration of immunoassays for the measurement of anti-TPO autoantibodies. It is proposed that the candidate preparation in ampoules coded 19/260 is established as

the 1st International Standard for anti-Thyroid Peroxidase antibodies with an assigned content of 555 IU/amp.

Introduction

Autoimmune thyroid disease is the most common autoimmune disease, and includes several inflammatory thyroid diseases, with Graves' Disease and Hashimoto's the most frequent forms [1-3]. The NIBSC Reference Reagent (NRR), 66/387, for anti-thyroid microsome serum, was produced in the 1960's from a pool of sera from three patients who showed autoimmune anti-thyroid microsome activity. The target for this anti-thyroid microsome autoimmunity has since been identified as thyroid peroxidase (TPO) [4-6], a membrane-bound enzyme that is responsible for iodine oxidation and iodination of the thyroglobulin molecule [2,6]. Autoantibodies to TPO are present in thyroid diseases such as Hashimoto's thyroiditis and hyperthyroidism [2,3].

The development of immunoassays to detect anti-TPO autoantibodies are an important component in the diagnosis of thyroid autoimmunity and disease, and the NRR 66/387 has been widely adopted for the calibration of these immunoassays [3,7]. However, stocks of the NRR 66/387 are now depleted. Given the widespread adoption of the NRR, there is an urgent need to provide a replacement standard for anti-TPO autoantibodies.

To prepare a candidate replacement standard (the 1st International Standard, IS) for anti-TPO antibodies, a bulk of human serum from three donors containing high anti TPO antibody titres was prepared and has been filled into ampoules (NIBSC code 19/260), following procedures recommended by WHO [8]. This batch of ampoules was evaluated in an international collaborative study with expert laboratories to determine the immunoreactivity of the candidate standard and assess its suitability to serve as a calibrant for immunoassays of anti-TPO antibodies. Human serum and plasma samples containing a range of anti TPO concentrations were included in the study in order to assess commutability of the candidate standard with native samples. It was intended to assign a value to the proposed 1st IS by immunoassay in units of IU/ampoule in terms of the NRR, 66/387.

The aims of the collaborative study were therefore:

1. To confirm the immunoreactivity of the 1st IS by immunoassay, and to assess the relationship of the activity of the 1st IS with the NRR, 66/387, and existing local standards.
2. To calibrate the candidate 1st IS for anti-TPO antibodies relative to the NRR, 66/387, by immunoassay.
3. To assess the commutability of the candidate 1st IS with native samples in immunoassays.
4. To assess the stability of candidate 1st IS by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study by immunoassay.

Participants

A total of seven laboratories from six countries participated in the collaborative study. These laboratories are listed alphabetically by country in table 1. Throughout the study, each of the seven participating laboratories 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

CHINA	Dr Nan Sun, National Institutes for Food and Drug Control, In vitro Diagnostic Laboratory 67095322, No 2. Tiantan Xili, Dongcheng District, Beijing, 100050
FRANCE	Gregory Barbero, Dr Beatrice Bourcier and Dr Boris Mirabella, Beckman Coulter Marseille – Immunotech, 130 Av Delattre de Tassigny, 13009 Marseille
GERMANY	Dr Michael Rottman, Roche Diagnostics GmbH, Nonnenwald 2, 82377 Penzberg
ITALY	Lorenzo Sangalli, Technogenetics s.r.l. Via della Filanda 24-26, 26900, Lodi
UNITED KINGDOM	Haf Saxby and Dr Rachel Morris, Siemens Healthcare Diagnostics Products Ltd, Glyn Rhonwy, Llanberis, LL55 4EL
USA	Dr Paul D'Agostino and Dr Hans Zou, Siemens Healthcare Diagnostics, 511 Benedict Avenue, Tarrytown, New York 10591
USA	John Rapp, Timothy Bliese and Dr Rushad Daruwala, Abbott Diagnostics, 326 Burwood Drive, Waukegan, IL 60085-8320

Materials and Methods

Bulk materials and processing

A batch of human serum containing high anti-TPO antibodies was produced by pooling defibrinated plasma from three donors, purchased from TCS Biosciences Ltd (Buckingham, UK) to produce a total volume of 1909 mL. This serum was buffered with 40 mM HEPES and aliquots of 1.0 ml were then dispensed into glass ampoules, lyophilised and sealed according to procedures recommended by WHO [8]. Bulk material processing was carried out on 28th February 2020 at NIBSC (Blanche Lane, Potters Bar, EN6 3QG, UK). Ampoules are stored at -20ºC in the dark at this address.

The bulk defibrinated plasmas were tested and found negative for anti-HIV 1 and 2, anti-HCV, HBSAg and syphilis, and negative for HIV 1 and HCV by PCR. assay HBsAg.

Product characterisation

A total number of 1839 ampoules, coded 19/260, were produced and are stored at -20ºC under temperature-controlled conditions at NIBSC (Potters Bar, UK). Check weights measured during filling demonstrated a mean fill weight of 1.0083 g, with a low CV of fill of 0.18% (n=78), a mean dry weight of 0.07764 g (CV 0.17%, n=6), mean residual moisture of 0.6058 %(CV 7.15%, n=12)

as determined by Karl Fischer titration and mean oxygen head space of 0.38% (CV 46.33%, n=12). No microbial contamination was detected in the pre-fill or post-filled or post freeze-dried material.

Collaborative study design for the value assignment of 19/260 by immunoassay

Materials

The collaborative study was organised by NIBSC. All participants were provided with a set of samples consisting of the NRR 66/387, duplicates of the candidate standard 19/260 and a panel of six human serum and six human plasma samples as described in Table 2 below. Samples of the candidate standard stored at elevated temperatures of +4º, +20º, +37º and +45º C were also provided to participants where assay space permitted.

Table 2. Preparations sent to participants

Anti TPO preparation	Ampoule content
NIBSC Reference Reagent for anti-TPO, 66/387	1000 IU per ampoule
Coded preparations of the candidate 1st IS, 19/260, stored at -20ºC	Nominally 800 IU per ampoule
Accelerated thermal degradation (ATD) samples of 19/260 stored at +4ºC, +20ºC, +37ºC and +45ºC, coded	Content assumed identical to 19/260 stored at -20ºC
Twelve human serum/plasma samples labelled TPOSerum1 to TPOSerum6 and TPOPlasma7 to TPOPlasma12	0.5ml aliquots human serum or human plasma

Methods contributed

Participants were requested to perform the immunoassay normally used in house for the measurement of anti-TPO. The immunoassay methods contributed, in alphabetical order, were: Abbott Architect; Beckman Access, Beijing Leadman C12000S; Diasorin Liaison; Maccura Biotech i3000; Mindray Biomedical cl2000i; Roche Elecsys, Siemens Immulite; Siemens ADVIA XPI; Siemens Atellica; Siemens Centaur XP; Snibe Maglumi and Technogenetics Zenit RA.

In order to assess commutability, participants were requested to test a set of common dilutions of each of the ampouled preparations provided (Table 2) in parallel with each set of the human serum/plasma samples (labelled TPOSerum 1-6 and TPOPlasma 7-12). The core dilutions were 800, 400, 200, 100, 50, 25 and 12.5 IU/ml, and a guide for their preparation was provided in the assay protocol (Appendix 1). Participants were requested to measure, in triplicate, the anti-TPO content of these and the set of human serum/plasma samples plus in house standard/controls, ensuring that a minimum of five points in the linear part of the dose response curve were included. These measurements formed one independent assay, and where possible, participants were requested to

perform at least two independent assays. A total of 7 laboratories contributed immunoassay data to the study, performing 13 different immunoassay methods.

Stability assessment

A thermally accelerated degradation (ATD) study was designed to assess the stability of the candidate standard 19/260. Coded samples that had been stored at elevated temperatures of +4º, +20º, +37º and +45º C for 7 months were assayed by participants in comparison with a coded ampoule of 19/260 stored at -20ºC. Stability data was provided by 4 participants using 5 different immunoassay methods.

Data and Statistical Analysis

Participants were asked to return all raw assay data in electronic form for central computation at NIBSC, plus participants' own estimates of activity as calculated by the method normally used in their laboratory.

Potency assignment of 19/260

Analysis was based on the results supplied by the participants, reported in IU/mL. To calculate laboratory reported potency estimates, first the mean $\log_{10}$ value at each dilution was calculated in each sample and assay run. Then a single estimate of $\log_{10}$ copies/mL was obtained for each sample within an assay run by correcting for the corresponding dilution factors and taking the arithmetic mean value across the results. A single estimate for each sample within the laboratory and assay method was then calculated as the arithmetic mean of the $\log_{10}$ estimates of IU/mL across assay runs.

Relative potencies where data was available across a range of doses for both reference and test sample were estimated using a parallel line model with $\log_{10}$-transformed IU/mL values as responses [9]. Calculations were performed using the R software package [10]. Estimates were calculated from this analysis, using an assigned or proposed value in International Units/mL (IU/mL) for the existing or candidate standard where appropriate. Estimates from all valid assays were combined to generate a geometric mean value for each laboratory and assay type.

Relative potencies for samples with only a single dilution were obtained by fitting a linear model to $\log_{10}$-transformed IU/mL against $\log_{10}$-dose and interpolating relative potency values for test samples from the model. Calculations were performed using the R software package [10]. Potency estimates were calculated from this analysis, using an assigned or proposed value in International Units/mL (IU/mL) for the existing or candidate standard where appropriate. Estimates from all valid assays were combined to generate a geometric mean value for each laboratory and assay type.

Overall analysis was based on the laboratory geometric mean estimate values. Overall sample estimates were calculated as the geometric means of all individual laboratories. Variation between laboratories (inter-laboratory) was expressed as % geometric coefficient of variation (GCV = $\{10^{s}-1\}\times100\%$ where s is the standard deviation of the $\log_{10}$ transformed estimates) of the estimates. To provide estimates that reduce the influence of more extreme laboratory potency estimates median value were calculated, as well as Huber's robust mean using the R package "WRS2" [11].

Assessment of commutability

Commutability of the candidate IS, 19/260, and the current NRR, 66/387, was assessed using a difference in bias approach. Geometric mean estimates for serum and plasma samples were calculated from reported estimates and estimates relative to both 19/260 and 66/387. Median values, calculated from $\log_{10}$ transformed estimates for analysis in order to achieve approximately constant scatter over the range of concentrations used, were used as the study consensus values for each sample in the analysis. Bias values were calculated as the difference between laboratory mean reported log estimates to the log study consensus value for the sample. In order to derive an acceptable bias range (for analysis of this study only), the standard deviation of the log transformed bias values was calculated within each laboratory, and a median value, s_P, was calculated across all laboratories. Criteria representing the maximum bias range were then set at ± 0.356 $\log_{10}$ (44% to 227%). Reference standards were to be concluded as commutable if the observed difference in bias was within the commutability criteria. A further range of ± 0.2 $\log_{10}$ (63% to 158%) is also included to help illustrate differences in tables and graphs. For this commutability assessment, the bias for plasma and serum samples has been assumed to be constant over the concentration range used.

Assessment of stability

Samples stored at elevated temperatures (+4, +20, +37, +45ºC) and a reference temperature (-20ºC), were analysed via immunoassay, with the intention of fitting an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay [12], and thus predict the degradation rates when stored at a range of temperatures.

Results

Data returned for analysis

A total of 32 immunoassays were returned for central analysis from 7 laboratories performing a total of 16 immunoassays using 13 different immunoassay methods. All assays included kit controls/standards and met the participants' associated acceptance criteria.

Validity criteria

The analysis of immunoassay data was based on the results from nominal concentrations 12.5 – 800 IU/ml for both 66/387 and 19/260 coded duplicates (19/260-A and 19/260-B). For reported estimate calculations, dilutional linearity (parallelism with kit standards) was considered acceptable if the slope of the fitted regression line for $\log_{10}$-transformed IU/mL against $\log_{10}$-dose was within the range of 0.80 to 1.25; results were excluded from further calculations if this was not the case. Fitted slope ratios for each laboratory are reported in Table A2.1 (Appendix 2) and demonstrate broadly acceptable parallelism for all laboratories, with the majority of slope ratios in the range 0.8 – 1.25. Exceptions were Lab 6 (assay 1, sample B), Lab 7g (Assay 1, sample B and Assay 2 samples A and B), and Lab 7i (assays 1 and 2, samples A and B).

For relative potency analyses model fit was assessed visually and by calculating an r^2 value from the fitted model. Samples with an r^2 value below 0.95 were considered non-linear and excluded from further analyses (marked NL in tables). Where relevant, non-parallelism was assessed by calculation of the ratio of fitted slopes for the test and reference samples under consideration. The samples were concluded to be non-parallel when the slope ratio was outside of the range 0.80 – 1.25 and in these

cases, no estimates are reported (marked NP in tables). For serum/plasma sample estimates (Tables A2.2, A2.3 and A2.4, Appendix 2), if the range of response values for a test sample did not fall within the range of response values for a reference, then no estimates is reported (marked RR in tables). Laboratory 2b reported potency estimates for coded duplicates A and B in assay 1 of 954 and 690. Laboratory 4 reported potency estimates for coded duplicates A and B in assay 1 of 468 and 590. The ratio of these values is outside of limits set at 0.80 – 1.25 for duplicate samples, as such the assay was excluded from further calculations due to having unreliable results.

Estimated potency of the candidate IS, 19/260

Potency estimates (in IU/ml) for 66/387 (calculated relative to kit standards) and 19/260 coded duplicates (calculated relative to kit standards and 66/387) are summarised in Table 3, and are expressed in IU/ml in order to compare dilutions between the two standards. Overall potency estimates were calculated as the geometric means of all individual laboratories, with median and Huber's robust mean also calculated to provide estimates that reduce the influence of more extreme laboratory potency estimates. Relative to kit standards, laboratory geometric means for the NRR 66/387 ranged from 594 IU/ml – 1133 IU/ml, with an overall geometric mean of 871 IU/ml (95% CI 800 – 948 IU/ml), a median of 872 IU/ml and robust mean of 879 IU/ml (Table 3). Laboratories were in reasonable agreement, with an inter-lab GCV of 17%. This estimated potency is very close to that expected of 800 IU/ml (used as the top concentration in the assay) giving an overall robust mean of 1099 IU/amp, when initial dilution of the ampoule is taken into account, which is in very good agreement with the assigned value to 66/387 of 1000 IU/amp. As expected, the content of the candidate standard was lower than that of the NRR, with laboratory geometric means for the candidate standard in terms of kits standards ranging from 283 IU/ml to 1065 IU/ml, and an overall geometric mean of 623 IU/ml (95% CI 521 – 745 IU/ml), a median of 637 IU/ml and a robust mean of 628 IU/ml (Table 3). Here there was a wider inter-laboratory GCV of 38%. When calculated relative to the NRR 66/387, estimates of the candidate standard 19/260 ranged from 408 IU/ml – 970 IU/ml, with an overall geometric mean of 571 IU/ml (95% CI 493 – 662 IU/ml), equivalent to an overall geometric mean of 571 IU/amp (95% CI 493 –662 IU/amp), a median of 533 IU/amp and a robust mean of 555 IU/amp. A slight improvement in agreement between laboratories was observed for the candidate standard when calculated relative to the NRR 66/387, with an inter-laboratory GCV of 32%.

Overall, the data indicates that the candidate standard, 19/260, is immunoreactive, and appears to behave in a similar manner to the NRR 66/387 in current immunoassays, indicating that its introduction as the 1st IS to replace the NRR will enable continued calibration of immunoassays for anti-TPO. To illustrate this, Figure 1, in addition to potency estimates of the two standards, shows the estimates of the serum and plasma samples measured by all laboratories both as reported by each laboratory, and when expressed relative to either 66/387 (to which immunoassays are currently calibrated) or to the candidate standard, 19/260.

Table 3. Potency estimates (IU/ml) for 66/387 and 19/260 (NL = Non-linear; NP = Non-parallel; GM = Geometric Mean; CI = Confidence Interval; GCV = Inter-lab geometric coefficient of Variation (%)* = Not used in further summary calculations)

Lab	Calculated relative to kit standards								Calculated relative to 66/387				
	66/387			19/260					19/260				
	Assay 1	Assay 2	Lab GM	Assay 1		Assay 2		Lab GM	Assay 1		Assay 2		Lab GM
				A	B	A	B		A	B	A	B	
1	573	615	594	271	276	291	293	283	410	421	396	404	408
2a	822	793	807	943	944	1014	1017	979	901	907	1052	1032	970
2b	No data	873	873	978*	979*	1101	1031	1065	No data	No data	982	928	955
3	759	750	755	464	521	467	482	483	481	543	495	510	507
4	No data	950	950	468*	590*	541	560	550	No data	No data	462	465	463
5	977	971	974	833	833	855	827	836	680	680	703	678	685
6	834	No data	834	638	NL	No data	No data	638	584	656	No data	No data	619
7a	1024	956	990	870	888	806	831	848	679	693	674	694	685
7b	1009	962	986	557	563	556	541	554	398	403	419	410	407
7c	1192	1077	1133	677	686	617	624	651	451	457	452	455	454
7d	741	799	770	588	515	580	569	562	639	560	580	571	587
7e	878	860	869	535	545	548	542	543	493	500	511	508	503
7f	928	1170	1042	606	631	663	651	637	505	523	445	447	479
7g	709	740	724	640	NL	NL	NL	640	764	NP	NP	NP	764
7h	849	891	870	515	505	516	526	515	459	450	436	443	447
7i	917	943	930	NL	NL	NL	NL	-	565	536	572	NP	561
GM			871					623					571
95% CI			800 – 948					521 – 745					493 – 662
GCV			17%					38%					32%
Median			872					637					533
Robust Mean			879					628					555

Figure 1 Potency estimates of 66/387 and 19/260, and estimates of serum and plasma samples as reported by laboratories and when expressed relative to either standard preparation

Commutability of the candidate IS, 19/260, and the current NRR, 66/387

The commutability of the candidate IS, 19/260, and the current NRR, 66/387, with human serum and plasma samples was assessed for all methods included in the study using a difference in bias approach. This approach measures calibration effectiveness [13], by examining the observed method bias for clinical samples in a range of laboratory methods and the impact on the bias for these samples when calibrated relative to a reference material. Where calibration is effective at reducing bias for clinical samples (harmonising results), this indicates that the reference material is commutable with these samples in the methods used. Data used for the assessment of commutability are shown in Appendix 2, Tables A2.5, A2.6 and A2.7. Table A2.5 shows the laboratory reported estimates for the samples in each laboratory, taken as the reported concentrations using whichever assay kits they use. Laboratory estimates calculated relative to 66/387 and 19/260 sample A by parallel line analysis are shown in Tables A2.6 and A2.7 respectively. For both reported and relative values, laboratory median values calculated using $\log_{10}$ transformed estimates are shown for each commutability sample (S01-S06, T07-T12) and have been used as the study consensus values for each sample.

Samples within each analysis set were ranked by increasing level of concentration for the purposes of plotting the data in this order for presentation. Bias values were calculated as the difference between laboratory mean reported log estimate and the log study consensus value for the sample. These bias values were anti-logged for presentation in Tables 4, 5 and 6 and are also represented graphically in Figures 2, 3, and 4. Criteria representing the maximum range of bias were then set at ± 0.356 $\log_{10}$ (44% to 227%) calculated using the median intra-laboratory variability of bias values across samples, shown in Table A2.2 (Appendix 2). A narrower range of ± 0.200 $\log_{10}$ (63% to 158%) was also arbitrarily chosen to illustrate differences.

Bias values for estimates relative to the current NRR 66/387 and candidate standard 19/260 (table 5, figure 3 and table 6, figure 4 respectively) indicate how close the laboratory estimate for a sample is to the consensus value, where 100% is no difference. The dark shaded cells show where the bias for a particular sample relative to 19/260 or 66/387 is outside the commutability criteria of 44% to 227% and the light shaded cells show where the bias for a sample is outside the narrower range of 63% to 158%. Serum/plasma samples, either as reported or expressed relative to either standard behaved in a similar manner in all laboratories, with the exception of laboratory 1, in which the majority of samples showed a negative bias as reported but then fell within the limits of commutability when expressed relative to either 66/387 or 19/260 (Table 4, 5 and 6). Similarly, in laboratories 2a and 2b, although within the limits of commutability, a negative bias for serum and plasma samples was observed when expressed relative to 19/260 in comparison to 66/387 or as reported (Table 6, Figure 4). All laboratory methods show bias values for the majority of serum samples are within the commutability criteria when expressed relative to either standard, suggesting that both 19/260 and 66/387 are commutable with the majority of methods used in this study. Although the majority of samples were within the commutability limits, there were a handful of samples that fell outside these limits when expressed relative to 66/387 (Table 5) and three samples that fell outside the limits when expressed relative to 19/260 (Table 6). In Lab 1 and Lab 4, there was 1 sample that fell below these limits when expressed relative to 66/387. In Labs 2a and 2b there were 3 and 2 samples respectively that fell outside the commutability limits (both above and below the set limits). Similarly, in Lab 7g, expressed relative to 66/387, there were 3 samples that were above the commutability limit set. Interestingly, when expressed relative to 19/260, there is a slight improvement in the bias shown by some of these samples, which then fall within the commutability limits (Table 6).

For comparison, a narrower range of 63% to 158% for commutability limits was also included. Relative to 66/387, there were 4/16 laboratory methods with bias for all sample potency estimates that were within this narrower range (Labs 3, 5, 7a and 7d), with the remaining laboratory methods showing a minority (1-4 samples) outside of this narrower range. Relative to 19/260, there were 7/16 laboratory methods with all sample potency estimates within the narrower range (Labs 3, 5, 6, 7a, 7d and 7e). With the exception of Laboratory 2b and 4, which both had 5 samples fall outside of this narrower range, in the remaining laboratory methods there were only a minority (1-4 samples) of samples outside this range for any given method, indicating that for both standards, the majority of samples showed a bias within the narrower commutability range, and that there was a slight improvement observed in samples relative to 19/260. For comparison, for reported sample potency estimates, there were 3/16 laboratory methods with bias for all sample potency estimates within the narrower range (Labs 3, 7d and 7e). With the exception of Lab 1, there were only 1-3 samples outside the range for any given method.

Also of interest are the results obtained by the same immunoassay methods used in different laboratories. Specifically, the assay method used in Lab 4 is the same as that in Lab 7f, in Lab 5 the method is the same as that in Lab 7a and in Lab 6, the method is the same as that in Lab 7i. In all

cases where the same immunoassay method is used, a very similar pattern is also seen in the bias in the sample estimates, either as reported, or relative to either standard (figures 2, 3 and 4).

Plots showing all bias estimates from Tables 4, 5 and 6 are given in Figures 5a and 5b, and a summary table of Inter-Quartile ranges in Table 7. These further illustrate that the study data demonstrate a slight improvement in harmonisation (less bias) across the laboratories and methods included when samples 66/387 or 19/260 A are used as a reference, indicating calibration effectiveness of the two reference materials.

Table 4. Bias in reported sample potency estimates (laboratory estimate as a percentage of study median value for sample)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	64%	87%	42%	33%	44%	38%	43%	50%	47%	78%	47%	47%
2a	79%	105%	113%	317%	112%	108%	84%	102%	136%	261%	29%	134%
2b	77%	108%	125%	280%	112%	101%	97%	101%	148%	234%	30%	126%
3	80%	93%	73%	80%	69%	76%	81%	75%	93%	85%	100%	85%
4	124%	53%	51%	144%	102%	142%	114%	120%	112%	70%	220%	114%
5	96%	125%	115%	141%	99%	112%	133%	103%	137%	131%	162%	109%
6	113%	86%	46%	94%	97%	117%	83%	74%	68%	84%	77%	44%
7a	101%	135%	128%	137%	96%	99%	118%	99%	134%	139%	159%	108%
7b	191%	84%	106%	96%	97%	142%	100%	162%	111%	103%	132%	100%
7c	105%	160%	147%	78%	101%	96%	123%	94%	90%	163%	122%	80%
7d	90%	98%	97%	96%	82%	88%	105%	80%	94%	106%	130%	77%
7e	99%	120%	97%	76%	103%	94%	69%	107%	116%	124%	100%	103%
7f	126%	55%	112%	159%	104%	115%		115%	107%	72%	228%	132%
7g	272%	102%		287%	240%	78%		122%	79%	89%		
7h	74%	117%	100%	50%	74%	65%	117%	74%	70%	97%	71%	60%
7i	136%	93%	80%	104%	108%	131%	100%	87%	75%	89%	75%	44%

Outside the range of 63% to 158%

Outside the range of 44% to 227%

Table 5. Bias in sample potency estimates calculated relative to Sample 66/387 by parallel line analysis (laboratory estimate as a percentage of study median value for sample)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	92%	114%	68%	43%	70%	52%		75%	67%	103%	69%	77%
2a	93%	101%		313%	133%	117%		121%	144%	253%	32%	152%
2b	86%	97%		256%	123%	104%		109%	144%	216%	32%	137%
3	94%	98%	94%	88%	88%	86%	89%	92%	107%	91%	118%	111%
4	134%	46%	44%	121%	102%	134%		128%	101%	63%	175%	100%
5	91%	101%	106%	119%	98%	100%		100%	122%	109%	145%	107%
6	111%	87%	67%	97%	121%	120%		75%	72%	83%	91%	58%
7a	94%	107%	121%	114%	94%	87%	95%	95%	117%	113%	140%	103%
7b	159%	67%	125%	79%	96%	118%	105%	144%	99%	83%	134%	117%
7c	82%	113%		56%	86%	72%		76%	68%	117%	96%	69%
7d	110%	99%	104%	103%	102%	100%		100%	105%	111%	143%	93%
7e	106%	108%	105%	73%	114%	95%	62%	118%	116%	115%	100%	112%
7f	133%	45%	82%	120%	96%	106%		118%	89%	61%	154%	98%
7g	335%	107%		263%	301%	93%		157%	93%	97%		
7h	72%	109%	117%	46%	82%	61%	120%	76%	69%	90%	74%	70%
7i	115%	85%	96%	96%	121%	117%	114%	76%	70%	77%	79%	52%

Outside the range of 63% to 158%

Outside the range of 44% to 227%

Table 6. Bias in sample potency estimates calculated relative to 19/260 Sample A by parallel line analysis (laboratory estimate as a percentage of study median value for sample)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	124%			63%	92%	80%		114%	97%	141%		
2a	56%	52%			57%	69%		72%	67%		14%	
2b	51%	47%			51%	60%		66%	64%		13%	
3	103%	129%	108%	106%	97%	109%	124%	113%	130%	106%	115%	139%
4	161%	64%	51%	152%	118%	182%		171%	128%	78%	172%	127%
5	67%	95%		103%	76%	87%		84%	106%	90%	104%	100%
6	99%	100%	73%	101%	116%	128%		75%	74%	83%	79%	64%
7a	72%	102%	104%	100%	74%	78%	100%	83%	104%	96%	100%	96%
7b	205%	108%		114%	128%	178%		213%	147%	117%	167%	
7c	98%	159%		73%	102%	99%		102%	89%	147%	100%	92%
7d	94%	106%		100%	90%	101%		98%	103%	105%	115%	97%
7e	114%	138%	116%	85%	121%	117%	83%	142%	136%	130%	94%	135%
7f	146%	59%	100%	150%	108%	136%		148%	111%	72%	159%	129%
7g	234%	78%				85%		133%	69%	69%		
7h	74%	170%		58%	100%	77%		93%	91%	117%	84%	108%
7i	101%	96%	98%	98%	115%	124%		75%	70%	75%	67%	57%

Outside the range of 63% to 158%

Outside the range of 44% to 227%

Figure 2. Bias in reported sample potency estimates (laboratory estimate as a percentage of study median value for sample)

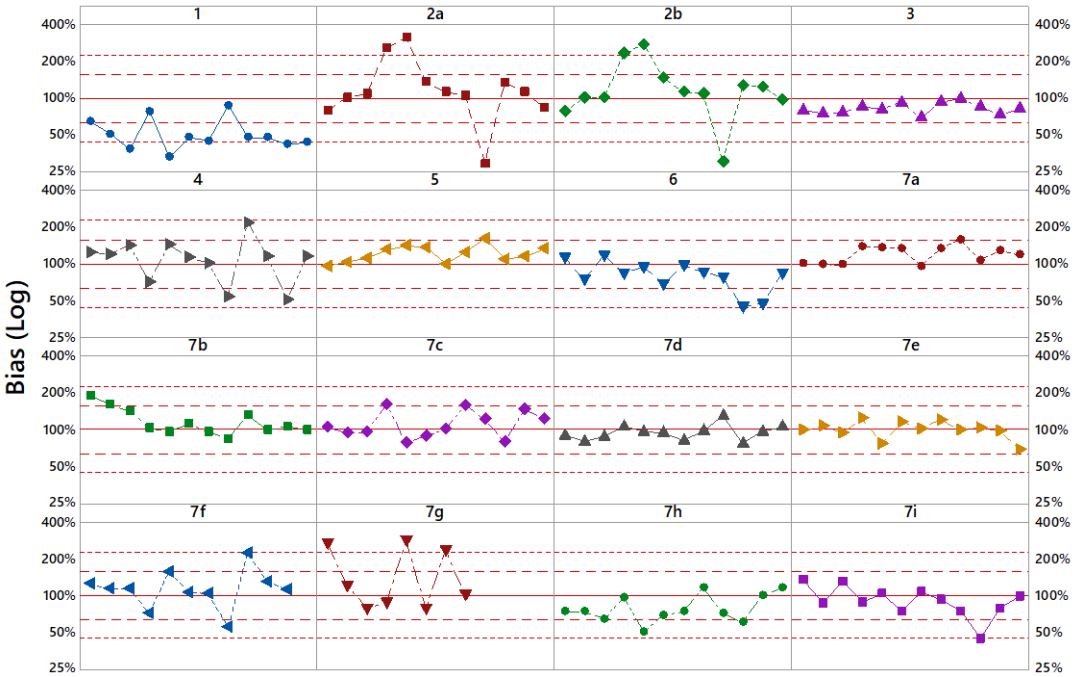


Figure 3. Bias in sample potency estimates calculated relative to Sample 66/387 by parallel line analysis (laboratory estimate as a percentage of study median value for sample)

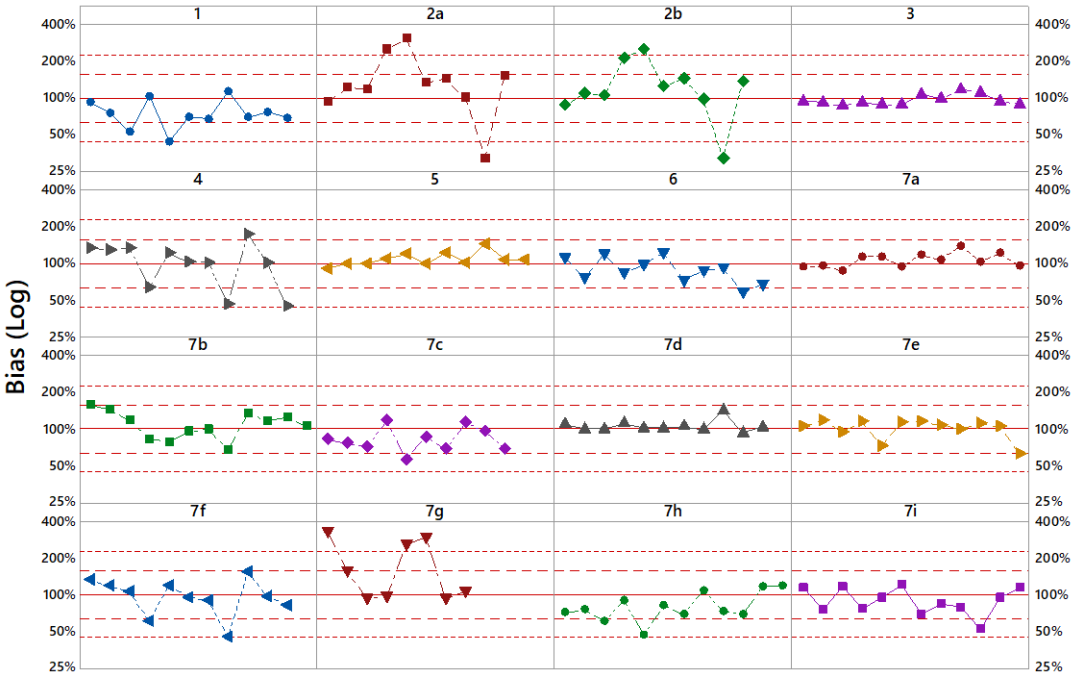


Figure 4. Bias in sample potency estimates calculated relative to 19/260 Sample A by parallel line analysis (laboratory estimate as a percentage of study median value for sample)

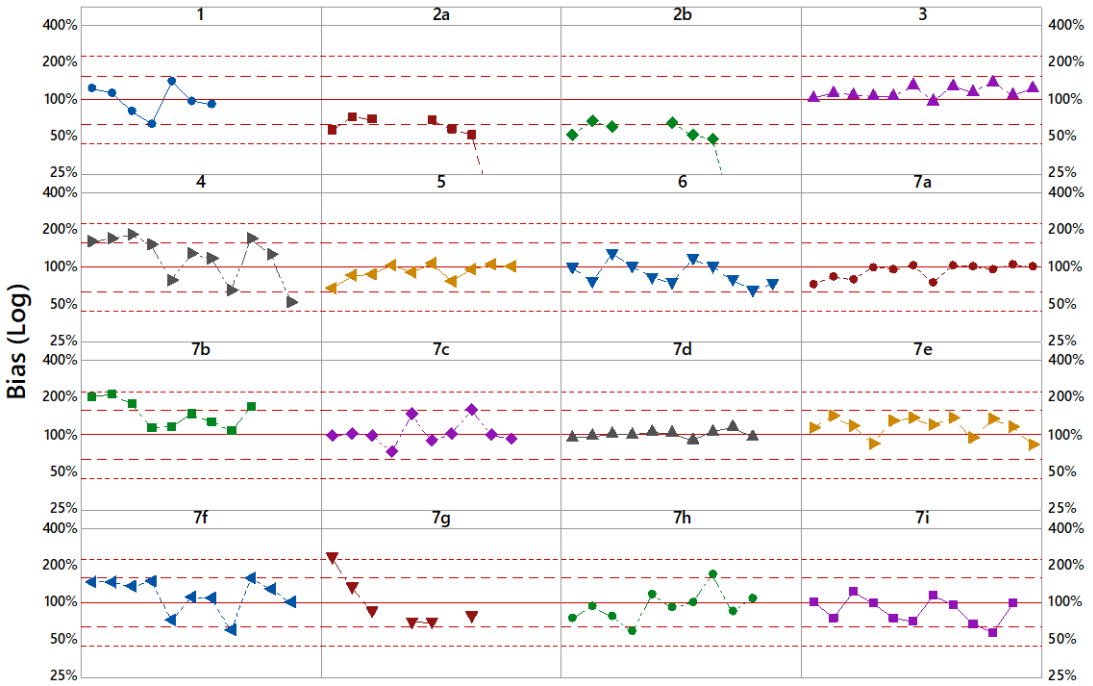


Table 7. Summary Statistics for Bias

Method	Lower Quartile	Upper Quartile	Inter-Quartile Range
Reported	80%	120%	41%
Relative to 66/387	83%	117%	34%
Relative to 19/210-A	78%	118%	40%

Figure 5a. All study bias estimates for samples (reported and relative via parallel line analysis, as percentage of study median value for sample) shown as an individual value plot

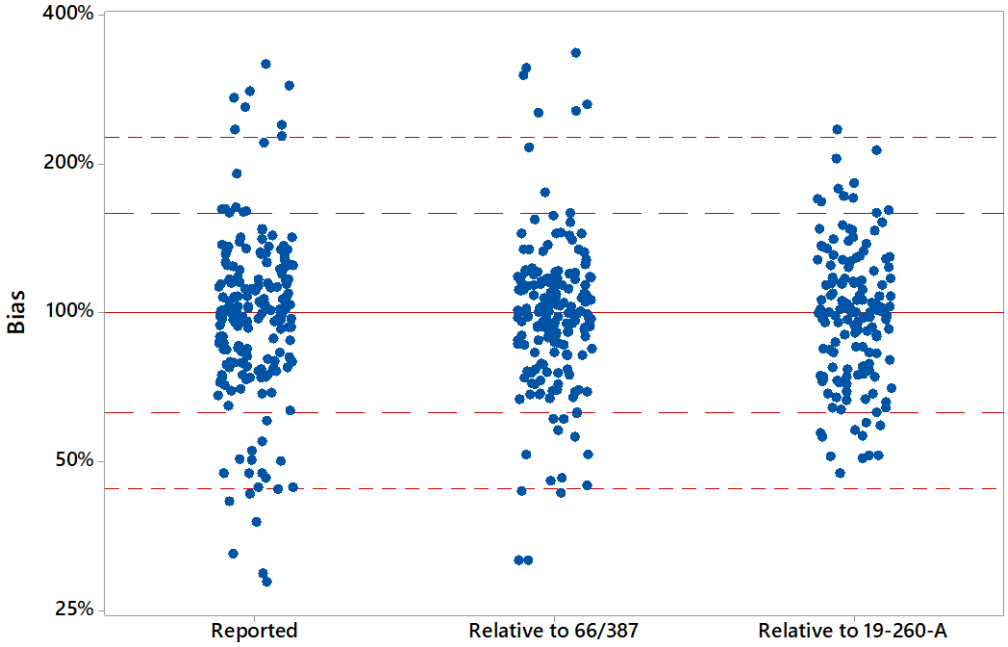
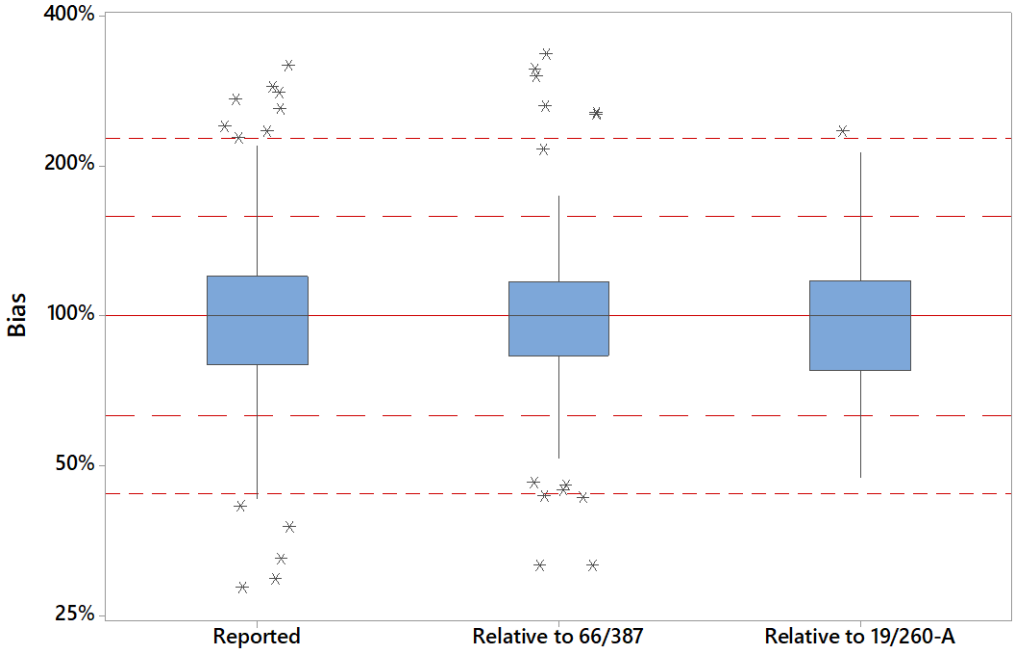


Figure 5b. All study bias estimates for samples (reported and relative via parallel line analysis, as percentage of study median value for sample) shown as a box-plot



Stability of 19/260

Ampoules of 19/260 stored at elevated temperatures of +4ºC, +20ºC, +37ºC and +45ºC for 7 months were analysed by 5 immunoassay methods from 4 laboratories. Data from 1 laboratory (Lab 2b) were not included in overall relative potency calculation due to lack of linearity with the -20ºC reference sample. Potencies of anti-TPO antibody content at each elevated temperature were calculated relative to that of ampoules stored at -20ºC, and the data is summarised in Table 8. At all storage temperatures there is a little loss in activity observed, which is increased at the storage temperatures of +37ºC and +45ºC with relative potencies of 0.942 and 0.957 respectively. This data was used to fit the Arrhenius model, which gave an estimated yearly loss of activity at -20ºC of 0.013% [12], indicating that the candidate standard is sufficiently stable to serve as an International Standard.

Table 8 Potencies (anti-TPO content by immunoassay) of ATD samples relative to the reference sample (stored at -20ºC).

ATD sample	Geometric Mean Relative Potency	95% Confidence Intervals
+4ºC	1.014	1.003 – 1.024
+20ºC	0.981	0.956 – 1.002
+37ºC	0.942	0.924 – 0.961
+45ºC	0.957	0.837 – 1.088

Discussion

The current NRR, 66/387, has been widely adopted for the calibration of immunoassays for anti-TPO autoantibodies. Stocks of this standard are now exhausted, and a replacement standard is urgently needed. This study describes the preparation and evaluation of a candidate 1st International Standard for anti-TPO autoantibodies, coded 19/260.

Immunoassay data from 7 laboratories (a total of 16 laboratory methods) were analysed to determine the potency of 19/260 in terms of the NRR 66/387. Estimates of the standard preparation 66/387, when calculated relative to kit standards, were in reasonable agreement with an overall geometric mean of 871 IU/ml (95% CI 800 – 948 IU/ml), a median of 872 IU/ml, a robust mean of 879 IU/ml and an inter-laboratory GCV of 17%. The overall robust mean is equivalent to 1099 IU/amp, which is in very good agreement with the assigned unitage of 1000 IU/amp. A wider range of potency estimates for 19/260 were obtained relative to kit standards, with an overall potency estimate of 623 IU/ml (95% CI 521 – 745 IU/ml), a median of 637 IU/ml, a robust mean of 628 IU/ml and an inter-laboratory GCV of 38%. When calculated relative to the NRR 66/387, the overall potency for 19/260 was 571 IU/ml (95% CI 493 – 662 IU/ml), a median of 533 IU/ml and a robust mean of 555 IU/ml, with an inter-laboratory GCV of 32%, indicating slightly improved agreement in potency estimates of the standard when expressed relative to the NRR 66/387. Due to the wider GCV for the estimated potencies of 19/260, the robust mean of 555 IU/ml, equivalent to 555 IU/amp is recommended as

the estimated potency of the candidate standard 19/260. Overall, the candidate standard has been demonstrated to be immunoreactive, and behaves in a very similar manner to 66/387 in the immunoassays included in this study, indicating that its introduction as the 1st IS to replace the NRR 66/387 will enable continued calibration of immunoassays for anti-TPO.

Both the candidate standard 19/260 and the NRR 66/387 were analyzed in comparison with patient samples by immunoassay to determine the calibration effectiveness, as an indicator of commutability, of the standards with patient samples in these assays using a difference in bias approach. Of the 16 immunoassay methods in which both 19/260 and 66/387 were analyzed, both reference standards demonstrated commutability with samples in all methods, with the majority of bias values for serum and plasma samples relative to either standard falling within the commutability limits set. Although both standards were found commutable with the majority of methods, there was a slight improvement in study bias estimates when reported relative to 19/260 in comparison with those relative to 66/387 or laboratory reported values, providing further evidence for the suitability of 19/260 as a calibrant for immunoassays for anti-TPO.

In summary, the candidate standard 19/260 exhibited the expected immunoreactivity and demonstrated similar behavior to the NRR 66/387 in the immunoassays contributed. In addition, prediction of the long-term stability during storage at -20ºC using the Arrhenius model gave estimated 0.013% loss of activity per year indicating that the candidate standard is sufficiently stable to serve as an International Standard.

Proposal

It is proposed that the candidate preparation in ampoules coded 19/260 is established as the 1st International Standard for anti-Thyroid Peroxidase antibodies with an assigned content of 555 IU/amp.

Comments from participants

Laboratory 1: gave a correction to their address which has been incorporated.

Laboratory 2: initial analysis of the study data indicated that data from Lab 2a and 2b be excluded due to non-linearity of the candidate standard doses relative to kit standards. Discussion with the laboratory led to exclusion of the top 3 dose points from the analysis of the laboratory's data, resulting in acceptable linearity and inclusion of the 19/260 data set.

Laboratories 3 to 7: no specific comments or corrections.

Acknowledgements

We gratefully acknowledge the important contribution of all invited participants, and in particular we would like to thank them for their continued support of this study during the Covid-19 pandemic. We would also like to acknowledge the important contributions of our NIBSC colleagues: the Standardisation Science Group for preparation of trial fill materials and the Standards Processing Division for the preparation and dispatch of ampouled materials.

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APPENDIX 1 PARTICIPANT STUDY PROTOCOL

INTERNATIONAL COLLABORATIVE STUDY TO ESTABLISH THE 1ST WHO INTERNATIONAL STANDARD FOR ANTI-THYROID PEROXIDASE ANTIBODIES

INTRODUCTION

Autoimmune thyroid disease is the most common autoimmune disease, and includes several inflammatory thyroid diseases, with Graves' Disease and Hashimoto's the most frequent forms [1-3]. The NIBSC reference reagent (NRR), 66/387, for anti-thyroid microsome serum, was produced in the 1960's from a pool of serum from three patients who showed autoimmune anti-thyroid microsome activity. The target for this anti-thyroid microsome autoimmunity has since been identified as thyroid peroxidase (TPO) [4-6]. Thyroid peroxidase is a membrane-bound enzyme that is responsible for iodine oxidation and iodination of the thyroglobulin molecule [2,6] and autoantibodies to TPO are present in thyroid diseases such as Hashimoto's thyroiditis and hyperthyroidism [2,3].

The development of immunoassays to detect anti-TPO autoantibodies are an important component in the diagnosis of thyroid autoimmunity and disease, and the NRR 66/387 has been widely adopted for the calibration of these immunoassays [3,7]. However, stocks of the NRR 66/387 are now depleted. Given the widespread adoption of the NRR, there is an urgent need to provide a replacement standard for anti-TPO autoantibodies.

A new preparation of anti TPO antibodies has been filled into ampoules (NIBSC code 19/260), following procedures recommended by WHO [8]. It is now intended to initiate an international collaborative study with expert laboratories to aid in the value assignment of the proposed 1st International Standard by immunoassay in units of IU/ampoule in comparison with the NRR, 66/387. Human serum and plasma samples containing a range of anti TPO concentrations will be included in the study in order to assess commutability of the candidate standard with native samples.

The aims of the collaborative study are therefore:

5. To confirm the immunoreactivity of the 1st IS by immunoassay, and to assess the relationship of the activity of the 1st IS with the NRR, 66/387, and existing local standards.
6. To calibrate the candidate 1st IS for anti-TPO antibodies relative to the 1st NRR, 66/387, by immunoassay.
7. To assess the commutability of the candidate 1st IS with native samples in immunoassays.
8. To assess the stability of ATD samples of the 1st IS by comparison with ampoules stored at elevated temperatures as part of an accelerated degradation stability study in immunoassays.

MATERIALS

Preparations supplied to participants in the collaborative study

The materials for the study are listed in Table 1. Each participant will be allocated a minimum set of samples consisting of the NRR, 66/387, duplicates of 19/260 and a panel of twelve human serum and plasma samples. Where assay capacity and sample availability allow, participants will receive an additional set of five coded ampoules to determine the stability of the candidate standard.

Table 1 – preparations for participants

Anti TPO preparation	Ampoule content
NIBSC Reference Reagent for anti-TPO, 66/387	1000 IU per ampoule
Coded preparations of the candidate 1st IS, 19/260, stored at -20ºC	Nominally 800 IU per ampoule
Accelerated thermal degradation (ATD) samples of 19/260 stored at +4ºC, +20ºC, +37ºC and +45ºC, coded	Content assumed identical to 19/260 stored at -20ºC
Twelve human serum/plasma samples labelled TPOSerum1 to TPOSerum6 and TPOPlasma7 to TPOPlasma12	0.5ml aliquots human serum or human plasma

NIBSC Reference Reagent, 66/387
Serum from three donors was pooled and diluted with veronal buffer (components/pH). Aliquots of 0.2 ml were then dispensed into glass ampoules, lyophilised and sealed according to procedures recommended by WHO [8] and stored at -20ºC in the dark at NIBSC.

The ampoules were tested and found negative for anti-HIV 1 and 2, HBsAg and HCV.

Candidate standard, 19/260
Defibrinated plasma from three donors was obtained from TCS Biosciences (Buckingham, UK), pooled and formulated with 40 mM HEPES to provide a bulk solution of 1909 ml. Aliquots of 1 ml were then dispensed into glass ampoules, lyophilised and sealed according to procedures recommended by WHO [8] and stored at -20ºC in the dark at NIBSC.

The bulk plasma were tested and found negative for anti-HIV 1 and 2, HBsAg and HCV by NAT assay.

Accelerated thermal degradation samples
Ampoules of the candidate standard which have been incubated at -20ºC, +4ºC, +20ºC, +37ºC and +45ºC for 7 months will be included in the study to assess the stability of the candidate standard.

Human samples
Serum samples are clinical remnants purchased from Cerba Specimen Services (Saint-Ouen l'Aumone, France) and diluted into normal human serum purchased from FirstLink (Wolverhampton, UK). Plasma samples were purchased from Abbaltis (Sittingbourne, UK). Samples are coded TPOSerum 1 to TPOSerum 6 and TPOPlasma 7 to TPOPlasma 12. Please note, clinical remnant serum samples obtained from Cerba Specimen Services **have not been tested** for blood borne pathogens and should be handled according to your own laboratory code of practice.

Plasma samples labelled TPOSerum3 and TPOPlasma7 are from high titre donors and contain >1000 IU/ml anti-TPO. Participants are requested to pre-dilute samples based on the approximate concentrations as necessary and in accordance with their in-house protocols. Please provide all details

of pre-dilution steps where necessary. A table of approximate anti-TPO content of each sample is provided in Appendix 1.

This material is to be used only for this study and in accordance with the Human Tissue Act or equivalent national legislation and is to be destroyed at the end of the collaborative study.

Handling of materials
Upon receipt, ampoules should be stored at -20ºC or below until use. Allow the contents to reach room temperature before opening. Reconstitute with a volume of appropriate assay diluent (e.g. your own assay buffer, PBS or saline, preferably with 0.05 – 0.1% added protein such as bovine serum albumin or human serum albumin to reduce adsorption). Leave at room temperature for a minimum of 10 minutes to fully dissolve. Dilutions should be prepared from this stock using your own assay diluent or PBS with protein cover as defined in common test sample concentrations below. A detailed protocol for reconstitution and dilution of the standards is provided in Appendix 1.

Please provide details of the reconstitution of the ampoules and the dilutions used to prepare the test samples.

Upon receipt, test samples of human serum/plasma should be stored at -20ºC or below until use. Allow contents to thaw and reach room temperature. Mix contents gently before measuring. Please do not re-freeze. Use a fresh aliquot for each run.

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.

TESTS REQUESTED
Participants are asked to perform **two independent runs** of the assay method(s) in use in their laboratory. An independent run should consist of the measurement of one set of dilutions prepared from each of the ampoules provided (66/387 and coded preparations 19/260-A and 19/260-B), one set of serum/plasma samples (n=12) which have been thawed specifically for that run and assay kit calibrators and controls where applicable. An independent run will use a single calibrated kit, integral or 96 well plate as required for your method.

An independent run of the accelerated thermal degradation samples will consist of the measurement of one set of dilutions prepared from each of the ampoules coded 19/260-C to 19/260-G.

Participants are asked to prepare dilutions of the ampouled preparations and measure, in triplicate, the anti-TPO concentration of these and the serum/plasma samples. The test concentrations are described in common test sample concentrations below, and in further detail in Appendix 1. A suggested ELISA plate layout is also provided in Appendix 1.

Common test samples concentrations
In order to assess commutability across different assay methods, participants are asked to measure a minimum number of dilutions of the coded 19/260 preparations and 66/387 that are common to all participants. **These core dilutions are 800, 400, 200, 100, 50, 25 and 12.5 IU/ml**. Where the top core dilution is above assay range, please disregard this dilution. Additional dilutions should be included in order to ensure that a minimum **of five points in the linear part of the dose response curve are measured**.

Data submission

Participants are requested to provide **all raw assay data** in an electronic spreadsheet format for central computation at NIBSC, along with participants' own estimates of activity as calculated by the method normally used in their laboratory, and all details of the reconstitution and dilution volumes used to prepare the test samples. A suggested reporting table is shown in Appendix 1, Table A4.

REPORT

A preliminary report will be prepared and circulated to all participants for comment before submission to the Expert Committee on Biological Standardisation 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.

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Appendix 1

Assay buffer

For dilution steps below, please use your appropriate assay buffer, ensuring protein cover is provided to prevent adsorption. PBS plus 0.1% bovine serum albumin (BSA) or 0.1% human serum albumin (HSA) may be used as an alternative.

Standard and sample processing

The following provides details on the reconstitution of the standards, along with example dilution steps to generate the working stock solution of the standards for use in the assay. These example dilution steps, or your own in-house dilution methods may be used, but please provide all details of reconstitution and dilution steps taken in your report.

A fresh ampoule of 66/387 and duplicates of 19/260 (coded CS684 19/260-A or CS684 19/260-B) should be used for each independent run.
For analysis of thermal degradation samples, a fresh ampoule of 19/260 CS684-C, 19/260 CS684-D, 19/260 CS684-E, 19/260 CS684-F and 19/260 CS684-G should be used for each independent run.

A. Reconstitution and dilution of ampoules of NRR 66/387

1. Before opening, ampoules should be brought to room temperature to minimize moisture uptake.
2. Reconstitute each ampoule in 1.25 ml diluent to provide a working stock solution of 800 IU/ml.
3. The working stock solution at 800 IU/ml will form dilution 1 and the solution from which serial dilutions should be made.
    - Prepare serial dilutions (1:2) of this working stock solution to provide dilutions 2 to 7. Table A1 below provides the full details of the dilutions and their expected concentrations.
    - To enable comparison across different immunoassays at the same dilution point, participants are asked to include the **7 core concentrations (dil 1-7) highlighted in bold** which should be included in **all assays**. Dilution 1 can be omitted if it is above the normal assay range. If assay space permits, additional concentrations can be included.

Table A1 66/387 and 19/260 dilution table

Dilution	66/387 and 19/260 concentration (IU/ml)
1	**800**
2	**400**
3	**200**
4	**100**
5	**50**
6	**25**
7	**12.5**

B. Reconstitution and dilution of ampoules of candidate standard 19/260 and ATD samples of 19/260

1. Before opening, ampoules should be brought to room temperature to minimize moisture uptake.
2. Reconstitute each ampoule in 1 ml diluent provide a working stock solution of approximately 800 IU/ml
3. The working stock solution at 800 IU/ml will form dilution 1 and the solution from which serial dilutions should be made.
    - Prepare 1:2 serial dilutions of this working stock solution to provide dilutions 2 to 7. Table A1 provides the full details of the dilutions and their expected concentrations.
    - To enable comparison across different immunoassays at the same dilution point, participants are asked to include the **7 core concentrations highlighted in bold** which should be included in **all assays**. Dilution 1 can be omitted if it is above the normal assay range. If assay space permits, additional concentrations can be included.

C. Preparation of serum and plasma samples TPOSerum1 to TPOSerum6 and TPOPlasma7 to TPOPlasma12

Upon arrival, please store all serum and plasma samples at -20ºC or below until use. Serum and plasma samples should be thawed at 37ºC and mixed well. Where required, serum and plasma samples should be diluted in your own assay buffer according to your standard assay protocol for measurement of anti-TPO. To aid with these dilutions Table A2 below provides the approximate anti-TPO content of each sample.

Table A2 Approximate serum/plasma sample anti-TPO content

Sample	Approx. antiTPO content (IU/ml)
TPOSerum1	35
TPOSerum2	220
TPOSerum3	1100
TPOSerum4	90
TPOSerum5	131
TPOSerum6	50
TPOPlasma7	1800
TPOPlasma8	60
TPOPlasma9	130
TPOPlasma10	200
TPOPlasma11	320
TPOPlasma12	420

D. Assay design and plate layout

Alongside local standards and controls, each assay/each plate should include **core dilutions of 66/387 and coded preparations of the candidate standard 19/260-A and 19/260-B, plus 1 set of serum/plasma samples, TPOSerum1-6 and TPOPlasma7-12.** All samples should be tested in triplicate if possible, according to the in-house method. If space does not permit triplicate readings, then duplicate readings may be performed. For an example, see 96 well ELISA plate layout, Figure A1.

To enable us to gather data regarding inter and intra-assay variability within each laboratory, participants are requested to perform two independent assays with the samples provided. Due to limited stocks, only 0.5 ml of each human serum/plasma sample can be provided and should be diluted in the appropriate assay buffer as per your usual assay protocol.

Figure A1 Suggested plate map for ELISA plate format*

Kit stnd 1	Kit stnd 1	66/387 dil 1	66/387 dil 1	19/260 A dil 2	19/260 A dil 2	19/260 B dil 3	19/260 B dil 3	TPO Serum 4	TPO Serum 4	TPO Plasma 12	TPO Plasma 12
Kit stnd 2	Kit stnd 2	66/387 dil 2	66/387 dil 2	19/260 A dil 3	19/260 A dil 3	19/260 B dil 4	19/260 B dil 4	TPO Serum 5	TPO Serum 5		
Kit stnd 3	Kit stnd 3	66/387 dil 3	66/387 dil 3	19/260 A dil 4	19/260 A dil 4	19/260 B dil 5	19/260 B dil 5	TPO Serum 6	TPO Serum 6		
Kit stnd 4	Kit stnd 4	66/387 dil 4	66/387 dil 4	19/260 A dil 5	19/260 A dil 5	19/260 B dil 6	19/260 B dil 6	TPO Plasma 7	TPO Plasma 7		
Kit stnd 5	Kit stnd 5	66/387 dil 5	66/387 dil 5	19/260 A dil 6	19/260 A dil 6	19/260 B dil 7	19/260 B dil 7	TPO Plasma 8	TPO Plasma 8		
Control1	Control1	66/387 dil 6	66/387 dil 6	19/260 A dil 7	19/260 A dil 7	TPO Serum 1	TPO Serum 1	TPO Plasma 9	TPO Plasma 9		
Control 2	Control 2	66/387 dil 7	66/387 dil 7	19/260 B dil 1	19/260 B dil 1	TPO Serum 2	TPO Serum 2	TPO Plasma 10	TPO Plasma 10		
Blank	Blank	19/260 A dil 1	19/260 A dil 1	19/260 B dil 2	19/260 B dil 2	TPO Serum 3	TPO Serum 3	TPO Plasma 11	TPO Plasma 11		

E. Data reporting

Estimates of the anti-TPO content of the candidate standard 19/260-A and 19/260-B and the NRR 66/387 should be calculated in comparison with the in-house assay kit standard. Participants are requested to provide details of the assay method used, including dilution steps, together with all the raw data e.g. counts for each sample, in electronic for (excel file) if possible. Please also clarify if/where serum sample dilutions have been taken into account when calculating the results. Participant's own calculated estimates of anti-TPO concentration are also requested. A sample reporting table is provided below in Table A3.

Table A3 Example data reporting table for recording anti-TPO content of test samples

Assay Run No.	***Platform:***				***Method:***			
	RLU/Absorbance Units/Counts				***Reported anti-TPO concentration (IU/ml)***			
****Sample***	***1***	***2***	***3***	***Avg***	***1***	***2***	***3***	***Avg***
Baselines								
Kit standard 1								
Kit standard 2								
Kit standard 3								
Kit standard 4								
Kit standard 5*								
66/387 dil 1								
66/387 dil 2 etc*								
Candidate A dil 1								
Candidate A dil 2 etc*								
Candidate B dil 1								
Candidate B dil 2 etc*								
TPOSerum1								
TPOSerum2								
TPOSerum3								
TPOSerum4 etc*								

* Final reporting table should be expanded according to finalised core dilutions of 66/387 and the candidate standard, serum sample numbers, thermal degradation sample core dilutions (where applicable) and assay specific kit standards and controls.

APPENDIX 2

Appendix Table A2.1 Fitted slope-ratios from immunoassays for parallelism assessment of different standards.

Lab	66/387 vs kit std		19/260 vs kit std				19/260 vs 66/387			
	Assay 1	Assay 2	Assay 1		Assay 2		Assay 1		Assay 2	
			A	B	A	B	A	B	A	B
1	0.95	0.93	0.95	0.89	0.88	0.89	1.03	1.00	1.00	1.03
2a	1.02	1.04	1.08	1.08	1.05	1.07	1.22	1.21	1.22	1.23
2b	No data	1.02	No data	No data	1.08	1.08	1.12	0.57†	1.22	1.21
3	0.98	0.98	0.95	0.95	0.99	0.99	0.96	0.97	1.02	1.01
4	No data	1.12	1.09	1.15	1.15	1.08	No data	No data	1.02	0.96
5	1.00	1.01	0.97	0.97	0.95	0.97	0.97	0.97	0.94	0.95
6	0.88	No data	0.83	0.78*	No data	No data	0.94	0.89	No data	No data
7a	1.01	1.01	0.98	0.98	0.98	0.97	0.97	0.97	0.97	0.96
7b	0.87	0.88	0.83	0.83	0.82	0.85	0.95	0.95	0.94	0.97
7c	1.00	0.96	0.97	0.98	0.99	0.97	0.98	0.98	1.02	1.01
7d	1.06	1.00	1.00	0.98	0.99	1.01	0.94	0.92	0.99	1.01
7e	1.02	1.01	1.03	1.01	1.00	1.02	1.01	0.99	0.99	1.00
7f	1.17	1.21	1.12	1.13	1.17	1.16	0.96	0.97	0.97	0.96
7g	1.06	1.04	1.20	1.27*	1.33*	1.34*	1.24	1.26†	1.31†	1.33†
7h	0.95	0.95	0.85	0.85	0.86	0.84	0.89	0.89	0.90	0.88
7i	0.83	0.83	0.76*	0.75*	0.78*	0.63*	0.92	0.89	0.96	0.79†

* Determined invalid due to lack of dilutional linearity

† Determined invalid due to reference and test samples being non-parallel

Appendix Table 2.2 Reported potency estimates of Samples S01 to T12 (IU/mL).

Lab	Assay	S01	S02	S03	S04	S05	S06	T07	T08	T09	T10	T11	T12
1	1	19	133	382	32	59	23	NL	24	58	81	96	197
	2	20	125	NL	42	59	21	560	25	57	84	126	200
2a	1	25	159	1049	365	150	64	1074	48	166	258	66	612
	2	25	153	1035	348	148	61	1120	51	165	300	67	516
2b	1	Excluded due to inconsistent results with coded duplicate samples											
	2	24	161	1147	315	150	59	1259	49	180	250	69	530
3	1	26	140	671	90	92	45	1095	37	113	95	235	365
	2	24	137	674	91	91	44	1025	36	112	87	226	345
4	1	Excluded due to inconsistent results with coded duplicate samples											
	2	39	78	465	161	135	83	1486	58	136	75	509	479
5	1	29	176	1085	163	132	66	1711	52	172	133	385	479
	2	31	196	1025	154	132	63	1755	48	161	147	364	439
6	1	35	127	426	105	129	68	1080	36	83	89	178	185
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7a	1	32	202	1221	158	125	52	1633	46	166	148	378	470
	2	31	199	1137	150	131	63	1457	50	161	148	358	434
7b	1	57	124	946	108	129	84	1326	81	137	113	312	416
	2	62	127	1011	107	128	81	1286	76	133	108	298	422
7c	1	33	245	1383	87	127	55	1672	42	107	174	277	339
	2	33	232	1323	88	142	56	1546	49	111	175	287	330
7d	1	32	135	856	114	116	53	1321	38	117	121	307	308
	2	25	157	923	103	101	49	1421	39	111	107	295	342
7e	1	31	178	893	85	137	55	888	52	138	132	229	436
	2	31	180	899	86	135	54	902	53	144	134	233	428
7f	1	36	80	1065	176	145	67	ND	58	133	77	486	532
	2	43	84	997	182	132	67	ND	54	128	77	570	578
7g	1	84	114	ND	270	283	38	ND	56	89	91	ND	ND
	2	86	203	ND	386	360	55	ND	63	105	99	ND	ND
7h	1	23	181	927	56	99	37	1546	37	82	106	168	247
	2	24	168	913	57	98	38	1489	35	88	102	162	259
7i	1	42	132	686	113	135	72	1199	40	87	88	166	174
	2	43	146	780	120	153	80	1406	45	95	102	181	198

ND = No Data
NL = Non-Linear

Appendix Table 2.3 Potency estimates of Samples S01 to T12, calculated relative to 66/387 (Assigned potency of 800 IU/mL).

Lab	Assay	S01	S02	S03	S04	S05	S06	T07	T08	T09	T10	T11	T12
1	1	26	182	531	42	80	30	NP	32	79	111	130	271
	2	25	162	NP	52	74	26	RR	30	71	107	162	263
2a	1	25	RR	RR	RR	RR	63	RR	47	RR	RR	64	RR
	2	27	RR	RR	RR	RR	63	RR	53	RR	RR	68	RR
2b	1	Excluded due to inconsistent results with coded duplicate samples											
	2	23	RR	RR	RR	RR	54	RR	45	RR	RR	62	RR
3	1	26	148	721	95	97	47	1174	39	120	100	251	393
	2	25	148	740	97	97	46	1118	37	120	93	246	378
4	1	Excluded due to inconsistent results with coded duplicate samples											
	2	37	69	339	132	112	72	RR	53	113	66	368	348
5	1	24	144	RR	133	108	54	RR	43	141	109	315	392
	2	26	161	821	126	108	53	RR	40	133	121	295	356
6	1	31	132	521	106	133	64	RR	31	81	88	192	201
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7a	1	25	157	938	123	97	41	1252	36	129	115	292	362
	2	26	166	936	125	109	53	1196	42	134	124	296	358
7b	1	41	98	912	83	103	63	1343	60	110	88	282	394
	2	47	106	1030	88	107	64	1357	59	112	88	282	420
7c	1	22	165	RR	58	85	37	RR	28	72	117	187	228
	2	23	176	RR	64	106	40	RR	35	82	131	219	254
7d	1	36	142	807	121	124	59	RR	43	125	128	308	309
	2	25	157	RR	103	101	49	RR	40	111	107	295	342
7e	1	29	161	800	78	125	51	787	48	125	120	206	387
	2	29	166	827	81	126	51	819	49	134	124	214	391
7f	1	37	72	739	143	121	62	ND	55	112	70	342	369
	2	36	63	553	120	92	52	ND	44	89	59	309	313
7g	1	92	124	ND	286	299	42	ND	62	97	100	ND	ND
	2	92	211	ND	RR	368	59	ND	68	111	106	ND	ND
7h	1	20	175	938	52	93	33	1603	33	76	99	162	243
	2	20	154	882	49	87	32	1477	30	78	91	148	243
7i	1	32	121	693	101	125	60	1333	30	75	75	158	168
	2	32	135	803	107	143	66	1615	33	81	88	174	194

ND = No Data
NL = Non-Linear
NP = Non-Parallel
RR = Non-overlapping response ranges

Appendix Table 2.4 Potency estimates of Samples S01 to T12, calculated relative to 19/260-A (Assumed potency of 600 IU/mL).

Lab	Assay	S01	S02	S03	S04	S05	S06	T07	T08	T09	T10	T11	T12
1	1	38	RR	RR	62	115	45	RR	47	114	158	RR	RR
	2	37	RR	RR	79	112	39	RR	45	108	RR	RR	RR
2a	1	18	78	RR	RR	75	38	RR	30	81	RR	39	RR
	2	16	68	RR	RR	66	33	RR	29	73	RR	36	RR
2b	1	Excluded due to inconsistent results with coded duplicate samples											
	2	16	67	RR	RR	63	31	RR	27	73	RR	35	RR
3	1	32	190	952	119	122	57	1538	47	152	126	327	520
	2	31	177	874	117	117	56	1327	46	145	112	292	447
4	1	Excluded due to inconsistent results with coded duplicate samples											
	2	49	90	427	170	145	94	RR	70	146	87	463	438
5	1	20	129	RR	119	95	47	RR	37	126	97	288	361
	2	20	142	RR	110	94	43	RR	32	116	105	272	332
6	1	30	142	613	113	144	66	RR	31	84	92	212	223
	2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7a	1	22	141	872	110	86	35	1172	31	115	103	267	334
	2	23	150	874	112	98	47	1125	37	121	111	273	332
7b	1	59	150	RR	127	158	94	RR	90	170	134	459	RR
	2	66	156	RR	128	158	91	RR	84	166	128	443	RR
7c	1	28	223	RR	77	114	48	RR	37	96	157	254	311
	2	32	229	RR	86	139	54	RR	48	108	172	284	327
7d	1	32	138	RR	116	119	54	RR	39	119	123	313	314
	2	26	163	RR	107	105	51	RR	41	115	111	308	357
7e	1	36	195	969	95	151	62	946	58	152	146	250	466
	2	34	196	979	95	148	59	963	58	158	146	255	467
7f	1	42	85	919	172	144	73	ND	64	133	82	423	459
	2	48	83	771	163	123	69	ND	57	120	78	432	437
7g	1	70	88	ND	RR	RR	37	ND	51	73	74	ND	ND
	2	73	137	ND	RR	RR	52	ND	58	84	81	ND	ND
7h	1	22	254	RR	64	125	39	RR	39	100	135	234	368
	2	23	229	RR	65	122	41	RR	37	108	128	220	382
7i	1	30	130	780	106	133	60	RR	28	77	77	173	184
	2	32	142	874	112	151	68	RR	33	84	91	186	208

ND = No Data
NL = Non-Linear
NP = Non-Parallel
RR = Non-overlapping response ranges

Appendix Table A2.5 Laboratory reported potency estimates (IU/mL)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	20	129	382	37	59	22	560	24	58	83	110	199
2a	25	156	1042	357	149	63	1097	49	166	278	66	562
2b	23	156	1106	303	144	57	1205	46	173	247	69	531
3	25	139	673	90	91	44	1060	36	113	91	230	355
4	39	78	465	161	135	83	1486	58	136	75	509	479
5	30	186	1055	158	132	65	1733	50	167	140	374	459
6	35	127	426	105	129	68	1080	36	83	89	178	185
7a	31	200	1178	154	128	57	1543	48	163	148	368	451
7b	60	125	978	108	129	83	1306	78	135	110	305	419
7c	33	238	1352	88	134	56	1608	46	109	175	282	334
7d	28	146	889	108	109	51	1370	39	114	114	301	325
7e	31	179	896	86	136	55	895	52	141	133	231	432
7f	39	82	1031	179	138	67		56	130	77	526	554
7g	85	152		323	319	46		59	96	95		
7h	23	174	920	57	98	38	1517	36	85	104	165	253
7i	43	139	732	117	144	76	1298	42	91	95	173	186
Median	31	149	920	112	133	57	1302	47	122	107	231	419
Rank	1	8	11	5	7	3	12	2	6	4	9	10

Appendix Table A2.6 Laboratory estimates for samples calculated relative to Sample 66/387 by parallel line analysis (IU/mL)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	25	171	531	47	77	28		31	75	109	145	267
2a	26	152		340	146	63		50	162	267	66	529
2b	24	147		278	135	56		45	162	228	66	478
3	26	148	731	96	97	46	1146	38	120	96	248	386
4	37	69	339	132	112	72		53	113	66	368	348
5	25	152	821	130	108	53		41	137	115	305	373
6	31	132	521	106	133	64		31	81	88	192	201
7a	26	161	937	124	103	47	1223	39	132	120	294	360
7b	44	102	969	86	105	63	1350	60	111	88	282	406
7c	23	170		61	95	39		31	77	124	202	241
7d	30	150	807	112	112	54		41	118	117	301	325
7e	29	163	813	79	125	51	803	49	130	122	210	389
7f	37	68	639	131	105	57		49	100	64	325	340
7g	92	162		286	332	50		65	104	103		
7h	20	164	909	50	90	33	1538	31	77	95	155	243
7i	32	128	746	104	133	63	1468	31	78	81	166	180
Median	27	151	776	109	110	54	1285	41	112	106	210	348
Rank	1	8	11	4	7	3	12	2	6	5	9	10

Appendix Table A2.7 Laboratory estimates for samples calculated relative to Sample A by parallel line analysis (IU/mL)

	Sample											
Lab	**S01**	**S02**	**S03**	**S04**	**S05**	**S06**	**T07**	**T08**	**T09**	**T10**	**T11**	**T12**
1	38			70	113	42		46	111	158		
2a	17	73			71	36		29	77		37	
2b	16	67			63	31		27	73		35	
3	31	183	912	118	119	57	1428	46	148	119	309	482
4	49	90	427	170	145	94		70	146	87	463	438
5	20	135		114	94	45		34	121	101	280	346
6	30	142	613	113	144	66		31	84	92	212	223
7a	22	145	873	111	92	41	1148	34	118	107	270	333
7b	62	153		127	158	92		87	168	131	451	
7c	30	226		81	126	51		42	102	164	268	319
7d	29	150		111	112	52		40	117	117	310	335
7e	35	196	974	95	150	61	954	58	155	146	252	467
7f	45	84	841	167	133	71		60	126	80	428	448
7g	71	110				44		54	78	78		
7h	23	241		65	124	40		38	104	131	227	375
7i	31	136	826	109	142	64		30	80	84	179	196
Median	30	142	841	111	124	52	1148	41	114	112	269	346
Rank	1	8	11	4	7	3	12	2	6	5	9	10

APPENDIX 3 DRAFT INSTRUCTIONS FOR USE

1st WHO International Standard for anti-thyroid peroxidase antibodies, 19/260
(version 1, dated XX/XX/XXXX)

1. INTENDED USE

The 1st International Standard for anti-thyroid peroxidase antibodies, coded 19/260, is intended for use in the calibration of immunoassays for anti-thyroid peroxidase. It replaces the NIBSC Reference Reagent, coded 66/387, for anti-thyroid microsome serum, produced in the 1960's. The target for anti-thyroid microsome activity has since been determined to be autoimmunity to the thyroid peroxidase (TPO) enzyme. Stocks of the NRR 66/387 are now exhausted. [The 1st IS was established by the Expert Committee on Biological Standardisation of the World Health Organisation in ___].

2. CAUTION

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

The preparation contains material of human origin.

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

Each ampoule of the International Standard contains **555 IU/ampoule** of anti-thyroid peroxidase antibodies**.**

4. CONTENTS

Country of original of biological material: Germany

Each ampoule contains the residue after freeze-drying of 1 mL of a solution that contained:

Human defibrinated plasma
HEPES, 40mM

5. STORAGE

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

6. DIRECTIONS FOR OPENING

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

7. USE OF MATERIAL

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

8. PREPARATION OF AMPOULES AND COLLABORATIVE STUDY

A batch of human serum containing high anti-TPO antibodies was produced by pooling defibrinated plasma from three donors, purchased from TCS Biosciences Ltd (Buckingham, UK) to produce a total volume of 1909 mL. This serum was buffered with 40 mM HEPES and aliquots of 1.0 ml were then dispensed into glass ampoules, lyophilised and sealed according to procedures recommended by WHO and stored at -20ºC in the dark at NIBSC, Potters Bar, UK.

This batch of ampoules, coded 19/260, was evaluated in a collaborative study to value assign the standard in International Units, by immunoassay, in terms of the NRR 66/387, and to assess its suitability to serve as an International Standard. The results of the study yielded an assigned content for 19/260 of 555 IU/ampoule.

An analysis of the commutability of 19/260 and 66/387 with native samples was also carried out during the collaborative study. In all immunoassays contributed, both standards were deemed to be commutable as assessed by a difference in bias approach.

An accelerated thermal degradation study was also performed. Data from immunoassays of accelerated thermal degradation samples of 19/260 stored at elevated temperatures of +4, +20, +37 and +45ºC were used to estimate the stability of the reference material at -20ºC, and gave a predicted loss in activity of 0.013%, indicating that the preparation 19/260 is sufficiently stable when stored at -20ºC to serve as an International Standard.

9. CITATION

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

10. LIABILITY AND LOSS

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

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

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

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

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

9.4 The Recipient shall defend, indemnify and hold NIBSC, its officers, employees and agents harmless against any loss, claim, damage or liability including reasonable legal costs and fees (of whatsoever kind or nature) made against NIBSC which may arise as a result of the wilful act, omission or negligence of the Recipient or its employees, the breach of any of the terms of the Contract, or the use, keeping, production or disposal of the Materials or any waste products arising from the use thereof by the Recipient or on its behalf.

11. REFERENCES

[1] Moore, M., Hockley, J. and Burns, C. (2021) WHO/BS/2021.2404: ECBS report to be referenced/linked

12. MATERIAL SAFETY SHEET

Physical properties (at room temperature)			
Physical appearance	Yellowish powder		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	Contains material of human origin, see caution, section 2		
Handling:	See caution, section 2		
Toxicological properties			
Effects of inhalation:	Not established, avoid inhalation		
Effects of ingestion:	Not established, avoid ingestion		
Effects of skin absorption:	Not established, avoid contact with skin		
Suggested First Aid			
Inhalation	Seek medical advice		
Ingestion	Seek medical advice		
Contact with eyes	Wash with copious amounts of water. Seek medical advice.		
Contact with skin	Wash thoroughly with water.		
Action on Spillage and Method of Disposal			
Spillage of ampoule contents should be taken up with absorbent material wetted with a virucidal agent. Rinse area with a virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.			