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WHO International Collaborative Study of the proposed 2nd International Standard for Thyroid Stimulating Antibody

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Summary

The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) has recognized (2006) the need for a replacement International Standard for Thyroid-stimulating antibody (TSAb) for the calibration of assays.

We report here the characterization of a candidate standard for TSAb in an International Collaborative Study carried out by 13 laboratories in 6 countries, and a comparison by receptor binding assay and bioassay with the existing International Standard coded 90/672.

The mean estimate of the TSAb content of the candidate standard, coded 08/204, using receptor binding assays is 0.113 IU per ampoule (95% confidence limits 0.106 – 0.120).

The results of this study also indicate that the candidate standard appears sufficiently stable to serve as an international standard, with a predicted yearly loss of 0.018% when stored at -20°C, on the basis of a thermally accelerated degradation study .

Introduction

Thyroid-stimulating hormone receptor (TSHR) autoantibodies (TRAb) are important in the pathogenesis of autoimmune hyperthyroidism and comprise thyroid-stimulating antibodies and thyroid-blocking antibodies (reviewed in 1). Thyroid-stimulating antibodies (TSAbs) can be detected in the vast majority of patients with hyperthyroidism caused by Graves' disease and have also been associated with a small portion of patients with toxic multinodular goitre. These autoantibodies interact with the TSH receptor on thyroid follicular cells and usually result in global stimulation of thyroid activity. Measurements of TSAbs are useful in the diagnosis and management of disease. For example, the detection of TSAbs has been used to predict relapse or remission in patients receiving treatment for Graves' disease, and also to predict neonatal hyperthyroidism in children of mothers with Graves' disease. The original preparation of 90/672, stocks of which are now exhausted, consisted of freeze-dried plasma proteins from a single human patient with high TSAb levels who was pregnant and whose plasma was regularly exchanged by plasmapheresis during pregnancy. This preparation was established by the WHO Expert Committee on Biological Standardization in 1995 and was itself calibrated in terms of MRC research standard B for long-acting thyroid stimulator (MRC LATS B, coded 65/122) and in terms of local standards. Although, the preparation was deemed suitable for the calibration of both bioassays and receptor assays for the measurement of autoantibodies to the TSH receptor, the significant differences between 90/672 and 65/122 in terms of bioassay and receptor assay unitage meant that the ampoule content of 90/672 did not represent a formal continuity of unitage with 65/122 and the relationship between the unitage of the two standards was dependent on the assay system employed. In addition it was noted that since 90/672 was derived from a single patient, it may not be qualitatively suitable to serve as a standard for all TSAb samples. Despite these limitations, 90/672 has been widely used for the calibration of assays to measure TSH receptor autoantibodies in human serum. In 2006, the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) recognized the need for a replacement International Standard for TSAbs for the calibration of these assays.

Since it has proved impossible to identify a similar source of plasma proteins of sufficient volume and autoantibody titre, a candidate material consisting of a human monoclonal thyroid stimulating autoantibody has now been filled into ampoules (NIBSC Code 08/204), following procedures recommended by WHO (2). This material, kindly donated by RSR Ltd (Cardiff, UK) has been produced from the lymphocytes of a patient with Graves' disease and exhibits the characteristics of serum TSH receptor autoantibodies. Thus, the human monoclonal antibody to the TSHR was able to stimulate cAMP production in TSHR-transfected CHO cells and importantly, TSHR autoantibodies with TSH agonist (stimulating) or TSH antagonist (blocking) from patient sera are effective inhibitors of monoclonal antibody binding to the TSHR (3, 4). It is intended that an international collaborative study is organised with expert laboratories to aid in the value assignment of the proposed International Standard (IS).

The aims of the study were, therefore:

- To calibrate the candidate standard (08/204) relative to the 1st IS for TSAb (90/672).
- To demonstrate the suitability of the preparation 08/204 to serve as the 2nd IS for TSAb by examining its behaviour in receptor binding assays and bioassay systems.
- To assess the relationships among the existing local standards and the proposed IS.
- To determine the stability of the preparation 08/204 by comparing them with ampoules that have been subjected to accelerated thermal degradation.

Participants

13 laboratories in 6 countries took part in the study and are listed alphabetically, by country, in Table 1. Throughout the study each participating laboratory is referred to by a code number. These code numbers were randomly assigned and do not reflect the order of listing.

Table 1: List of participants.

Dr. Catherine Massart, UF d'Hormonologie, CHU Pontchaillou, rue Henri le Guilloux, Rennes FRANCE.
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Dr. Bernard Rees-Smith and Dr. Jane Sanders, RSR Ltd, Avenue Park, Pentwyn, Cardiff, UK.
Mr Richard Tiplady, NIBSC, Biotherapeutics, Blanche Lane, South Mimms, Potters Bar, EN6 3QG, UK.
Ms. Carol Preissner, Mayo Clinic, 702 Hilton Building, Mayo Clinic, 200 First St SW, Rochester, MN, USA.

Materials

Bulk materials and preparation of ampoules of thyroid-stimulating antibody.

A preparation of a human monoclonal antibody to the thyroid-stimulating hormone (TSH) receptor (3, 4) in phosphate buffered saline, was generously donated to WHO by RSR Ltd (Cardiff, UK). A volume of 0.6 ml (5mg/ml) of this sterile solution was added to 2875 ml of human serum (Golden West Biologicals Inc, USA), which had been tested and found to be negative for HIV, Hepatitis C and Hepatitis B). 125 ml HEPES was added and the solution was stirred gently prior to filling into ampoules at 1.0 ml per ampoule (nominally 1 µg TSAb). Ampoules containing TSAb were lyophilised and sealed under nitrogen according to procedures described by WHO (2) and stored at -20°C in the dark at NIBSC. A final total of 3000 ampoules were obtained on 10th October 2008, each coded 08/204, with a mean oxygen content of 0.26% (CV 44.1%) assessed using electrochemical detection using the Orbisphere Pharmapack 3600, a mean fill weight of 1.006 g (CV 0.11%), a mean dry weight of 0.09 g (CV 0.62%) and a residual moisture content of 0.19% (CV 10.96%) assessed by coulometric Karl Fischer..

The preparations for this study, the majority of which were identified only by code letter, are listed in Table 2. Where possible, each participant was allocated the core preparations (the

duplicate coded candidate standard ampoules, stored at -20°C, and the 1st IS coded 90/672), and a further selection of samples based on assay capacity and sample availability (some thermally accelerated degradation samples were only available in limited numbers). In addition, participants were asked to include their own in-house standards in the assays.

Table 2: Preparations supplied to participants in collaborative study.

Code	TSAb preparation	Ampoule unitage and nominal content
Not coded	The 1st I.S. for TSAb (90/672) stored at -20°C.	0.1 IU per ampoule.
C and E Duplicates	TSAb candidate standard (08/204) stored at -20°C.	Nominally 1 µg (approx 0.1 IU) per ampoule.
D, A, F and B	Accelerated thermal degradation (ATD) samples of TSAb candidate standard (08/204) stored respectively at +4°C, +20°C, +37°C and +45°C for 28 weeks.	Contents assumed identical to C and E.

Study design and assay methods contributed

Receptor binding assay and bioassay of candidate standard 08/204

Participants were requested to carry out the assay(s) normally in use in their laboratory and, where possible, to perform at least two independent assays, using fresh ampoules, each assay to include all of the preparations allocated at preferably no fewer than five dose levels in the linear part of the dose-response curve. Handling instructions for the materials were included in the study protocol. In instances where there was not a fresh ampoule for subsequent assays, it was suggested that fresh dilutions be made from frozen stock solutions. Where dilutions of a stored stock solution were used, participants were asked to provide details of its storage and identification of the initial preparation. Participants were asked to ensure that all assays included their local standard where possible and to provide details of the assay method used, including dilution steps, together with all raw assay data in the form of clearly annotated optical densities, counts, etc. for central computation at NIBSC. Participants' own estimates of activity as calculated by the method normally used in their laboratory were also requested.

Assay methods contributed

Summaries of the methods used are given in Table 3. In the thirteen laboratories contributing data to the study, 16 different assays were used, four of which were bioassays based on cAMP production in transfected cells. All laboratories performed receptor-binding assays (RBA) of which there were 11 different assays from 6 manufacturers. One laboratory performed an in-house RBA.

Table 3 Assay methods used.

Lab No.	Assay type	Comments
1	Receptor binding assay	Roche Elecsys Anti-TSHR (No raw data supplied. Results reported IU/L)
2	Receptor binding assay Bioassay	a. Kronus (raw data as cpm) b. Roche Elecsys Anti-TSHR (No raw data supplied. Results reported as IU/L) c. DHI/Quidel Thyretain TSI Reporter BioAssay – cAMP reporter assay in transfected cells (raw data as RLU). d. cAMP reporter assay in transfected cells (raw data as RLU)
3	Bioassay Receptor binding assay	a. Bioassay - cAMP reporter assay in transfected cells (raw data as OD) b. TRAb ^{125}I TSH receptor coated tube assay (raw data as % inhibition) c. TRAb ELISA based on M22 biotin binding to TSH receptor coated wells (raw data as OD).
4	Receptor binding assay	TRAK ELISA based on biotin labelled TSH binding to TSH receptor coated wells (raw data as OD).
5	Receptor binding assay	a. Roche Elecsys Anti-TSHR b. TRAK human Radio receptor assay – BRAHMS (raw data as cpm)
6	Receptor binding assay	a. T.R.A ELISA based on biotin labelled TSH binding to TSH receptor coated wells (raw data as OD). b. TRAb clone ELISA based on M22 biotin binding to TSH receptor coated wells (raw data as OD). c. TRAb Radio receptor assay based on ^{125}I TSH binding to receptor coated tubes.
7	Receptor binding assay	Roche Elecsys Anti-TSHR (raw data as cpm).
8	Receptor binding assay	a. TRAb clone ELISA based on M22 biotin binding to TSH receptor coated wells (raw data as OD). b. TRAK human Radio receptor assay – BRAHMS (raw data as cpm)
9	Receptor binding assay	a. TRAK human Radio receptor assay – BRAHMS (raw data as cpm) b. TRAK – BRAHMS (raw data as cpm)
10	Receptor binding assay	Roche Elecsys Anti-TSHR adapted for use on Modular Analytics E170 (raw data as RLU)
11	Receptor binding assay	a. sTRAb-DERA (raw data as RLU) b. TRAK human LIA – BRAHMS (raw data as RLU)
12	Receptor binding assay Bioassay	a. TRAK human Radio receptor assay – BRAHMS (raw data as cpm) b. Bioassay – cAMP reporter assay in transfected cells
13	Receptor binding assay	TRAb ELISA based on M22 biotin binding to TSH receptor coated wells (raw data as OD).

Statistical analysis

An independent statistical analysis of data was performed at NIBSC. Where raw assay data were available, potency estimates relative to IS 90/672 were calculated by fitting a parallel-line model comparing assay response to log concentration (5). All data were plotted and assay validity was assessed both visually and by analysis of variance with deviations from the model considered significant at the 1% level. Where significant deviations from the model appeared to result from underestimation of the residual error, measured by replicate responses included in the analysis, linearity was confirmed visually and non-parallelism was assessed for significance by using the deviations from linearity as an alternative residual error.

In laboratories 3b, 4, 5a, 5b, 9a, 9b, 11b, 12a, 12b and 13, assay responses were transformed to percentages relative to the estimated upper and lower limits of the dose-response curve and an in-house program (6) was used to provide weighted regression of logit response on log dose and produce estimates of relative potency. In other cases, untransformed responses (laboratories 2a, 2c, 2d, 3c, 8a and 8b) or log-transformed responses (laboratories 3a, 7, 10 and 11a) were used and parallel-line analysis was carried out on the linear section of the dose-response curve. Laboratories 1 and 2b did not provide any raw data for analysis and the reported results in IU/ml at each dilution were used to confirm parallelism and calculate mean potency estimates. Laboratory 6 also did not provide raw data and reported only mean results for each sample which are shown in this report. All results relative to local standards have been calculated directly from the results reported by the laboratories in IU with the exception of laboratory 3a.

All mean potencies given in this report are unweighted geometric mean (GM) potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\}\times100\%$ where s is the standard deviation of the log transformed potency estimates).

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 (7) and hence predict the degradation rates when stored at -20°C.

Results

Data returned for analysis.

In total 81 assays were performed. Data were contributed by thirteen laboratories, eight of which used more than one method (laboratories 2, 3, 5, 6, 8, 9, 11 and 12). Where this was the case, the laboratory code has been subdivided for method differences, for example 2a, 2b and 2c. Mean estimates for 08/204 are summarised in Table 4 and Figure 1 (estimates calculated relative to 90/672) and Table 5 (reported estimates relative to local standards). Results from individual assays are given in appendix 1 (Tables A1 and A2). Slopes of fitted dose-response lines are also shown in appendix 1 (Table A3).

Assay validity

The majority of assays allowed statistically valid estimates to be calculated, although some samples were excluded from further analysis due to non-linearity. These were sample A (assay 6 by laboratory 2c), B (assay 1 by laboratory 2a), E (assay 3 by laboratory 2c) and 90/672 (assay 2c by laboratory 3a and assay 2 by laboratory 12a). Non-parallelism resulted in the removal of samples D and E in assay 1 by laboratory 4, sample C in assay 2 by laboratory 4 and the

rejection of assay 3 by laboratory 4, assay 2 by laboratory 8a, assay 2 by laboratory 8b and assay 3 by laboratory 12a.

Parallelism of dose-response lines

In addition to the assessment of non-parallelism within each assay, a review of the fitted slopes (Table A3) for the dose-response lines of 08/204 (samples coded C & E) and 90/672 was carried out and no consistent slope difference between the two preparations was detected, with the fitted slope for 08/204 being less than that for 90/672 in 60% of assays. The average within-assay slope ratio of 0.983 (95% confidence limits 0.961 – 1.005; CV 8.9%) for 08/204 relative to 90/672 was in agreement with the expected value of 1.0 for parallel dose-response lines. Similar slope differences were observed for identical samples of 08/204 relative to each other with an average within-assay slope ratio for C relative to E of 1.001 (95% confidence limits 0.975 – 1.028; CV 9.3%).

Potency of 08/204 calculated relative to IS 90/672

The geometric mean potency calculated from all laboratories (Table 4) is 0.128 IU per ampoule (n=25; 95% confidence limits 0.112 – 0.146). Excluding bioassays the mean is 0.113 IU per ampoule (n=21; 95% confidence limits 0.106 – 0.120) and for bioassays only is 0.242 IU per ampoule (n=4; 95% confidence limits 0.166 – 0.353). Between-laboratory variability is greater for bioassays (GCV 26.8%) compared to receptor binding assays (GCV 14.5%). Variability within laboratories, assessed by GCV's for samples coded C and E in laboratories where at least three potency estimates were obtained, is significantly lower than that between laboratories with GCV's ranging from 8.3% to 17.9% for bioassays and 1.1% to 11.9% for other assays.

Stability of 08/204 based on thermally accelerated degradation samples.

Estimates of the potency of ampoules stored at elevated temperatures for a period of 28 weeks are summarized in Table 4. Expressing the potencies relative to ampoules stored continuously at -20°C and excluding the results from bioassays gives a predicted yearly loss of 0.018% when stored at -20°C.

Potency of 08/204 and 90/672 calculated relative to local standards

The geometric mean potency of 08/204 calculated from all laboratories (Table 5) is 0.113 IU per ampoule (n=18; 95% confidence limits 0.105 – 0.121; GCV 15.2%). Excluding bioassays by laboratory 12b, the mean is 0.112 IU per ampoule (n=17; 95% confidence limits 0.104 – 0.121; GCV 15.6%) which is in agreement with the value calculated relative to 90/672 directly. This is not unexpected since, with the exception of assays from laboratory 3a (which have been excluded from the mean potency calculations), all estimates of the potency of ampoules of 08/204 relative to local/kit standards are reported in terms of 90/672 (all of the manufacturer's kits used in this study were calibrated against the 1st IS). The laboratory mean estimate of the potency of 08/204 (ampoules coded C and E) in assays from laboratory 3a was reported in terms of ng M22 IgG/ampoule and was 1189.7 ng/ampoule (GCV 36.4%); which is slightly higher than the nominal mass of M22 IgG filled (1000 ng/ampoule). The geometric mean potency of 90/672 calculated from all laboratories is 0.096 IU per ampoule (n=18; 95% confidence limits 0.087 – 0.106; GCV 21.7%). Excluding bioassays by laboratory 12b, the geometric mean recovery of 90/672 in receptor binding assays (0.098 IU/ampoule; n=17; 95% confidence limits 0.090 – 0.108) was close to 100% (the assigned unitage is 0.100 IU/ampoule).

Conclusions and recommendations

The clinical measurement of stimulatory autoantibodies to the TSHR is useful for the confirmation of Graves' disease and Graves' opthalmopathy, the differential diagnosis of Graves' diseases from other causes of hyperthyroidism and the prediction of neonatal thyrotoxicosis in pregnant women with Graves' disease. Although functional bioassays are the gold standard in basic science research and in some rare clinical cases, the second and third generation receptor binding assays based on the competition of patient autoantibodies and labelled TSH/M22 for binding sites on the TSHR are more widely used in clinical diagnosis. Indeed the high sensitivity and specificity of these assays has allowed clinically accepted cut-offs for the diagnosis of Graves' disease to be determined. These cut-offs are quoted in international units of TSAb activity calibrated in terms of the 1st IS 90/672. Stocks of this international standard are now exhausted and this report describes a collaborative study to establish a replacement international standard. The 1st IS consisted of freeze-dried plasma proteins from a single human Graves' disease patient with high TSAb levels who was pregnant and whose plasma was regularly exchanged by plasmapheresis during pregnancy. The most desirable material for a replacement for 90/672, is a pool of plasma or serum with high autoantibody titre from patients with Graves' disease. If a preparation such as this were available it would be likely to reflect the heterogeneous nature of TRAbs encountered in the clinical diagnosis of the disease. However, since it has proved impossible to source a sufficient volume of plasma or serum with high autoantibody titre for the preparation of a replacement international standard, the candidate standard (coded 08/204) prepared and calibrated in this study is a human monoclonal thyroid stimulating autoantibody.

Analysis of the fitted slopes for the dose-response lines of 08/204 (samples coded C and E) and 90/672 in receptor binding assays and bioassays showed no consistent slope differences, demonstrating that the candidate standard 08/204 fulfils the requirement of a replacement international standard in terms of parallelism of assay response with this plasma preparation of autoantibodies from a patient with Graves' disease.

The geometric mean potency of 08/204 from receptor binding assays alone is 0.113 IU per ampoule (n=21; 95% confidence limits 0.106 – 0.120) which is some 13% higher than the expected unitage based on the combination of filling 1 µg of IgG per ampoule and the manufacturers data suggesting that 1 µg IgG is equivalent to 0.100 IU. However, this higher result is supported by the laboratory mean estimate of the potency of 08/204 in assays from laboratory 3a which was reported in terms of ng M22 IgG/ampoule and was 1189.7 ng/ampoule (GCV 36.4%) which is some 19% higher than the nominal mass of M22 IgG filled (1000 ng/ampoule).

The geometric mean potency calculated from bioassays alone is 0.242 IU per ampoule (n=4; 95% confidence limits 0.166 – 0.353) which is significantly higher than the potency calculated by receptor binding assays ($p<0.001$ in unpaired two-sided t-test of log transformed laboratory geometric mean estimates) and would exclude the combination of results from both methods. These data also suggest that the candidate standard 08/204 is approximately two-fold more potent in these bioassays than the 1st IS. This is perhaps not surprising since the candidate standard is known to consist solely of thyroid stimulatory immunoglobulins (8), rather than the combination of both thyroid stimulatory and thyroid inhibitory immunoglobulins present in the 1st IS. As a result, the establishment of 08/204 as the 2nd IS for TSAb would not represent a formal continuity of unitage for the calibration of bioassays. It is worth noting that this is not a problem encountered solely as a result of the use of a monoclonal TSAb as a candidate standard, since it is also highly likely that the functional heterogeneity of patient autoantibodies would

mean that any two plasma- or serum-derived preparations, including pooled patient materials, would have different potencies in functional TRAb bioassays. Indeed this was the case with the replacement of the MRC LATS B standard (65/122) with the 1st IS. Taking these data together with the finding that the between-laboratory variability is also greater for bioassays (GCV 26.8%) compared to receptor binding assays (GCV 14.5%), it is proposed that the candidate standard be value-assigned on the basis of receptor binding assays alone and that the assigned unitage of 0.113 IU per ampoule should reflect formal continuity of unitage with 90/672 for these assays alone. This is unlikely to be a significant restriction to users of bioassays since these are more commonly reported as a percentage increase over reference control serum rather than in terms of the 1st IS.

Although, in accordance with WHO guidelines, every effort should be made to ensure that the IU defined by a replacement reference standard is as similar as possible to the IU defined by the old reference standard so that continuity of the IU is maintained, this is not always possible. Although it can be argued that the monoclonal nature of the candidate standard and the single epitope on the TSHR to which it binds, may not be fully representative of the heterogeneous mixture of TRAbs present in patient serum, a number of reports now suggest that thyroid-stimulating antibodies and thyroid-blocking antibodies bind to very similar or even overlapping epitopes within the TSHR (8, 10-12). Therefore, although it is important to recognise that a monoclonal autoantibody with thyroid-stimulating activity is clearly not an identical replacement reference standard for 90/672, the calibration of 08/204 in terms of 90/672 in this study has demonstrated its suitability to serve as an International Standard for the calibration of receptor binding assays for the measurement of TSAbs. Furthermore, the establishment of 08/204 as the 2nd IS for TSAbs has the additional advantage that it would be possible to replace such a preparation with an identical material, when stocks become exhausted. Finally, it should also be noted that the monoclonal nature of the preparation 08/204 may render it unsuitable as an experimental standard for TSAbs in some basic research milieu since it may not truly reflect the action of a mixed population of thyroid-stimulating antibodies in these settings. With these limitations in mind, the establishment of a reference preparation for TRAbs, derived from pooled patient plasma, should be retained as a priority for the WHO standardisation programme.

The candidate preparation 08/204 appears to be sufficiently stable to serve as an international standard since the predicted yearly loss of TSAb potency at -20°C is 0.018% based on the thermally accelerated degradation samples assayed in this study. These results indicate that 08/204 is likely to be highly stable under long terms storage conditions at -20°C.

Proposal

With the agreement of all the study participants, it is recommended that the preparation in ampoules (2800 in total), coded 08/204, be established as the Second International Standard for Thyroid stimulating antibody, with an assigned content of 113 mIU per ampoule.

Acknowledgements

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Table 4. Laboratory mean estimates (IU/ampoule) calculated relative to 90/672

Lab	C, E (-20°C)		D (4°C)	A (20°C)	F (37°C)	B(45°C)
	GM	GCV	GM	GM	GM	GM
1	0.132	.	.	.	.	.
2a	0.118	5.9%	0.110	0.117	0.104	0.096
2b	0.127	5.0%	0.122	0.123	0.112	0.107
2c	0.171	14.5%	.	.	.	.
2d	0.261	8.3%	.	.	.	.
3a	0.293	8.4%	0.286	0.264	0.269	0.210
3b	0.128	4.3%	0.127	0.116	0.115	0.104
3c	0.112	5.0%	0.108	0.110	0.100	0.085
4	0.115	6.3%	.	0.111	0.095	0.104
5a	0.120	1.6%	0.119	0.116	0.109	0.098
5b	0.120	11.1%	0.123	0.113	0.120	0.108
6a	0.103	.	0.099	0.103	.	0.087
6b	0.106	.	0.103	0.100	.	0.091
6c	0.090	.	0.087	0.097	.	0.072
7	0.120	1.1%	0.121	0.124	0.110	0.100
8a	0.104	.	0.104	0.109	.	0.077
8b	0.109	.	0.112	0.118	.	0.098
9a	0.109	.	0.107	0.110	0.097	0.088
9b	0.138	4.4%	0.134	0.138	0.119	0.103
10	0.105	.	0.098	0.109	0.089	0.090
11a	0.081	4.6%	.	.	0.076	.
11b	0.095	6.8%	.	.	0.081	.
12a	0.119	.	.	.	.	.
12b	0.262	17.9%	.	.	.	.
13	0.139	11.9%	.	.	.	.
All assays						
GM	0.128		0.118	0.119	0.109	0.098
GCV	38.0%		29.9%	24.9%	34.4%	25.3%
95% CI	0.112 – 0.146		0.102 – 0.135	0.106 – 0.133	0.092 – 0.129	0.088 – 0.110
n	25		16	17	14	17
Receptor binding						
GM	0.113		0.111	0.113	0.101	0.094
GCV	14.5%		12.2%	8.9%	15.4%	12.5%
95% CI	0.106 – 0.120		0.104 – 0.118	0.108 – 0.118	0.093 – 0.110	0.088 – 0.100
% of C,E	100		98.2	100	89.4	83.2
n	21		15	16	13	16
Bioassays:						
GM	0.242		0.286	0.264	0.269	0.210
GCV	26.8%		.	.	.	.
95% CI	0.166 – 0.353		.	.	.	.
n	4		1	1	1	1

Figure 1. Laboratory mean estimates (IU/ampoule) for 08/204 calculated relative to 90/672

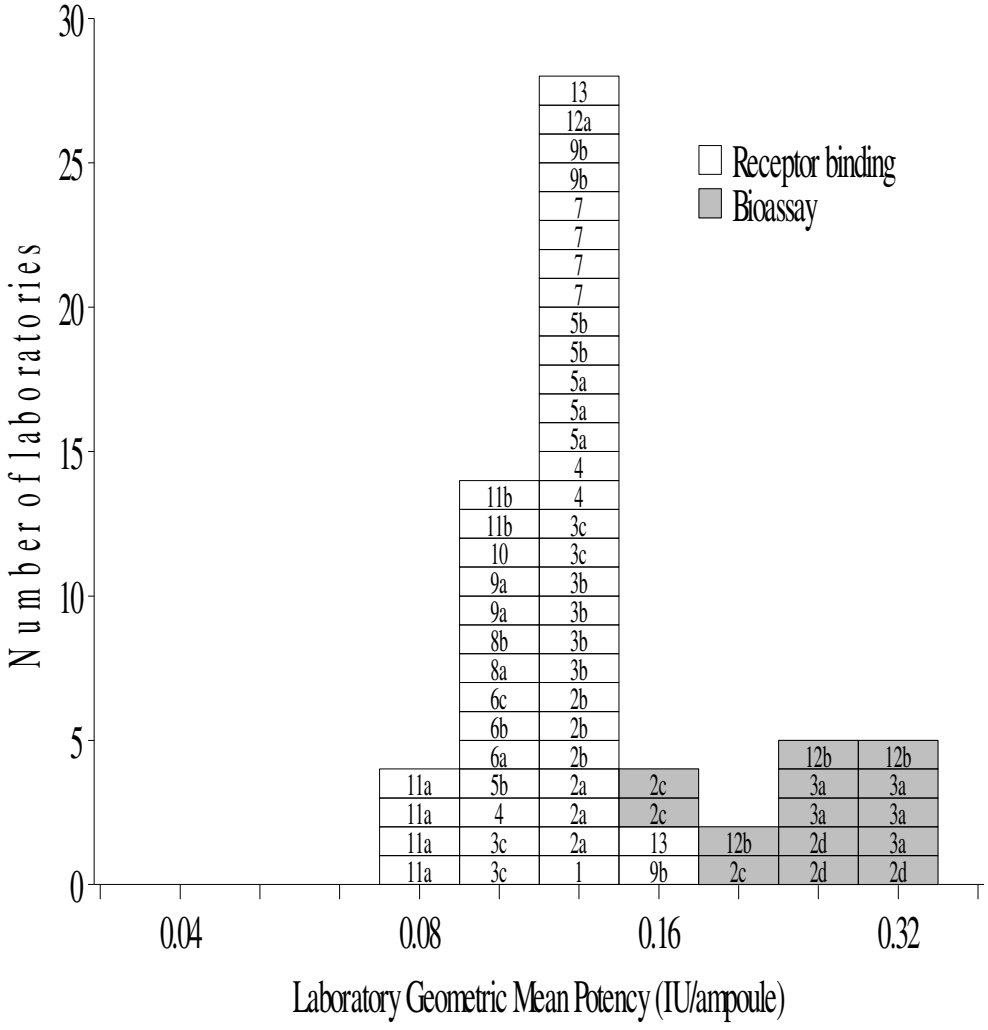

Table 5. Laboratory mean estimates (IU/ampoule) calculated relative to local (manufacturer's kit) standards.

Lab	C, E (-20°C)		D (4°C)	A (20°C)	F (37°C)	B(45°C)	90/672
	GM	GCV	GM	GM	GM	GM	GM
1	0.134	.	.	.	.	.	0.102
2a	0.102	8.2%	0.088	0.102	0.084	0.078	0.084
2b	.	.	.	.	.	.	.
2c	.	.	.	.	.	.	.
2d	.	.	.	.	.	.	.
3a	.	.	.	.	.	.	.
3b	0.111	38.8%	0.127	0.109	0.087	0.100	0.089
3c	0.113	3.5%	0.110	0.108	0.100	0.083	0.100
4	0.120	34.0%	.	0.130	0.109	0.123	0.117
5a	0.114	1.8%	0.114	0.111	0.104	0.094	0.095
5b	0.111	9.3%	0.110	0.104	0.102	0.089	0.087
6a	.	.	.	.	.	.	.
6b	.	.	.	.	.	.	.
6c	.	.	.	.	.	.	.
7	0.118	2.4%	0.118	0.122	0.109	0.099	0.099
8a	0.110	.	0.108	0.108	.	0.080	0.104
8b	0.123	.	0.122	0.129	.	0.094	0.100
9a	0.105	.	0.108	0.103	0.103	0.085	0.090
9b	0.088	2.3%	0.086	0.087	0.078	0.066	0.065
10	0.130	.	0.125	0.126	0.117	0.105	0.112
11a	0.079	7.3%	.	.	0.075	.	0.102
11b	0.131	16.2%	.	.	0.110	.	0.160
12a	0.105	.	.	.	.	.	0.097
12b	0.122	6.3%	.	.	.	.	0.068
13	0.136	1.6%	.	.	.	.	0.094
All assays:							
GM	0.113		0.110	0.111	0.097	0.090	0.096
GCV	15.2%		13.8%	12.4%	15.9%	17.4%	21.7%
95% CI	0.105–0.121		0.101–0.120	0.103–0.119	0.089–0.107	0.082–0.100	0.087–0.106
n	18		11	12	12	12	18
Receptor binding:							
GM	0.112						0.098
GCV	15.6%						19.9%
95% CI	0.104–0.121						0.090–0.108
n	17						17

APPENDIX 1: INDIVIDUAL ASSAY RESULTS

Table A1. Potency estimates (IU/ampoule) calculated relative to 90/672

Lab	Assay	A	B	C	D	E	F	C:E ratio
1	1	.	.	0.133	.	0.131	.	1.016
2a	1	0.115	.	0.113	0.113	0.114	0.105	0.986
2a	2	0.120	0.096	0.128	0.113	0.124	0.109	1.031
2a	3	0.115	0.096	0.119	0.105	0.111	0.098	1.070
2b	1	0.131	0.112	0.131	0.128	0.134	0.119	0.981
2b	2	0.121	0.106	0.132	0.122	0.130	0.113	1.017
2b	3	0.116	0.104	0.119	0.117	0.120	0.105	0.994
2c	1	.	.	0.192	.	0.143	.	1.344
2c	2*	0.115	0.086	0.100	0.095	0.083	.	1.211
2c	3	.	.	0.196	.	.	.	.
2c	4*	0.097	0.076	0.100	0.087	0.075	.	1.329
2c	5	.	.	0.175	.	0.156	.	1.126
2c	6*	.	0.087	0.100	0.090	0.086	.	1.159
2d	1	.	.	0.294	.	0.277	.	1.063
2d	2*	0.112	0.096	0.100	0.113	0.085	.	1.172
2d	3	.	.	0.268	.	0.246	.	1.093
2d	4*	0.116	0.083	0.100	0.110	0.086	.	1.160
2d	5	.	.	0.247	.	0.241	.	1.028
2d	6*	0.111	0.085	0.100	0.112	0.097	.	1.034
3a	1a	0.265	0.212	.	.	.	.	.
3a	1b	.	.	0.265	0.264	.	.	.
3a	1c	.	.	.	.	0.295	0.257	.
3a	2a	0.240	0.202	.	.	.	.	.
3a	2b	.	.	0.312	0.304	.	.	.
3a	2c	.	.	.	.	.	.	.
3a	3a	0.289	0.214	.	.	.	.	.
3a	3b	.	.	0.320	0.292	.	.	.
3a	3c	.	.	.	.	0.274	0.282	.
3b	1a	0.113	0.106	.	.	.	.	.
3b	1b	.	.	0.125	0.113	.	.	.
3b	1c	.	.	.	.	0.128	0.114	.
3b	2a	0.119	0.103	.	.	.	.	.
3b	2b	.	.	0.136	0.143	.	.	.
3b	2c	.	.	.	.	0.123	0.116	.
3c	1a	0.112	0.087	.	.	.	.	.
3c	1b	.	.	0.113	0.115	.	.	.
3c	1c	.	.	.	.	0.118	0.108	.
3c	2a	0.108	0.083	.	.	.	.	.
3c	2b	.	.	0.105	0.102	.	.	.
3c	2c	.	.	.	.	0.111	0.093	.
4	1	0.111	.	0.123	.	.	.	.

4	2	.	0.109	.	.	0.113	0.101	.
4	3	.	.	.	.	.	.	.
4	4	.	0.100	0.056**	.	0.110	0.089	.
5a	1	0.117	0.097	0.118	0.121	0.121	0.109	0.980
5a	2	0.116	0.098	0.117	0.117	0.120	0.108	0.975
5a	3	.	.	0.120	.	0.122	.	0.987
5b	1	0.119	0.113	0.132	0.134	0.137	0.136	0.963
5b	2	0.107	0.103	0.107	0.114	0.106	0.107	1.006
5b	3	.	.	0.122	.	0.121	.	1.006
6a	1	0.103	0.087	0.104	0.099	0.102	.	1.019
6b	1	0.100	0.091	0.106	0.103	0.106	.	1.000
6c	1	0.097	0.072	0.093	0.087	0.087	.	1.076
7	1	0.120	0.100	0.119	.	.	.	.
7	2	.	.	.	0.119	0.118	0.105	.
7	3	0.129	0.101	0.121	.	.	.	.
7	4	.	.	.	0.123	0.121	0.115	.
8a	1	0.109	0.077	0.105	0.104	0.104	.	1.013
8a	2	.	.	.	.	.	.	.
8b	1	0.118	0.098	0.111	0.112	0.108	.	1.026
8b	2	.	.	.	.	.	.	.
9a	1	0.110	0.087	0.114	0.113	0.110	0.102	1.036
9a	2	0.110	0.089	0.106	0.102	0.107	0.093	0.991
9b	1	0.141	0.099	0.142	0.138	0.147	0.126	0.966
9b	2	0.135	0.100	0.136	0.130	0.130	0.113	1.046
9b	3	0.137	0.110	0.138	0.133	0.134	0.118	1.030
10	1	0.109	0.090	0.112	0.098	0.098	0.089	1.140
11a	1	.	.	0.080	.	0.087	0.082	0.920
11a	2	.	.	0.075	.	0.081	0.080	0.919
11a	3	.	.	0.083	.	0.084	0.075	0.988
11a	4	.	.	0.080	.	0.081	0.070	0.992
11b	1	.	.	0.091	.	0.090	0.081	1.012
11b	2	.	.	0.103	.	0.099	0.081	1.043
12a	1	.	.	0.121	.	0.118	.	1.032
12a	2	.	.	.	.	.	.	.
12a	3	.	.	.	.	.	.	.
12b	1	.	.	0.270	.	0.280	.	0.964
12b	2	.	.	0.220	.	0.209	.	1.053
12b	3	.	.	0.312	.	0.300	.	1.040
13	1	.	.	0.144	.	0.148	.	0.974
13	2	.	.	0.150	.	0.118	.	1.271

*potencies relative to sample C
**excluded from further calculations

Table A2. Potency estimates[§] calculated relative to local standards.

Lab	Assay	90/672	A	B	C	D	E	F
1	.	0.102	.	.	0.135	.	0.133	.
2a	1	.	.	.	.	.	.	.
2a	2	0.085	0.105	0.080	0.110	0.093	0.104	0.091
2a	3	0.083	0.098	0.077	0.103	0.084	0.091	0.077
2b*	1	.	.	.	.	.	.	.
2b*	2	.	.	.	.	.	.	.
2b*	3	.	.	.	.	.	.	.
2c[#]	1	.	.	.	.	.	.	.
2c[#]	2	.	.	.	.	.	.	.
2c[#]	3	.	.	.	.	.	.	.
2c[#]	4	.	.	.	.	.	.	.
2c[#]	5	.	.	.	.	.	.	.
2c[#]	6	.	.	.	.	.	.	.
2d[#]	1	.	.	.	.	.	.	.
2d[#]	2	.	.	.	.	.	.	.
2d[#]	3	.	.	.	.	.	.	.
2d[#]	4	.	.	.	.	.	.	.
2d[#]	5	.	.	.	.	.	.	.
2d[#]	6	.	.	.	.	.	.	.
3a[$]	1a	.	1293.400	870.600	.	.	.	.
3a[$]	1b	.	.	.	864.200	897.000	.	.
3a[$]	1c	.	.	.	.	.	1136.000	831.800
3a[$]	2a	.	709.000	555.400	.	.	.	.
3a[$]	2b	.	.	.	1369.400	1097.600	.	.
3a[$]	2c	.	.	.	.	.	1266.700	1135.000
3a[$]	3a	.	954.400	628.600	.	.	.	.
3a[$]	3b	.	.	.	1950.000	1059.200	.	.
3a[$]	3c	.	.	.	.	.	854.000	728.000
3b	1a	0.095	0.107	0.101	.	.	.	.
3b	1b	0.137	.	.	0.171	0.155	.	.
3b	1c	0.091	.	.	.	.	0.116	0.104
3b	2a	0.093	0.110	0.100	.	.	.	.
3b	2b	0.074	.	.	0.100	0.105	.	.
3b	2c	0.064	.	.	.	.	0.078	0.074
3c	1a	0.099	0.111	0.086	.	.	.	.
3c	1b	0.102	.	.	0.115	0.117	.	.
3c	1c	0.098	.	.	.	.	0.115	0.105
3c	2a	0.097	0.105	0.080	.	.	.	.
3c	2b	0.102	.	.	0.107	0.104	.	.
3c	2c	0.103	.	.	.	.	0.114	0.096
4	1	0.120	0.130	.	0.142	0.156	0.156	.
4	2	0.116	.	0.123	0.088	.	0.128	0.117
4	3	0.117	0.130	.	0.135	0.133	0.146	.

4	4	0.114	.	0.123	0.067	.	0.128	0.102
5a	1	0.095	0.109	0.093	0.111	0.115	0.115	0.104
5a	2	0.097	0.113	0.096	0.113	0.113	0.116	0.105
5a	3	0.094	.	.	0.112	.	0.116	.
5b	1	0.082	0.105	0.091	0.120	0.112	0.127	0.112
5b	2	0.095	0.103	0.087	0.103	0.108	0.101	0.093
5b	3	0.084	.	.	0.107	.	0.107	.
6a[#]	1	.	.	.	.	.	.	.
6b[#]	1	.	.	.	.	.	.	.
6c[#]	1	.	.	.	.	.	.	.
7	1	0.099	0.118	0.099	0.116	.	.	.
7	2	0.099	.	.	.	0.115	0.115	0.104
7	3	0.099	0.126	0.100	0.120	.	.	.
7	4	0.100	.	.	.	0.122	0.120	0.113
8a	1	0.086	0.087	0.063	0.088	0.084	0.085	.
8a	2	0.125	0.134	0.102	0.142	0.139	0.138	0.116
8b	1	0.099	0.119	0.087	0.106	0.105	0.103	.
8b	2	0.102	0.141	0.102	0.146	0.142	0.145	0.124
9a	1	0.088	0.105	0.081	0.101	0.109	0.105	0.103
9a	2	0.091	0.102	0.089	0.103	0.108	0.113	0.103
9b	1	0.061	0.084	0.060	0.086	0.086	0.087	0.078
9b	2	0.069	0.088	0.070	0.088	0.086	0.087	0.074
9b	3	0.065	0.090	0.070	0.092	0.087	0.088	0.082
10	1	0.112	0.126	0.105	0.130	0.125	0.130	0.117
11a	1	0.100	.	.	0.075	.	0.080	0.077
11a	2	0.095	.	.	0.069	.	0.075	0.074
11a	3	0.106	.	.	0.084	.	0.084	0.076
11a	4	0.108	.	.	0.083	.	0.082	0.073
11b	1	0.150	.	.	0.115	.	0.116	0.103
11b	2	0.170	.	.	0.146	.	0.153	0.119
12a	1	0.088	.	.	0.108	.	0.105	.
12a	2	0.106	.	.	0.104	.	0.114	.
12a	3	0.098	.	.	0.089	.	0.111	.
12b	1	0.064	.	.	0.126	.	0.123	.
12b	2	0.077	.	.	0.130	.	0.124	.
12b	3	0.063	.	.	0.120	.	0.109	.
13	1	0.094	.	.	0.135	.	0.138	.
13	2	.	.	.	.	.	.	.

[§]All data are expressed as IU/ampoule (calibrated vs 90/672) with the exception of lab 3a which are expressed as ng IgG/ampoule and are excluded from the overall calculations.

*Assay results non-parallel with local standard; [#]No data for local standard provided; [$]Assay results expressed relative to in house M22 IgG preparation.

Table A3. Slopes of fitted dose response lines

Lab	Assay	90/672	A	B	C	D	E	F
1	.	1.043	.	.	1.012	.	0.965	.
2a	1	-7844	-7688	.	-7616	-7460	-7460	-7255
2a	2	-5688	-5450	-6037	-6271	-6209	-5871	-6535
2a	3	-5431	-4933	-5032	-5657	-5799	-5611	-5602
2b	1	1.593	1.499	1.595	1.498	1.535	1.485	1.596
2b	2	1.602	1.551	1.566	1.431	1.503	1.424	1.55
2b	3	1.361	1.353	1.366	1.335	1.342	1.316	1.384
2c	1	15197	.	.	19033	.	16666	.
2c	2	.	15056	15446	14819	16528	15687	.
2c	3	15554	.	.	14939	.	.	.
2c	4	.	15087	13659	15759	14284	14872	.
2c	5	14907	.	.	14096	.	14621	.
2c	6	.	.	14785	14607	12971	13643	.
2d	1	21545	.	.	22311	.	23864	.
2d	2	.	29537	25716	20299	21085	24027	.
2d	3	16717	.	.	17235	.	16146	.
2d	4	.	17366	18577	18140	17527	18419	.
2d	5	23842	.	.	28368	.	27214	.
2d	6	.	25187	25169	23182	27011	27928	.
3a	1a	-0.2551	-0.2344	-0.2644	.	.	.	.
3a	1b	-0.2555	.	.	-0.2404	-0.2350	.	.
3a	1c	-0.2344	.	.	.	.	-0.2505	-0.2389
3a	2a	-0.2570	-0.2574	-0.2289	.	.	.	.
3a	2b	-0.2546	.	.	-0.2135	-0.2165	.	.
3a	2c	.	.	.	.	.	.	.
3a	3a	-0.2447	-0.2679	-0.2449	.	.	.	.
3a	3b	-0.2622	.	.	-0.2442	-0.2485	.	.
3a	3c	-0.2362	.	.	.	.	-0.2389	-0.2475
3b	1a	1.219	1.189	1.049	.	.	.	.
3b	1b	1.014	.	.	1.023	0.993	.	.
3b	1c	1.200	.	.	.	.	1.274	1.202
3b	2a	1.267	1.192	1.122	.	.	.	.
3b	2b	1.294	.	.	1.165	1.182	.	.
3b	2c	1.126	.	.	.	.	1.176	1.051
3c	1a	-1.374	-1.376	-1.397	.	.	.	.
3c	1b	-1.398	.	.	-1.374	-1.335	.	.
3c	1c	-1.274	.	.	.	.	-1.338	-1.309
3c	2a	-1.363	-1.354	-1.324	.	.	.	.
3c	2b	-1.364	.	.	-1.358	-1.326	.	.
3c	2c	-1.266	.	.	.	.	-1.313	-1.336
4	1	-1.380	-1.350	.	-1.361	-1.051	-1.135	.
4	2	-1.471	.	-1.313	-1.800	.	-1.370	-1.408

4	3	-1.718	-1.492	.	-1.417	-1.434	-1.362	.
4	4	-2.017	.	-1.685	-1.772	.	-1.663	-1.986
5a	1	-1.396	-1.417	-1.396	-1.386	-1.389	-1.382	-1.382
5a	2	-1.337	-1.308	-1.307	-1.322	-1.342	-1.307	-1.330
5a	3	-1.483	.	.	-1.450	.	-1.417	.
5b	1	-2.640	-2.620	-2.303	-2.495	-2.351	-2.478	-2.164
5b	2	-2.280	-2.474	-2.106	-2.492	-2.416	-2.565	-2.177
5b	3	-2.559	.	.	-2.514	.	-2.364	.
6a	1	.	.	.	.	.	.	.
6b	1	.	.	.	.	.	.	.
6c	1	.	.	.	.	.	.	.
7	1	-1.265	-1.382	-1.276	-1.397	.	.	.
7	2	-1.291	.	.	.	-1.352	-1.371	-1.301
7	3	-1.202	-1.375	-1.267	-1.347	.	.	.
7	4	-1.246	.	.	.	-1.360	-1.340	-1.350
8a	1	-1.066	-0.924	-1.185	-1.086	-1.117	-1.084	.
8a	2	-1.030	-0.816	-1.085	-0.902	-0.837	-0.817	-0.777
8b	1	-6903	-6624	-6226	-6872	-6551	-6456	.
8b	2	-7108	-6706	-6326	-5633	-5889	-6598	-6166
9a	1	-1.821	-1.782	-1.713	-1.705	-1.733	-1.868	-1.742
9a	2	-1.703	-1.692	-1.668	-1.627	-1.658	-1.749	-1.757
9b	1	-2.122	-1.847	-1.729	-1.708	-1.762	-1.661	-1.672
9b	2	-2.314	-1.858	-1.778	-1.723	-1.876	-1.861	-1.959
9b	3	-1.722	-1.560	-1.511	-1.572	-1.549	-1.568	-1.665
10	1	-1.390	-1.439	-1.338	-1.409	-1.424	-1.374	-1.368
11a	1	1.017	.	.	0.999	.	1.007	0.989
11a	2	1.046	.	.	1.036	.	1.022	1.027
11a	3	1.019	.	.	0.981	.	0.989	0.975
11a	4	1.041	.	.	1.018	.	1.019	1.024
11b	1	-1.259	.	.	-1.356	.	-1.375	-1.400
11b	2	-1.349	.	.	-1.227	.	-1.305	-1.307
12a	1	-1.550	.	.	-1.474	.	-1.541	.
12a	2	.	.	.	.	.	.	.
12a	3	-1.259	.	.	-1.458	.	-1.304	.
12b	1	-1.146	.	.	-1.167	.	-1.112	.
12b	2	-1.123	.	.	-1.137	.	-1.155	.
12b	3	-1.332	.	.	-1.419	.	-1.354	.
13	1	-1.467	.	.	-1.280	.	-1.387	.
13	2	-1.588	.	.	-1.129	.	-1.531	.

APPENDIX 2: Study Protocol

Replacement of the 1st WHO International Standard for Thyroid-Stimulating Antibody (90/672)

INTRODUCTION

Thyroid-stimulating antibodies (TSAbs) can be detected in approximately 75% of patients with hyperthyroidism caused by Graves' disease and have also been associated with a small portion of patients with toxic multinodular goitre. These autoantibodies interact with the TSH receptor on thyroid follicular cells and usually result in global stimulation of thyroid activity. Measurements of TSAbs are useful in the diagnosis and management of disease. For example, the detection of TSAbs has been used to predict relapse or remission in patients receiving treatment for Graves' disease, and also to predict neonatal hyperthyroidism in children of mothers with Graves' disease. The original preparation of 90/672, stocks of which are now exhausted, consisted of freeze-dried plasma proteins from a single human patient with high TSAb levels. A number of the current users of 90/672 have been approached regarding a suitable replacement preparation and a candidate material has now been filled into ampoules (NIBSC Code 08/204), following procedures recommended by WHO (1). It is intended that an international collaborative study is organised with expert laboratories to aid in the value assignment of the proposed International Standard (IS).

AIMS OF THE STUDY

- To calibrate the candidate standard (08/204) relative to the 1st IS for TSAb (90/672).
- To demonstrate the suitability of the preparation 08/204 to serve as the 2nd IS for TSAb by examining its behaviour in receptor binding assays and bioassay systems.
- To assess the relationships among the existing local standards and the proposed IS.
- To determine the stability of the preparation 08/204 by comparing them with ampoules that have been subjected to accelerated thermal degradation.

MATERIALS

Preparations supplied to participants in collaborative study.

A bulk preparation of a monoclonal autoantibody to the TSH receptor, produced from lymphocytes from a patient with Graves' disease, was generously donated by Dr B.Rees Smith, RSR Ltd, Cardiff. The material was added to a pool of healthy human serum (tested and found to be negative for both TSAbs and HIV, Hepatitis B and Hepatitis C), containing 40mM HEPES, before being dispensed (1ml aliquots) into glass ampoules, lyophilised and sealed.

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

Table 1.

Thyroid stimulating antibody preparation	**Ampoule content**
1st International Standard TSAb (90/672)	0.1IU/ampoule
Candidate standard 08/204 stored at -20°C	Nominally 1µg (approx 0.1IU)/ampoule
Accelerated thermal degradation (ATD) samples of 08/204 stored at +4°C, +20°C, +37°C , +45°C	Content assumed identical to 08/204

TESTS REQUESTED

Participants are requested to carry out the assay(s) normally in use in their laboratory and, where possible, to perform at least two independent assays, using fresh ampoules (not a stored aliquot), each assay to include all of the preparations allocated **at preferably no less than five dose levels in the linear part of the dose-response curve** to provide information on parallelism. In instances where there is not a fresh ampoule for subsequent assays, it is suggested that fresh dilutions are made from frozen stock solutions, and where this is the case, participants are requested to provide details of freeze-thaw steps. **Participants are also asked to ensure that all assays include their local standard where possible.**

The ampoule contents of the test preparations are listed in Table 1. The candidate standard 08/204 and its degradation samples will be coded, in random order, by letter in the final protocol. On receipt ampoules should be stored at -20°C until use. It is recommended that the contents of each ampoule are reconstituted in 1ml distilled H_2O and appropriate dilutions made from this stock.

Participants are asked to provide details of the assay methods used, including dilution steps, together with all **raw assay data (**in electronic format if possible) in the form of clearly annotated optical densities, counts, etc. for central computation at NIBSC. Participants' own estimates of activity as calculated by the method normally used in their laboratory are also requested.

REPORT

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

REFERENCES

1. WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards (revised 2004). In: WHO TRS, No. 932, 2006, Annex 2

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Participants in the study are advised to take note of the disclaimers in the 'Instructions for Use' which accompany the samples and of the prohibitions against (i) use in humans (ii) further transfer (iii) use for commercial purposes, and (iv) use for any purpose other than the establishment of a reference standard. They are also requested not to publish or circulate information concerning the candidate material without the prior agreement of the NIBSC on behalf of WHO. After agreement by all participants on the final report and after submission to the ECBS, this reservation no longer applies.

Appendix 3: Draft Instructions for use.

Proposed 2nd WHO International Standard for Thyroid Stimulating Antibody 08/204

Instructions for Use (June 2010, first version)

This material is not for *in vitro* diagnostic use

1. INTRODUCTION

The first International Standard (IS) for Thyroid-stimulating antibody (TSAb) 90/672, stocks of which are now exhausted, consisted of freeze-dried plasma proteins from a single human patient with high TSAb levels. A candidate replacement material has now been filled into ampoules (NIBSC Code 08/204) and has been characterized by receptor binding assays in an international collaborative study with expert laboratories to aid in the value assignment of the proposed 2nd International Standard.

2. AMPOULE CONTENTS

Each ampoule contains the freeze-dried residue of 1ml of a solution containing a monoclonal autoantibody to the TSH receptor which had been added to a pool of healthy human serum, containing 40mM HEPES.

3. UNITAGE

For the calibration of receptor-binding assays the assigned content is 113 mIU TSAb per ampoule.

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS
The preparation does not contain material of human origin.
A safety data sheet is included in the last page of these instructions.

5. USE OF AMPOULES

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution. For practical purposes each ampoule contains the same quantity of TSAb. The entire content of each ampoule should be completely dissolved in an accurately measured amount of diluent. No attempt should be made to weigh out portions of the freeze-dried powder. Suitable diluents are PBS, saline and most assay buffers. If the contents are to be diluted extensively, the addition of 0.05 – 0.1% protein (HSA or BSA) is recommended to minimise adsorption. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat. A fresh ampoule should be used for each assay as repeated freeze-thawing may lead to loss of potency, although if required, users should conduct their own investigations.

Suitable precautions should be taken in the use and disposal of the ampoule and its contents: see **MATERIAL SAFETY SHEET**.

6. DIRECTIONS FOR OPENING AMPOULE

Tap the ampoule gently to collect the material at the bottom (labeled) 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. STABILITY

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. Stability studies at elevated temperatures have shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned value. Reference Materials should be stored on receipt as indicated on the label. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. NIBSC follows the policy of WHO with respect to its reference materials. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

8. CITATION

In all publications (or data sheets for kits) in which this preparation is used as an assay calibrant, it is important that the title of the preparation, ampoule code and the name and address of NIBSC are cited and cited correctly.

9. PRODUCT LIABILITY

9.1 Information emanating from NIBSC is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but is provided without liability in its application and use.

9.2 This product is intended for use as a standard or reference material in laboratory work in relation to biological research, manufacturing or quality control testing of biological products or in the field of in vitro diagnostics. It is the responsibility of the user to ensure that he/she has the necessary technical skills to determine the appropriateness of this product for the proposed application. Results obtained from this product are likely to be dependent on the conditions of use and the variability of materials beyond the control of NIBSC.
NIBSC accepts no liability whatsoever for any loss or damage arising from the use of this product, whether loss of profits, or indirect or consequential loss or otherwise, including, but not limited to, personal injury other than as caused by the negligence of NIBSC. In particular, NIBSC accepts no liability whatsoever for :-

i) results obtained from this product; and/or
ii) non-delivery of goods or for damages in transit.

9.3 In the event of any replacement of goods following loss or damage a customer accepts as a condition of receipt of a replacement product, acceptance of the fact that the replacement is not to be construed as an admission of liability on NIBSC's behalf.

10. MATERIAL SAFETY SHEET

Proposed 2nd WHO International Standard for Thyroid Stimulating Antibody 08/204

Physical Properties (at room temperature)	
Physical appearance *Freeze-dried powder*	
Fire hazard *None*	
Chemical Properties	
Stable *Yes*	Corrosive *No*
Hygroscopic *Yes*	Oxidising *No*
Flammable *No*	Irritant *No*
Other (specify)	
Handling: *See precautions in section 4*	
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 viricidal agent. Rinse area with a viricidal agent followed by water. *Absorbent material used to treat spillage should be treated as biologically hazardous waste.*	

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