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WHO International Collaborative Study of the proposed 5th International Standard for Chorionic Gonadotrophin

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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 Chorionic Gonadotrophin (CG) for the calibration of assays to control the quality and potency of CG used in the treatment of infertility, and for the calibration of assays used in the diagnosis of pregnancy and a range of other clinical conditions.

We report here the characterization of two candidate standards for CG in an International Collaborative Study carried out by 19 laboratories in 11 countries, and their comparison by immunoassay and bioassay with the existing International Standard coded 75/589, calibrated in International Units, and the WHO 1st Reference Reagent for intact CG coded 99/688, calibrated in molar units.

Estimates of the CG content of the candidate standard, coded 07/364, by immunoassay indicated that it would be suitable to serve as a reference preparation for immunoassay with an assigned content of 179 IU per ampoule. Additionally, by comparison with 99/688, immunoassay estimates of the CG content of the candidate standard in molar units indicated a content of 0.39 nmol per ampoule. Furthermore, estimates by bioassay indicated that it would be suitable to serve as a reference preparation for bioassay with an assigned content of 162 IU per ampoule. The results of this study also indicate that the candidate standard appears sufficiently stable, on the basis of a thermally accelerated degradation study, to serve as an international standard.

Introduction
Chorionic Gonadotrophin (CG) is a glycoprotein hormone produced by the developing embryo in pregnancy. Its role is to support the corpus luteum and thereby maintain the levels of progesterone that are required for pregnancy. The 3rd International Standard for Chorionic Gonadotrophin (ampoules coded 75/537) was established by the WHO Expert Committee on Biological Standardization in 1986. When this preparation was exhausted, a second batch of ampoules coded 75/589, containing the same bulk preparation of CG as that in the 3rd IS and prepared at the same time as the 3rd IS using identical procedures, was established as the 4th International Standard for Chorionic Gonadotrophin in 1999. Both standards were purified from urine but contained small amounts of the nicked and ß-subunit forms of CG. Both standards have been widely used for the calibration of assays to control the quality and potency of CG used in the treatment of infertility, and for the calibration of assays used in the diagnosis of pregnancy and a range of other clinical conditions. Both applications require approximately equal numbers of ampoules. Stocks of the 4th IS are rapidly depleting and the standard needs to be replaced.

In 2001, in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), WHO established a panel of 6 CG preparations (WHO Reference Reagents) including intact, nicked and sub-unit forms, for the intended purpose of investigating and characterizing the specificity of existing CG assays (1-3). The intact CG (ampoules coded 99/688) was highly purified from urine to remove other forms of CG particularly the nicked forms and free subunits. The IFCC have agreed that some of the intact CG be made available to WHO, to be filled into ampoules and included as a candidate preparation in an international study to establish the 5th WHO International Standard for Chorionic Gonadotrophin. With the increasing availability of recombinant CG and allowing for the possibility that therapeutic and diagnostic manufacturers may make increasing use of recombinant in-house reference materials, a recombinant preparation of CG, kindly donated to WHO by Merck Serono (Geneva, Switzerland) and previously filled into ampoules at NIBSC (NIBSC Code 96/608) following procedures recommended by WHO (4), was also included in the study.

Although currently, clinical hCG results are reported in IU and therapeutic preparations of hCG are assigned potency in IU, the purity of the preparation of intact hCG coded 99/688, established as part of the WHO Reference Reagent panel, enabled assignment of its unitage in substance concentrations (nmol/ampoule). As the diagnostic community may wish to calibrate assays for hCG in substance concentrations in the future, it is the intention here to provide a unitage for the ampoule contents of the candidate standards in both IU and nmol.

The aims of the study were, therefore:

1) to compare, by immunoassay and bioassay, the ampouled preparations of intact and recombinant CG with local standards presently in use,
2) to calibrate the preparation of intact CG or recombinant CG for use as a potential International Standard,
3) to assess the stability of the proposed International Standard after accelerated thermal degradation

Participants

19 laboratories in 11 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

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Materials

Bulk materials and preparation of ampoules of native and recombinant CG

A bulk preparation of intact CG, highly purified to remove other forms of CG, was donated to the IFCC as part of a project to establish a panel of reference reagents for the characterisation of the specificity of CG assays (1-3). 129 mg of this lyophilised bulk material (tested and found to be negative for HIV, Hepatitis C and Hepatitis B) was dissolved in 6.336 litres of sterile water containing 50mM sodium phosphate (pH7.4), 2 mg/ml human serum albumin (tested and found to be negative for HIV, Hepatitis C and Hepatitis B) and 10 mg/ml trehalose and was then ampouled at 1.0 ml per ampoule (nominally 20 µg CG). Highly purified recombinant human (rh)CG, expressed in CHO cells, was kindly donated to WHO by Merck Serono (Geneva, Switzerland). The preparation was received as a sterile liquid which, after formulation with 0.89% (w/v) NaCl and 1% (w/v) human serum albumin (tested and found to be negative for HIV, Hepatitis C and Hepatitis B), had been previously ampouled at 1.0 ml per ampoule (nominally 8.6 µg/ml CG). Both the native (urinary, non-recombinant) CG and the recombinant CG were lyophilised and sealed according to procedures described by WHO for International Biological Standards (4) and stored at -20°C in the dark at NIBSC. A final total of 6000 ampoules of intact native CG, each coded 07/364, was obtained, with a mean fill weight of 1.007 g (CV 0.10%), a mean dry weight of 0.02 g (CV 4.17%) and a residual moisture content of 2.49% (CV 14.29%). A final total of 3425 ampoules of rhCG, each coded 96/608, was obtained, with a mean fill weight of 1.011 g (CV 0.08%), a mean dry weight of 0.02 g (CV 0.004%) and a residual moisture content of 0.70% (CV 7.04%).

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, the 4th IS and the IRR 96/688), 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

Ampoule code	CG preparation	Ampoule unitage and nominal content
Not coded	The 4th I.S. for CG (75/589) stored at -20°C	650 IU ampoule
G, H Duplicates	Intact CG candidate standard (07/364) stored at -20°C	Nominally 200 IU ampoule
A, C, I and B	Accelerated thermal degradation (ATD) samples of CG candidate standard (07/364) stored respectively at +4°C, +20°C, +37°C and +45°C for 6 months	Contents assumed identical to 07/364
F, K Duplicates	Recombinant human (rh)CG candidate standard (96/608) stored at -20°C	Nominally 200 IU ampoule
D, L, E and J	Accelerated thermal degradation (ATD) samples of rhCG candidate standard (96/608) stored respectively at +4°C, +20°C, +37°C and +45°C for 145 months	Contents assumed identical to 96/608
Not coded	1st WHO Reference Reagent, intact CG (99/688) stored at -20°C	1.88 nmol/ampoule

Study design and assay methods contributed

Immunoassay and Bioassay of candidate standards, 07/364 and 96/608

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/vials, 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 fourteen laboratories contributing immunoassay data to the study, 29 different assays were used. 10 of these were in-house assays and the remaining 19 assays used 13 different kits from 7 manufacturers. Five laboratories contributed bioassay data to the study, 4 of which were based on seminal vesicle weight gain in rats and one was based on uteri weight gain in mice. In these 5 laboratories, all performed bioassays on three doses of the samples, with the exception of laboratory 19, where the bioassays were performed on five doses of the samples.

Table 3 Assay methods used

Lab No.	Assay type	Comments
1	Immunoassay	Abbott Architect total β-hCG (RLU)
2	Immunoassay	Siemens ADVIA Centaur total hCG (RLU)
3	Immunoassay	a. TOSOH ST AIA-PACK intact hCG (Rate) b. TOSOH ST AIA-PACK total β-hCG (Rate)
4	Immunoassay	a. In-house radioimmunoassay for intact hCG (cpm) b. In-house radioimmunoassay for total β-hCG (cpm) c. In-house immunoradiometric assay for intact hCG (cpm) d. In-house immunoenzymometric assay for intact hCG (OD) e. In-house ELISA for intact hCG (OD) f. In-house chemiluminescent immunoassay for intact hCG (RLU) g. In house time-resolved fluoroimmunoassay for intact hCG (cps)
5	Immunoassay	Beckman Coulter Access Total β-hCG(RLU)
6	Bioassay	Uteri weight gain in mice
7	Bioassay	Seminal vesicle weight gain in rats
8	Immunoassay	In-house ELISA for intact hCG (OD)
9	Immunoassay	Biomerieux Vidas intact hCG (RFV)
10	Immunoassay	Siemens Immulite 2000 total hCG (rate)
11	Bioassay	Seminal vesicle weight gain in rats
12	Immunoassay	a. Perkin Elmer (AutoDELFIA) intact hCG assay (counts) b. In-house DELFIA intact hCG immunoassay (counts)
13	Immunoassay	a. Siemens Dimension VISTA total β-hCG (kct) b. Siemens Dimension clinical chemistry total hCG (kct)
14	Immunoassay	a. Beckman Coulter Access Total β-hCG(RLU) b. Roche Modular total hCG (counts)
15	Immunoassay	a. TOSOH AIA-PACK total hCG (Rate) b. TOSOH AIA-PACK intact hCG (Rate) c. Siemens Immulite total hCG (Rate)
16	Immunoassay	a. AutoDelfia fluoroimmunometric assay for intact hCG (Rate) b. Delfia Xpress fluoroimmunometric assay for intact hCG (Rate)
17	Immunoassay	a. Roche Elecsys intact hCG assay (counts) b. Roche Elecsys total hCG assay (counts)
18	Immunoassay Bioassay	In-house ELISA for intact hCG (OD) Seminal vesicle weight gain in rats
19	Bioassay	Seminal vesicle weight gain in rats

Statistical Analysis

An independent statistical analysis of all raw data was performed at NIBSC. Potencies of the candidate standards and degradation samples were calculated relative to 75/589 or 99/688 using the principles of multiple parallel line analysis comparing transformed assay response to log dose. Analysis of variance was used to determine the significance of any deviations from the fitted model (non-linearity and non-parallelism) by comparing them to the within-assay error measured by duplicate responses included in the analysis.

For the majority of immunoassays a log transformation was used and analysis was carried out on the linear section of the dose-response curve. For laboratory 4e a square root transformation was used as it appeared to give better linearity. The data from laboratories 2, 4a, 4b, 8 and 10 were best described using a four-parameter logistic function so assay responses were transformed to percentages relative to the estimated upper and lower limits of the dose-response curve and an in-house programme (5) was used to provide weighted regression of logit response on log dose. For the bioassays used by laboratories 6, 7 and 19, organ weights were expressed as proportion of body weight and log transformed for parallel line analysis. For the bioassays used by laboratories 11 and 18b, log transformed organ weights were used.

All mean potencies given in this report are unweighted geometric mean potencies. Variability between assays and laboratories has been expressed using geometric coefficients of variation (%GCV)

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

Results

Laboratory mean potency estimates (IU/ampoule) calculated relative to the 4th IS (75/589) are summarised in Table 4 and represented graphically in Figures 1 (for 07/364) and 2 (for 96/608). Estimates (nmol/ampoule) calculated relative to the purified intact hCG WHO Reference Reagent (99/688) are summarised in Table 5. Estimates of the relative potency of ampoules stored at elevated temperatures are shown in Table 6. Individual assay relative potency estimates are shown in Appendix 1, Table A1.1.

Data returned for analysis

A total of 88 immunoassays and 17 bioassays were analysed. Data were contributed by 19 laboratories, 9 of which used more than one method. Where this was the case, the laboratory code has been subdivided for method differences, for example 3a and 3b.

Assay validity

Although the majority of assays allowed statistically valid estimates to be calculated, some data were excluded from further analysis. In assay 1 from laboratory 3a, samples C, H and I were found to be non-parallel to the other samples. In assay 2 by this laboratory, sample I was non-parallel. In assay 2 by laboratory 4c, samples F and G were found to be non-linear. For assay 1 by laboratory 6, sample F was found to be non-parallel to the other samples. In laboratory 16 (a and b), sample K was found to be non-linear in assay 2. Assay 3 from laboratory 18b, was rejected because of non-linearity of 75/589.

Assessment of intra-assay variability using coded duplicate samples

Coded duplicates of 07/364 (G & H) and 96/608 (F & K) were included in the study to provide an independent measure of the minimum level of variability present in these assay systems. Individual assay potency estimates of the coded duplicates relative to one another ranged from 0.87 to 1.44 with geometric means of 1.01 for 07/364 (G relative to H) and 1.02 for 96/608 (F relative to K). These values are in agreement with their expected value of 1. Geometric coefficients of variation for these means were found to be 7.4% and 6.9% respectively, indicating a lower level of intra-assay variability compared to that observed between laboratories.

Potency estimates for the Candidate Standards 07/364 and 96/608 in terms of the 4th International Standard for CG (75/589) and the 1st WHO Reference Reagent for intact hCG (99/688)

The geometric mean result calculated from immunoassay estimates alone (Table 4), for the potency in IU (in terms of the 4th IS, 75/589) of 07/364 is 179.1 IU per ampoule (n=29; 95% confidence limits 170.5 – 188.2). Although this is slightly higher than the geometric mean result calculated from bioassay estimates of 162.0 IU per ampoule (n=5; 95% confidence limits 132.4 – 198.4), this difference was not significant (P=0.133). The overall geometric mean result, calculated from both immunoassay and bioassay estimates (Table 4), for the potency of 07/364 is 176.5 IU per ampoule (n=34; 95% confidence limits 168.3 – 185.2). Figure 1 demonstrates the spread of laboratory geometric mean estimates for 07/364. Figure 2 shows the bioassay data expanded, showing considerable spread in individual bioassay estimates across laboratories. For the recombinant preparation 96/608, the potency in IU is 183.8 IU per ampoule (n=28; 95% confidence limits 177.4 – 190.5) and the spread of laboratory means is shown in figure 3. The overall geometric mean result, calculated from immunoassay estimates (Table 5), for the potency in nmol (in terms of the 1st WHO RR, 99/688) of 07/364 is 0.39 nmol per ampoule (n=29; 95% confidence limits 0.38 – 0.41). For 96/608, the potency is 0.40 nmol per ampoule (n=27; 95% confidence limits 0.38 – 0.42).

Stability based on thermally accelerated degradation samples

Estimates of the relative potency of ampoules stored at elevated temperatures for periods of 6 months (07/364) and 145 months (96/608) are summarized in Table 6. No significant loss in immunoreactivity or bioactivity was found for 07/364 at any of the elevated temperatures. For 96/608, the predicted loss of activity per year when stored at -20°C is less than 0.001%.

Conclusions and recommendations

Reference preparations for CG are used for the calibration of assays to control the quality and potency of CG used in the treatment of infertility, and for the calibration of assays used in the diagnosis of pregnancy and a range of other clinical conditions. This study, to establish a replacement for the 4th IS for CG, was designed to allow the assignment of dual unitage (IU and nmol) to the candidate replacement standards and to meet both the current requirement for an International Standard calibrated in IU and the potential future requirement from the diagnostic community to calibrate assays for CG in molar units. The use of International System of Units (SI units) is desirable for immunoassay standardisation and is feasible when a standard can be isolated in a pure form. The purity of the bulk material used to prepare the candidate standard 07/364 had previously been demonstrated in the study to establish the WHO International Reference Reagent panel of hCG forms (1,2). This intact hCG Reference Reagent (99/688) was value assigned by amino acid analysis and, as a result, the comparison of the candidate standards with this reference reagent allowed their calibration in molar units in addition to IU.

By comparison with the 4th IS for CG (75/589), the mean potency calculated from immunoassay estimates for 07/364 was 179.1 IU per ampoule. The mean potency calculated from bioassay estimates for 07/364 was 162 IU per ampoule. For the recombinant preparation 96/608, the overall mean potency was 183.8 IU per ampoule. Comparisons with the 1st WHO Reference Reagent (99/688) by immunoassay demonstrated that 07/364 contained 0.39 nmol per ampoule and 96/608 contained 0.40 nmol per ampoule.

Both candidate standards have been shown to exhibit immunological activity and, although the data from bioassays are more limited, they both also exhibit the expected bioactivity. Since urinary preparations of CG are widely used in the treatment of infertility and the measurement of native CG in urine or serum is the basis of pregnancy testing, the adoption of the candidate standard, 07/364, as the 5th International Standard for CG would adhere to the guidelines of WHO which require International Standards that match, as closely as possible, the analytes to which they are being compared. This study has also demonstrated the suitability of a recombinant preparation of CG to serve as an International Standard for the assay of CG, should this be required for future standardisation activities.

Almost all CG assays are currently calibrated against either the 3rd IS 75/537 or the 4th IS 75/589, which are essentially identical. Since the new standard is calibrated against the 4th IS, if established as the new International Standard 07/364 will maintain a continuity of unitage (in IU) for assays of CG. In addition, in view of the ongoing efforts of the IFCC Working Group on hCG to improve the between-method (immunoassay) agreement for reported hCG results (1-3) and the potential future requirement by the diagnostic immunoassay community for a universally accepted International Standard of sufficient purity to enable calibration in molar units, assignment of a molar unitage to 07/364 provides an excellent opportunity to improve the calibration and standardisation of hCG assays. The impact of adoption of 07/364 as the primary reference preparation for the calibration of hCG immunoassays is currently under investigation in a much larger study run in tandem with this international collaborative study and organised by UKNEQAS/IFCC. This study in which a panel of over 400 specimens, including a large number of diluted patient samples, will be assayed against both the 4th IS 75/589 and the proposed 5th IS 07/364, will provide a comprehensive dataset from which the effect of recalibration of assays using 07/364 will be determined.

The candidate preparation 07/364 appears to be sufficiently stable to serve as an International Standard since no significant loss in immunoreactivity or bioactivity was found at any of the elevated temperatures. This suggests that 07/364 is likely to be highly stable under long term storage conditions at -20°C. However, it is noted that because of the short duration of this study and the lack of detectable degradation, it is impossible to predict the degradation rate of the proposed standard. As a result, it will be a future requirement to complete the assessment of CG immunoreactivity and bioactivity in the residual ampoules that have remained stored at elevated temperatures.

Proposals

With the agreement of all the study participants, it is recommended that the preparation in ampoules coded 07/364 be established as the Fifth International Standard for chorionic gonadotrophin with an assigned content of 179 IU per ampoule for the calibration of immunoassays. For those users wishing to calibrate immunoassays in molar units, it is also recommended that the preparation be assigned a unitage in molar terms of 0.39 nmol per ampoule.

Based on comments from participating laboratories, it is further proposed that, for the calibration of bioassays to control therapeutic preparations of CG, the preparation be assigned a content of 162 IU per ampoule, based on the mean potency calculated from bioassay estimates alone.

Acknowledgements

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Table 4. Laboratory geometric mean potencies (IU/ampoule) calculated relative to 75/589 (650 IU/ampoule). Bioassay contributions are shown as shaded rows:

Lab	07/364	96/608	99/688
1	164.3	169.2	809.5
2	135.8	.	565.5
3a	184.2	197.3	978.6
3b	183.8	193.2	978.8
4a	184.7	192.8	774.3
4b	181.2	211.7	812.6
4c	236.9	195.7	1026.4
4d	216.8	230.1	961.5
4e	204.1	205.8	846.3
4f	203.5	161.5	994.1
4g	196.0	201.4	921.2
5	145.3	163.2	701.4
6	171.1	175.4	.
7	133.3	.	.
8	140.3	179.4	865.1
9	198.6	178.9	946.9
10	189.5	167.4	895.0
11	202.6	.	.
12a	181.1	190.1	862.0
12b	187.4	190.9	869.6
13a	196.1	198.9	866.6
13b	192.7	189.6	852.7
14a	148.2	166.6	682.4
14b	162.5	166.7	773.6
15a	191.3	185.8	868.4
15b	184.6	183.3	875.6
15c	184.9	180.3	872.8
16a	172.3	179.1	1027.8
16b	173.7	172.8	1021.5
17a	185.8	188.5	900.3
17b	153.7	151.1	734.9
18a	157.6	.	.
18b	144.0	.	.
19	168.0	.	.

Geometric Mean (All assays)	176.5	183.8	859.9
GCV	14.7%	9.6%	14.6%
Lower 95% confidence limit	168.3	177.4	815.6
Upper 95% confidence limit	185.2	190.5	906.7
n	34	28	28
Geometric Mean (Immunoassays)	179.1	184.1	.
GCV	13.8%	9.8%	.
Lower 95% confidence limit	170.5	177.4	.
Upper 95% confidence limit	188.2	191.0	.
n	29	27	.
Geometric Mean (Bioassays)	162.0	175.4	.
GCV	17.7%	.	.
Lower 95% confidence limit	132.4	.	.
Upper 95% confidence limit	198.4	.	.
n	5	1	.

Figure 1. Laboratory geometric mean potencies (IU/ampoule) of 07/364 calculated relative to 75/589 (650 IU/ampoule)

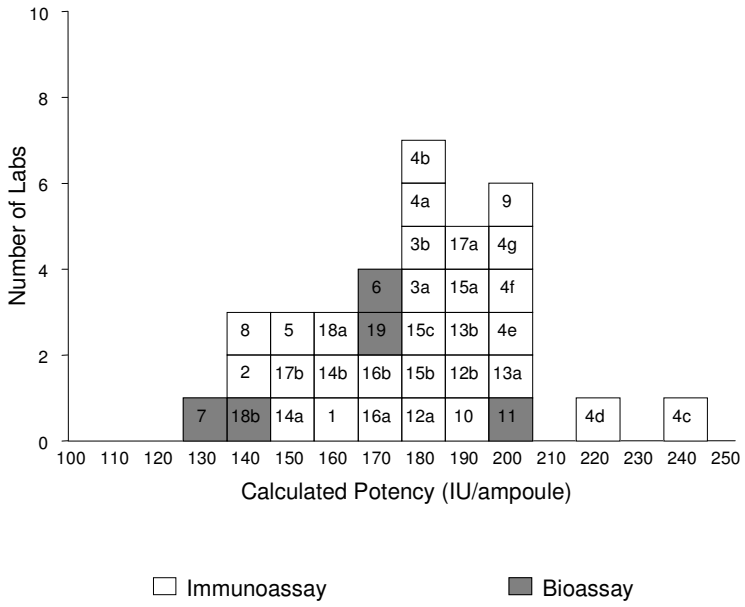

Figure 2. Individual laboratory bioassay estimates of the potency of 07/364 (IU/ampoule) calculated relative to 75/589 (650 IU/ampoule)

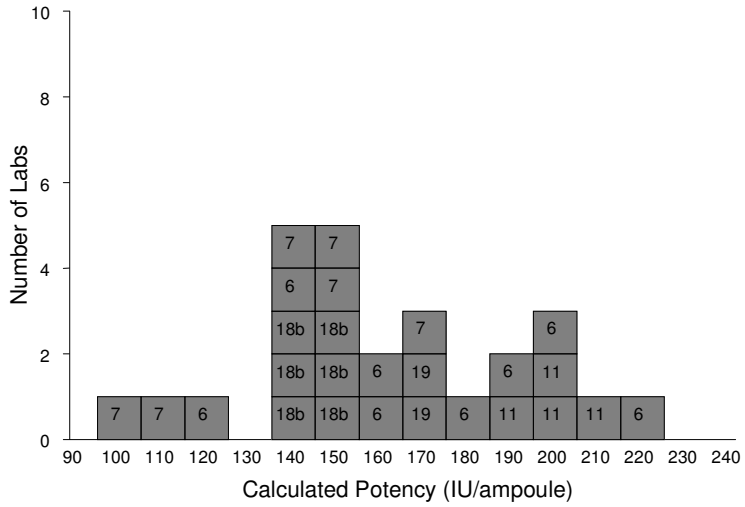

Figure 3. Laboratory geometric mean potencies (IU/ampoule) of 96/608 calculated relative to 75/589 (650 IU/ampoule)

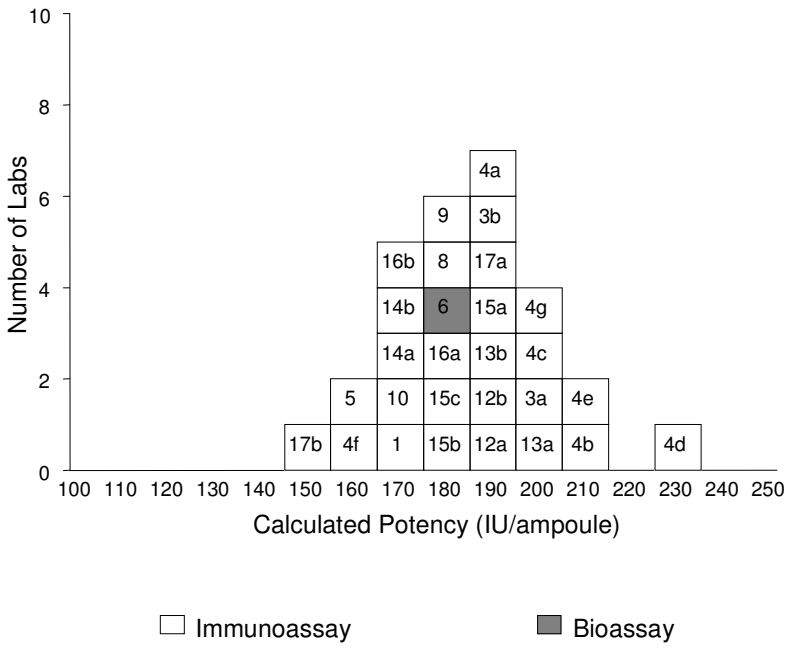

Table 5. Laboratory geometric mean potencies (nmol/ampoule) calculated relative to 99/688 (1.88 nmol/ampoule):

Lab	07/364	96/608
1	0.38	0.39
2	0.45	.
3a	0.35	0.38
3b	0.35	0.37
4a	0.45	0.47
4b	0.42	0.49
4c	0.44	0.36
4d	0.42	0.45
4e	0.45	0.46
4f	0.38	0.31
4g	0.40	0.41
5	0.39	0.44
6	.	.
7	.	.
8	0.30	0.43
9	0.39	0.36
10	0.40	0.35
11	.	.
12a	0.40	0.41
12b	0.41	0.41
13a	0.43	0.43
13b	0.42	0.42
14a	0.41	0.46
14b	0.39	0.41
15a	0.41	0.40
15b	0.40	0.39
15c	0.40	0.39
16a	0.32	0.33
16b	0.32	0.32
17a	0.39	0.39
17b	0.39	0.39
18a	0.39	.
18b	.	.
19	.	.
Geometric Mean	0.39	0.40
GCV	10.8%	12.3%
Lower 95% confidence limit	0.38	0.38
Upper 95% confidence limit	0.41	0.42
n	29	27

Table 6. Laboratory geometric mean potencies for degradation samples expressed relative to relevant samples stored at -20°C (bioassay contributions are shown as shaded rows):

Lab	07/364				96/608			
	A (+4)	C (+20)	I (+37)	B (+45)	D (+4)	L (+20)	E (+37)	J (+45)
1	1.00	.	0.99	.	0.99	.	0.83	.
2	1.00	.	0.98	.	.	.	.	.
3a	1.06	0.96	.	1.02	.	.	.	.
3b	1.01	.	1.11	.	.	.	.	.
5	0.99	1.03	0.99	0.99	.	.	.	.
7	.	0.99	.	1.22	.	.	.	.
8	0.95	0.94	0.93	0.96	0.95	0.90	0.66	0.46
9	1.02	.	1.05	.	0.97	.	0.78	.
10	.	.	.	.	.	.	.	.
11	1.00	.	1.00	.	.	.	.	.
12a	1.00	0.99	1.00	0.98	0.99	0.97	0.87	0.60
12b	1.03	1.01	1.00	1.00	1.01	0.96	0.84	0.54
15a	1.00	1.02	1.00	0.97	0.99	1.01	0.86	0.42
15b	1.00	1.01	1.00	0.97	0.99	0.98	0.83	0.41
15c	0.98	1.02	0.98	0.98	0.97	1.01	0.82	0.41
17a	.	1.03	.	1.01	.	0.98	.	.
17b	.	1.06	.	1.05	.	1.00	.	.
18a	.	0.96	.	0.94	.	.	.	.
18b	.	0.99	.	.	.	.	.	.
Geometric Mean	1.00	1.00	1.00	1.01	0.98	0.98	0.81	0.47
GCV	2.5%	3.3%	4.2%	6.9%	2.0%	4.0%	9.5%	17.5%
Lower 95% confidence limit	0.99	0.98	0.98	0.96	0.97	0.94	0.75	0.40
Upper 95% confidence limit	1.02	1.02	1.03	1.05	1.00	1.01	0.87	0.55
n	13	13	12	12	8	8	8	6

Appendices

Appendix 1: Individual assay results. Table A1.1. Potencies (ampoule/ampoule) relative to 75/589

Lab	Assay	07/364						96/608						99/688	G:H ratio	F:K ratio
		G -20°C	H -20°C	A +4°C	C +20°C	I +37°C	B +45°C	F -20°C	K -20°C	D +4°C	L +20°C	E +37°C	J +45°C			
1	1	0.254	0.25	0.25	.	0.249	.	0.258	0.26	0.254	.	0.211	.	1.243	1.016	0.993
1	2	0.252	0.256	0.256	.	0.253	.	0.263	0.26	0.263	.	0.219	.	1.248	0.985	1.01
2	1	0.172	0.175	0.172	.	0.171	.	.	.	.	.	.	.	0.847	1.01	.
2	2	0.176	0.172	0.179	.	0.169	.	.	.	.	.	.	.	0.841	1.029	.
3a	1	0.245	NP	0.293	NP	NP	0.264	0.3	0.307	.	.	.	.	1.509	.	0.978
3a	2	0.313	0.296	0.288	0.294	NP	0.295	0.314	0.293	.	.	.	.	1.502	1.059	1.073
3b	1	0.241	0.276	0.282	.	0.306	0.264	0.286	0.301	.	.	.	.	1.488	0.875	0.95
3b	2	0.319	0.3	0.287	.	0.321	0.298	0.31	0.292	.	.	.	.	1.524	1.063	1.062
4a	1	0.287	0.299	.	.	.	.	0.332	0.288	.	.	.	.	1.091	0.959	1.152
4a	2	0.279	0.272	.	.	.	.	0.289	0.28	.	.	.	.	1.301	1.026	1.03
4b	1	0.257	0.279	.	.	.	.	0.426	0.299	.	.	.	.	1.159	0.92	1.425
4b	2	0.293	0.288	.	.	.	.	0.289	0.307	.	.	.	.	1.348	1.015	0.94
4c	1	0.377	0.359	.	.	.	.	0.327	0.291	.	.	.	.	1.566	1.049	1.124
4c	2	NL	0.357	.	.	.	.	NL	0.287	.	.	.	.	1.592	.	.
4d	1	0.339	0.331	.	.	.	.	0.36	0.359	.	.	.	.	1.514	1.027	1.003
4d	2	0.338	0.327	.	.	.	.	0.343	0.353	.	.	.	.	1.445	1.033	0.971
4e	1	0.319	0.283	.	.	.	.	.	0.298	.	.	.	.	1.377	1.126	.
4e	2	0.33	0.326	.	.	.	.	.	0.336	.	.	.	.	1.231	1.011	.
4f	1	0.332	0.322	.	.	.	.	0.255	0.259	.	.	.	.	1.623	1.03	0.984
4f	2	0.298	0.302	.	.	.	.	0.241	0.24	.	.	.	.	1.441	0.988	1.003
4g	1	0.292	0.328	.	.	.	.	0.315	0.312	.	.	.	.	1.472	0.89	1.01
4g	2	0.321	0.269	.	.	.	.	0.319	0.293	.	.	.	.	1.364	1.191	1.088

5	1	0.222	0.22	0.225	0.222	0.229	0.218	0.246	0.247	.	.	.	.	1.072	1.008	0.994
5	2	0.227	0.224	0.219	0.236	0.215	0.223	0.256	0.256	.	.	.	.	1.086	1.014	0.999
6	1	0.221	0.192	.	.	.	.	.	.	.	.	.	.	.	1.154	.
6	2	0.344	0.239	.	.	.	.	0.212	.	.	.	.	.	.	1.443	.
6	3	0.314	0.295	.	.	.	.	0.292	.	.	.	.	.	.	1.066	.
6	4	0.25	0.284	.	.	.	.	0.317	.	.	.	.	.	.	0.88	.
7	1	0.223	.	.	0.181	.	.	.	.	.	.	.	.	.	.	.
7	2	.	0.148	.	.	.	0.194	.	.	.	.	.	.	.	.	.
7	3	.	0.265	.	0.318	.	.	.	.	.	.	.	.	.	.	.
7	4	0.173	.	.	.	.	0.196	.	.	.	.	.	.	.	.	.
7	5	0.234	0.209	.	.	.	.	.	.	.	.	.	.	.	1.117	.
8	1	0.186	0.2	.	.	.	.	.	.	.	.	.	.	1.179	0.93	.
8	2	0.199	0.207	.	.	.	.	.	.	.	.	.	.	1.26	0.961	.
8	3	0.204	.	0.191	0.189	0.187	0.195	.	.	.	.	.	.	.	.	.
8	4	0.197	.	0.191	0.188	0.188	0.189	.	.	.	.	.	.	.	.	.
8	5	.	.	.	.	.	.	0.261	0.275	.	.	.	.	1.172	.	0.951
8	6	.	.	.	.	.	.	0.279	0.28	.	.	.	.	1.238	.	0.997
8	7	.	.	.	.	.	.	0.283	.	0.266	0.255	0.184	0.127	.	.	.
8	8	.	.	.	.	.	.	0.279	.	0.265	0.248	0.184	0.129	.	.	.
8	9	0.24	0.245	.	.	.	.	.	.	.	.	.	.	1.568	0.976	.
8	10	0.239	0.251	.	.	.	.	.	.	.	.	.	.	1.645	0.953	.
9	1	0.311	0.313	0.313	.	0.327	.	0.279	0.276	0.27	.	0.211	.	1.453	0.994	1.012
9	2	0.302	0.296	0.313	.	0.313	.	0.271	0.276	0.263	.	0.218	.	1.46	1.019	0.982
10	1	0.278	0.274	.	.	.	.	0.257	0.249	.	.	.	.	1.343	1.016	1.035
10	2	0.296	0.321	.	.	.	.	0.255	0.27	.	.	.	.	1.412	0.922	0.945
11	1	0.306	0.297	0.308	.	0.311	.	.	.	.	.	.	.	.	1.029	.
11	2	0.33	0.315	0.316	.	0.314	.	.	.	.	.	.	.	.	1.047	.

12a	1	0.275	0.272	0.277	0.273	0.275	0.273	0.286	0.292	0.285	0.284	0.251	0.173	1.343	1.01	0.981
12a	2	0.285	0.283	0.28	0.278	0.282	0.274	0.297	0.295	0.295	0.284	0.258	0.179	1.31	1.01	1.006
12b	1	0.284	0.286	0.291	0.288	0.286	0.284	0.291	0.295	0.291	0.283	0.243	0.154	1.35	0.992	0.987
12b	2	0.291	0.292	0.301	0.293	0.29	0.295	0.298	0.291	0.301	0.28	0.249	0.164	1.325	0.995	1.026
13a	1	0.295	0.308	.	.	.	.	0.309	0.303	.	.	.	.	1.333	0.957	1.019
13b	1	0.294	0.299	.	.	.	.	0.292	0.291	.	.	.	.	1.312	0.984	1.005
14a	1	0.227	0.227	.	.	.	.	0.254	0.261	.	.	.	.	1.03	1.002	0.975
14a	2	0.229	0.229	.	.	.	.	0.26	0.251	.	.	.	.	1.07	1.001	1.034
14b	1	0.252	0.254	.	.	.	.	0.263	0.257	.	.	.	.	1.199	0.995	1.025
14b	2	0.246	0.248	.	.	.	.	0.253	0.253	.	.	.	.	1.181	0.988	0.998
15a	1	0.303	0.299	.	.	.	.	0.285	0.294	.	.	.	.	1.377	1.012	0.967
15a	2	0.29	0.285	.	.	.	.	0.28	0.285	.	.	.	.	1.296	1.019	0.983
15a	3	1.000*	0.984	1.017	1.027	1.003	1.006	.	.	.	.	.	.	.	1.017	.
15a	4	1.000*	1.023	0.99	1.011	1.001	0.948	.	.	.	.	.	.	.	0.978	.
15a	5	.	.	.	.	.	.	1.000*	0.989	1.019	1.02	0.875	0.483	.	.	1.011
15a	6	.	.	.	.	.	.	1.000*	1.013	0.965	1.004	0.856	0.373	.	.	0.987
15b	1	0.29	0.288	.	.	.	.	0.286	0.284	.	.	.	.	1.375	1.007	1.01
15b	2	0.28	0.278	.	.	.	.	0.279	0.279	.	.	.	.	1.319	1.006	0.998
15b	3	1.000*	0.976	1.012	1	1.001	0.973	.	.	.	.	.	.	.	1.025	.
15b	4	1.000*	1.017	0.984	1.008	0.989	0.965	.	.	.	.	.	.	.	0.983	.
15b	5	.	.	.	.	.	.	1.000*	1.011	1.022	0.971	0.867	0.483	.	.	0.989
15b	6	.	.	.	.	.	.	1.000*	0.995	0.968	0.995	0.795	0.345	.	.	1.005
15c	1	0.297	0.291	.	.	.	.	0.285	0.274	.	.	.	.	1.37	1.019	1.041
15c	2	0.274	0.276	.	.	.	.	0.278	0.274	.	.	.	.	1.316	0.992	1.014
15c	3	1.000*	0.974	1.009	1.028	0.971	0.992	.	.	.	.	.	.	.	1.027	.
15c	4	1.000*	1.025	0.959	1.005	0.992	0.972	.	.	.	.	.	.	.	0.976	.
15c	5	.	.	.	.	.	.	1.000*	0.951	0.959	1	0.799	0.444	.	.	1.051
15c	6	.	.	.	.	.	.	1.000*	0.959	0.943	0.969	0.803	0.364	.	.	1.042

16a	1	0.289	0.274	.	.	.	.	0.272	.	.	.	.	.	1.611	1.055	.
16a	2	0.237	0.263	.	.	.	.	0.296	0.260	.	.	.	.	1.552	0.901	1.138
16b	1	0.285	0.281	.	.	.	.	0.263	.	.	.	.	.	1.613	1.014	.
16b	2	0.235	0.271	.	.	.	.	0.288	0.248	.	.	.	.	1.531	0.867	1.161
17a	1	0.288	0.284	.	0.295	.	0.29	0.291	0.289	.	0.285	.	.	1.385	1.016	1.005
17b	1	0.236	0.237	.	0.25	.	0.248	0.237	0.228	.	0.233	.	.	1.131	0.998	1.036
18a	1	0.243	0.221	.	.	.	.	.	.	.	.	.	.	.	1.097	.
18a	2	0.226	0.231	.	.	.	.	.	.	.	.	.	.	.	0.981	.
18a	3	0.247	0.24	.	0.238	.	0.238	.	.	.	.	.	.	.	1.029	.
18a	4	0.26	0.238	.	0.249	.	0.231	.	.	.	.	.	.	.	1.093	.
18a	5	0.241	0.239	.	0.222	.	0.225	.	.	.	.	.	.	.	1.007	.
18a	6	0.276	0.252	.	0.244	.	0.232	.	.	.	.	.	.	.	1.099	.
18a	7	.	.	.	0.227	.	0.228	.	.	.	.	.	.	.	.	.
18a	8	.	.	.	0.215	.	0.228	.	.	.	.	.	.	.	.	.
18a	9	0.21	0.212	.	.	.	.	.	.	.	.	.	.	1.000*	0.993	.
18a	10	0.206	0.193	.	.	.	.	.	.	.	.	.	.	1.000*	1.07	.
18a	11	0.21	0.213	.	0.211	.	0.191	.	.	.	.	.	.	1.000*	0.986	.
18a	12	0.218	0.21	.	0.191	.	0.189	.	.	.	.	.	.	1.000*	1.037	.
18a	13	0.214	0.206	.	0.188	.	0.197	.	.	.	.	.	.	1.000*	1.036	.
18a	14	0.213	0.181	.	0.21	.	0.204	.	.	.	.	.	.	1.000*	1.173	.
18a	15	.	.	.	0.188	.	0.206	.	.	.	.	.	.	1.000*	.	.
18a	16	.	.	.	0.19	.	0.197	.	.	.	.	.	.	1.000*	.	.
18b	1	0.213	0.226	.	0.22	.	.	.	.	.	.	.	.	.	0.943	.
18b	2	0.221	0.226	.	0.219	.	.	.	.	.	.	.	.	.	0.981	.
18b	3	NL	NL	.	NL	.	.	.	.	.	.	.	.	.	.	.
18b	4	0.22	0.224	.	.	.	.	.	.	.	.	.	.	.	0.983	.
19	1	0.259	.	.	.	.	.	.	.	.	.	.	.	.	.	.
19	2	0.258	.	.	.	.	.	.	.	.	.	.	.	.	.	.

NL = no estimate due to non-linearity
NP = no estimate due to non-parallelism
* Potencies calculated relative to indicated preparation as 75/589 not included in assay.

Appendix 2: Study protocol

INTERNATIONAL COLLABORATIVE STUDY IN CONJUNCTION WITH IFCC TO ESTABLISH THE 5TH WHO INTERNATIONAL STANDARD FOR CHORIONIC GONADOTROPHIN

STUDY PROTOCOL

INTRODUCTION

The 3rd International Standard for Chorionic Gonadotrophin (CG) in ampoules coded 75/537 was established by the WHO Expert Committee on Biological Standardization in 1986. When this preparation was exhausted, a second batch of ampoules coded 75/589, containing the same bulk preparation of CG as that in the 3rd IS and prepared at the same time as the 3rd IS using identical procedures, was established as the 4th International Standard for Chorionic Gonadotrophin in 1999. Both standards have been widely used for the calibration of assays to control the quality and potency of CG used in the treatment of infertility, and for the calibration of assays used in the diagnosis of pregnancy and a range of other clinical conditions. Both applications require approximately equal numbers of ampoules. Stocks of the 4th IS are rapidly depleting and the standard needs to be replaced.

In 2001, in collaboration with the IFCC, WHO established a panel of 6 CG preparations (WHO Reference Reagents) including intact, nicked and sub-unit forms, for the intended purpose of investigating and characterizing the specificity of existing CG assays. The intact CG (ampoules coded 99/688) was highly purified to remove other forms of CG particularly the nicked forms and free subunits. The IFCC have agreed that some of the intact CG be made available to WHO, to be filled into ampoules and included as a candidate preparation in an international study to establish the 5th WHO International Standard for Chorionic Gonadotrophin. With the increasing availability of recombinant CG and allowing for the possibility that therapeutic and diagnostic manufacturers may make increasing use of recombinant in-house reference materials, a recombinant preparation of CG, previously filled into ampoules at NIBSC (96/608), will also be included in the study.

The aims of the study would be, therefore:

1) to compare, by immunoassay and bioassay, the ampouled preparations of intact and recombinant CG with local standards presently in use,
2) to calibrate the preparation of intact CG or recombinant CG for use as a potential International Standard,
3) to assess the stability of the proposed International Standard after accelerated thermal degradation

MATERIALS

Ampoules of the current IS 75/589 contain 650 IU (approximately 70 µg) purified CG which is far in excess of the amount needed for immunoassay or bioassay. For the proposed standard, we have filled 6000 ampoules (approximately 200 IU intact CG per ampoule). The recombinant preparation (96/608) also contains approximately 200 IU ampoule. In addition to these materials, we have included ampoules of the intact CG (99/688) preparation, which has been formally assigned a unitage in nmol/ampoule and will provide information on the CG content of the proposed standard in nmol. The materials, most of which will be identified by code letter only, 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: Preparations for inclusion in collaborative study

CG preparation	Ampoule contents
The 4th I.S. for CG (75/589)	650 IU ampoule
Highly purified intact CG (07/364)	Nominally 200 IU ampoule
*Accelerated thermal degradation (ATD) samples of (07/364) stored each at +4°C, +20°C, +37°C, +45°C for 6 months	As above
Recombinant human (rh)CG (96/608)	Nominally 200 IU ampoule
*Accelerated thermal degradation (ATD) samples of rhCG (96/608) stored each at +4°C, +20°C, +37°C and +45°C.	As above
1st WHO Reference Reagent, intact CG (99/688)	1.88 nmol (approx 800IU)/ampoule

*In order to derive stability data, samples undergo accelerated thermal degradation at various temperatures for at least 6 months prior to the start of the study.

TESTS REQUESTED

Immunoassay and Bioassay

Participants will receive a selection of coded samples, distributed on the basis of assay capacity, sample availability and study design. Participants are requested to carry out the assay(s) normally in use in their laboratory and, where possible, to perform at least two independent runs, using fresh ampoules, each run to include all of the preparations allocated **at preferably no less than five dose levels in the linear part of the dose-response curve**. If there is a need to sub-aliquot or freeze/thaw the ampoule contents, participants are requested to provide details where appropriate. **Participants are also asked to ensure that all assays include their local standard where possible,** and to provide details of the assay method used, 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.

The ampoule contents of the test preparations are listed in Table 1. On receipt ampoules should be stored at -20°C until use. Before opening, ampoules should be brought to room temperature to minimise moisture uptake. It is recommended that the contents of each ampoule are reconstituted in a suitable assay diluent e.g. PBS or saline (not water) and appropriate dilutions made from this stock solution. Since there may be extensive dilutions to achieve required assay doses, protein cover (typically 0.1% BSA or HSA) to prevent surface adsorption should be provided. There is some evidence that if the initial dilution is carried out in a relatively large volume, variation resulting from the numerous dilution steps is minimized. If practicable, an initial dilution volume of 5ml is therefore suggested.

REPORT

A preliminary report will be prepared and circulated to all participants and the IFCC for comment before submission to the Expert Committee on Biological Standardization of WHO.
For further information, please contact:

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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: Instructions for use

Proposed 5th WHO INTERNATIONAL STANDARD FOR CHORIONIC GONADOTROPHIN
NIBSC CODE 07/364
Instructions for Use (June 2009, first version)
This material is not for *in vitro* diagnostic use

1. INTRODUCTION

The 3rd International Standard for Chorionic Gonadotrophin in ampoules coded 75/537 was established by the WHO Expert Committee on Biological Standardization in 1986. When this preparation was exhausted, a second batch of ampoules coded 75/589, containing the same bulk preparation of CG as that in the 3rd IS and prepared at the same time as the 3rd IS using identical procedures, was established as the 4th International Standard for Chorionic Gonadotrophin in 1999. Both standards have been widely used for the calibration of assays to control the quality and potency of CG used in the treatment of infertility, and for the calibration of assays used in the diagnosis of pregnancy and a range of other clinical conditions. When stocks of the 4th IS were depleted it became necessary to establish a replacement preparation. A preparation of purified intact CG, coded 07/364, has been ampouled and has been evaluated for its suitability to serve as a replacement International Standard for CG.

2. CONTENTS

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

10 mg/ml trehalose
50 mM sodium phosphate (pH7.4)
2 mg/ml human serum albumin
Purified human CG

3. UNITAGE

An assigned CG content of 179 IU per ampoule for the calibration of immunoassays. (For the calibration of CG immunoassays in molar units, this preparation has an assigned unitage of 0.39 nmol per ampoule).

An assigned CG content of 162 IU per ampoule for the calibration of bioassays.

4. CAUTION

THIS PREPARATION IS NOT FOR ADMINISTRATION TO HUMANS

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. However, as with all preparations of human origin, this material cannot be assumed to be free from infectious agents. Suitable precautions should be taken in the use and disposal of the ampoule and its contents. 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.

5. DIRECTIONS FOR OPENING THE DIN AMPOULE

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.

6. USE OF AMPOULED MATERIAL

For practical purposes each ampoule contains the same quantity of CG. The entire content of each ampoule should be completely dissolved in an accurately measured amount of buffer solution. No attempt should be made to weigh out portions of the freeze-dried powder. The use of water to reconstitute ampoule contents is not recommended. The material has not been sterilized and the ampoules contain no bacteriostat.

7. STABILITY

Materials should be stored on receipt as indicated on the label. The candidate preparation 07/364 appears to be sufficiently stable to serve as an international standard since no significant loss in immunoreactivity or bioactivity was found at any of the elevated temperatures at which samples were stored during an accelerated degradation study. These results indicate that 07/364 is likely to be highly stable under long term storage conditions at -20°C.

8. PREPARATION OF AMPOULES

Bulk material
A bulk preparation of intact CG, highly purified to remove other forms of CG, was donated to the IFCC as part of a project to establish a panel of WHO International Reference Reagents for the characterisation of the specificity of CG assays (1-2).

Distribution into ampoules
129 mg of this lyophilised bulk material (tested and found to be negative for HIV, Hepatitis C and Hepatitis B) was dissolved in 6.336 litres of sterile water containing 50mM sodium phosphate (pH7.4), 2mg/ml human serum and 10mg/ml trehalose and was then distributed into ampoules as 1ml aliquots, which were lyophilised and sealed according to procedures described by WHO for International Biological Standards (3) and stored at -20°C in the dark.

9. CITATION

In all publications (or data sheets for immunoassay 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.

10.PRODUCT LIABILITY

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.

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 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.

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.

11.REFERENCES

1. Birken S., Berger P., Bidart J.M., Weber M., Bristow A., Norman R, et al. (2003). Preparation and characterization of new WHO reference reagents for human chorionic gonadotrophin and metabolites. *Clin Chem* 49: 144-54.
2. Bristow A., Berger P., Bidart J.M., Birken S., Norman R., Stenman U.H. and Sturgeon C. (2005). Establishment, value assignment and characterization of new WHO reference reagents for six molecular forms of human chorionic gonadotrophin. *Clin Chem* 51:177-82.
3. WHO Technical Report Series No.800, 1990; 181-214

12.MATERIAL SAFETY SHEET

NIBSC Code 07/364, Chorionic Gonadotrophin
(June 2009, First Version)

Physical properties (at room temperature)			
Physical appearance	Freeze dried powder		
Fire hazard	None		
Chemical properties			
Stable	Yes	Corrosive:	No
Hygroscopic	No	Oxidising:	No
Flammable	No	Irritant:	No
Other (specify)	Can react with oxidising materials. Avoid contact with acids and alkalis		
Handling:	See caution, 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 materials used to treat spillage should be treated as biologically hazardous waste.			