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RECOMBINANT HUMAN LUTEINIZING HORMONE
Proposed International Standard

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Summary

The proposed international standard for recombinant luteinizing hormone (LH) (in ampoules coded 96/602; proposed IS), and LH 96/816 and LH 96/820 (prepared in the same way as the proposed IS and from the same LH preparation), were compared with the fourth International Standard for Human Urinary FSH and LH (IS 98/704) and the second International Standard for Human Pituitary LH (IS 80/552) by 9

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laboratories in 9 countries using the seminal weight gain LH bioassay. These LH preparations were also compared by one laboratory in one LH immunoassay system. Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 and in terms of IS 80/552 were not homogeneous in the majority of laboratories, and were not homogeneous overall. However, unweighted analysis of variance indicated that there were no significant differences between estimates from the different laboratories. The combined unweighted geometric mean estimate of LH content of the proposed IS (with 95% fiducial limits) in terms of IS 98/704 was 189 (175 – 204) IU/ampoule, and in terms IS 80/552 was 247 (220 – 278) IU/ampoule. The combined unweighted geometric mean bioassay estimates (with 95% fiducial limits) of the laboratory mean LH contents of LH 96/816 and LH 96/820, as % of that of the proposed IS, were 96 (86-107) and 103 (94-112), respectively.

Estimates of the LH content of ampoules of the proposed IS, LH 96/816 and LH 96/820 kept at elevated temperatures suggested that all three preparations would be adequately stable under normal storage conditions.

Significant differences were found in this study between the biological properties of these pituitary, urinary and recombinant LH preparations, indicating differences between their isoform compositions, and hence the need for a separate standard for recombinant LH. Thus, the slopes of log dose-response lines in the bioassay for the urinary LH preparation IS 98/704 differed significantly from those of each of the IS 80/552 and the proposed IS, which were not significantly different from one another. Furthermore, there was between-assay and between-laboratory heterogeneity of estimates of the LH potencies of these three preparations relative to one another, which is unusual for estimates from this type of bioassay and was not found in previous collaborative assays where preparations of pituitary LH were compared with one another, and where preparations of urinary LH were compared with one another. However, the differences found in this study between the magnitude of the IU of pituitary LH and that of urinary LH, namely that 1 IU of pituitary LH is equivalent to approximately 0.80 IU of urinary LH, appear to be due mainly to the differences between the procedures used to calibrate the standards which define these units.

On the basis of these results, the proposed IS appears to be suitable to serve as the international standard for recombinant LH for bioassay. It is recommended that the contents of each ampoule of the proposed IS be assigned an activity of 189 International Units of recombinant LH on the basis of their calibration in terms of IS 98/704, so as to maintain continuity with the international units of urinary LH, which have been, and still are being, used to calibrate LH-containing therapeutic products.

Introduction

Luteinizing hormone (LH) activity, mostly as human chorionic gonadotrophin (hCG), is used widely, in combination with follicle stimulating hormone (FSH), for the treatment of infertility in women, and sometimes also in men. Until recently, the only form of LH available for treatment purposes has been human menopausal gonadotrophin. This consists of a purified extract of the urine of post-menopausal women, and contains both FSH and LH. However, recently, recombinant human LH

(lutropin alfa; rLH) became licensed for therapeutic use. This LH preparation has the advantage of being more highly purified than the LH in human menopausal gonadotrophin, and also provides the possibility, in the treatment of infertility, of replacing hCG with LH, which differ in their pharmacokinetics and pharmacodynamics. Thus the results of a recent clinical study suggested that rLH could be as effective as hCG in inducing follicular maturation and ovulation in in-vitro fertilization procedures, and that its use could be associated with a lower incidence of the ovarian hyperovulation syndrome [1].

The LH content of therapeutic products is controlled by in-vivo bioassays. This is because the intrinsic heterogeneity of LH, as well as the degree of purity of the urinary-derived products, has until now precluded their control by physicochemical methods or by in-vitro assays. The LH bioassays used to control these therapeutic products have been calibrated in terms of international standards for urinary FSH and LH, currently the fourth International Standard for Human Urinary FSH and LH [2].

At its 45th meeting, the Expert Committee on Biological Standardization of the World Health Organization (WHO) noted that the current international standard for urinary FSH and LH, at that time the third International Standard for Urinary FSH and Urinary LH [3], might not be appropriate for the assay of rLH, which was then under development as a therapeutic product [4]. It also noted that one manufacturer had offered a quantity of rLH as a candidate reference material. The Committee therefore requested the National Institute for Biological Standards and Control (NIBSC) to distribute this material into ampoules and organize a collaborative study.

The donated rLH preparation was subsequently distributed into ampoules as the proposed international standard for recombinant LH (proposed IS), and was subjected to an international collaborative study. The objectives of this study were: to compare by LH bioassay the proposed IS with the fourth International Standard for Human Urinary FSH and LH (IS 98/704) and the second International Standard for Human Pituitary LH (IS 80/552); to calibrate the proposed IS by in-vivo LH bioassays in terms of the IS 98/704 and the IS 80/552; and to estimate the LH bioactivities of accelerated thermal degradation samples of the proposed IS, so as to assess the stability of the proposed IS.

Participants

The 9 laboratories from 9 countries, which took part in the study, are listed below. Throughout this report, each participant has been identified by a number between 1 and 9, but these numbers do not relate to the order of listing which is alphabetical by country.

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Materials

1. The fourth International Standard for Human Urinary FSH and LH (IS 98/704)

Each ampoule (coded 98/704) contains approximately 0.967mg of an extract from the urine of post-menopausal women with a defined activity of 72 International Units of human urinary FSH and a defined activity of 70 International Units of human urinary LH [2].

2. The second International Standard for Human Pituitary LH (IS 80/552)

Each ampoule (coded 80/552) contains approximately 5.8μg of highly purified pituitary LH with a defined activity of 35 International Units of human pituitary LH [5].

3. Proposed international standard for recombinant human luteinizing hormone (proposed IS)

This consists of a batch of ampoules (coded 96/602) containing rLH. Two other batches of ampoules (coded 96/816 (LH 96/816) and 96/820 (LH 96/820)) were prepared from the same batch of bulk rLH as the proposed IS and under identical conditions.

Bulk rLH

This consisted of approximately 118mg of highly purified rLH (Batch no BLBA9410), synthesized in Chinese hamster ovary (CHO cells), and generously donated to WHO by Serono, through the good offices of Dr A Eshkol. It was

received as seven 2-ml aliquots of a frozen solution stated to contain 8.42 mg/ml of rLH. The rLH of Batch no BLBA9410 was stated to have a specific activity of 17,760 IU/mg as estimated by the seminal vesicle weight gain (SVW) assay [6]. Analyses by Serono showed that the identity and purity of the rLH of Batch no BLBA9410 as assessed by SDS-PAGE, isoelectric focusing and size-exclusion HPLC conformed to those of the Serono in-house interim reference preparation.

Distribution into ampoules

The proposed IS, LH 96/816 and LH 96/820 were prepared in September 1996, January 1997 and February 1997, respectively, using the same conditions. Some 3.66ml of the combined solution from two aliquots of bulk, containing 30.8mg rLH, were diluted to a volume of 3500ml with a solution containing 1%(w/v) lactose, 0.89%(w/v) sodium chloride and 0.2%(w/v) human plasma albumin (Zenalb, Bio Products Laboratory, Elstree; batch no ABC 0183 for the proposed IS, batch no ABC 0183 for LH 96/816, and batch no ABC 0183 for LH 96/820). The solution was distributed into ampoules as approximately 1.0ml aliquots. The solution of rLH was kept at 4°C throughout. The ampoule contents were freeze-dried, secondarily desiccated and sealed under nitrogen [7;8]. The proposed IS consisted of 3401 ampoules, LH 96/816 of 3455 ampoules and LH 96/820 of 3464 ampoules. The mean weight of filling solution in 67 weighed ampoules of the proposed IS, 66 weighed ampoules of LH 96/816 and 67 weighed ampoules of LH 96/820 were found to be 1.0072g for the proposed IS, 1.00797g for LH 96/816, and 1.0079g for LH 96/820, with a coefficient of variation of 0.65% for the proposed IS, 0.11% for LH 96/816, and 0.147% for LH 96/820, and a range as % of the mean of 1.0072 for the proposed IS, 0.536 for LH 96/816, and 0.704 for LH 96/820.

Each ampoule of the proposed IS, LH 96/816 and LH 96/820 contains about 8.8µg of rLH, 10mg of lactose, 8.9mg of sodium chloride and 2mg of human plasma albumin.

Activity of ampoule contents

Preliminary estimates at NIBSC of the LH potency (with 95% confidence limits) by SVW assay in terms of the 2nd International Standard for Urinary FSH and LH (in ampoules coded 71/223 (IS 71/223); [9;10] was 209(175–249)IU/ampoule for the proposed IS, 194(171–220)IU/ampoule for LH 96/816, and 188(164–216) IU/ampoule for LH 96/820. Preliminary estimates at NIBSC of the LH potency of the proposed IS (with 95% confidence limits) by SVW assay in terms of LH 80/552 was 272(218–339) IU/ampoule. Comparison of these estimates of potency of the contents of the ampouled preparations with the stated specific activity of the bulk rLH (156IU/8.8µg) by LH in-vivo bioassays indicated that there had been no significant loss of LH activity during the distribution of the bulk rLH into ampoules.

4. Accelerated thermal degradation samples of the proposed IS

Ampoules of the proposed IS, LH 96/816 and LH 96/820 which had been kept at +20°C or +37°C for several years were also included in the study.

Methods

Design of the study

Participants were asked to contribute in-vivo and in-vitro bioassays which were, as far as possible, specific for LH. In particular they were asked, if possible, to contribute LH estimates by in-vivo bioassays using the SVW method and the ovarian ascorbate depletion method [11]. Participants were informed that preliminary estimates suggested that an assumed LH potency of 200 IU/ampoule would be appropriate for assays of the proposed IS, LH 96/816 and LH 96/820 and their accelerated thermal degradation samples. Ampoules of the proposed IS, LH 96/816 and LH 96/820 and their accelerated thermal degradation samples sent to participants had been coded as follows:

Proposed IS as LH A;
LH 96/816 as LH E;
LH 96/820 as LH C;
the proposed IS kept for 2067 days at +4°C as LH F;
the proposed IS kept for 2067 days at +20°C as LH D;
the proposed IS kept for 2067 days at +37°C as LH B;
the proposed IS kept for 2067 days at +45°C as LH G;
LH 96/816 kept for 1928 days at +20°C as LH H;
LH 96/820 kept for 1920 days at +20°C as LH J.

Participants were asked to provide full results of their assays, including all raw data, as well as their own calculated estimates of potency.

Statistical methods

Data from in-vivo bioassays were examined for consistency where possible, and a preliminary assessment of data for homogeneity of variances and outliers was carried out using an in-house program (SCAN)[12]. Ancillary information (initial and final body weights for the majority of assays) was also assessed [13]. Assay data were analysed using the methods of parallel line assays, for example [14], and statistical validity was assessed. Assays showing no significant deviations from homogeneity, linearity, or parallelism ($p > 0.1$) were accepted as valid. Assays not meeting these criteria were further examined to determine where possible the source of the deviations, and their effect on potency estimation. Any values omitted from the final analysis are listed, together with the reasons for omission. Assays were analysed as multiple parallel line assays except that comparisons among the existing and candidate standards were made separately from comparisons of thermally accelerated degradation samples with the same sample. Calculations were carried out using both log of organ weight and log of ratio of organ weight to final body weight as response. Estimates of potency were calculated as the displacement of parallel log dose – log response lines with weights obtained as the reciprocal of variance of log potency. Log potency estimates were tested for homogeneity using the chi-squared test. Homogeneous estimates have been combined as weighted geometric means except as otherwise noted. Heterogeneous estimates have been combined as unweighted geometric means with variance determined as the variance of the log estimates combined.

Data for in-vitro assays (enzyme-linked immunoassays (ELISA) assays carried out in one laboratory (laboratory 1) were also analysed using the methods of parallel line assays. For these assays an in-house program (WRANL) [15] was used for calculation.

Results and discussion

Data contributed

Data from a total of 39 SVW in-vivo bioassays were contributed to the study. In laboratory 4, the 'assays' comprised a single large experiment with replications of several preparations in the course of the experiment. This experiment has been treated as a single large assay for preliminary analysis, although for subsequent comparisons replicated portions of the experiment have been analysed as separate assays. In all other cases, the individual assays appeared to comprise separate experiments carried out independently at separate times. Where more than one existing and / or candidate standard were included in the same assay with degradation samples the assay has been separated into two parts, identified by the same initial assay code, followed by a second number. All participants provided initial and final body weights, with the exception of both assays in laboratory 3 where only initial weights combined for the two assays were supplied, and one assay in laboratory 1 where the initial weights were not available. The body weights were identified with individual rat and treatment group, except in laboratory 3.

One participant (laboratory 1) also supplied data from several ELISA assays.

Preliminary analysis

For each SVW assay, initial and final body weights and weight change over the course of the assay were assessed, and any anomalous values identified. In laboratory 1, assay 2 one value of final weight was reported as 44, with an initial weight of 50. All other final weights in this assay were greater than 54, and this was the only rat in the assay to show a weight loss. However, the organ weight of this rat was consistent with others in the same group, This final weight has been replaced by the value 69, the initial weight plus the mean weight gain in this assay. Other anomalous values have been noted, but did not disproportionately affect the final results and have not been excluded or replaced.

Initial and final body weights and changes in weight over the course of the assay differed significantly between laboratories, and between assays within laboratories. The mean initial and final weights and weight changes over the course of each assay are summarized in Table 1. The distribution of initial weights within assays more closely approximated a uniform than a normal distribution. This is likely to reflect selection of rats according to assay procedures which customarily specify the allowable weight range for the assay. In contrast, the final body weights and weight changes over the course of the assay did not deviate significantly from a normal distribution in the majority of assays. Initial and final weights were significantly correlated ($r \sim 0.85$ considered overall). There was no indication that weight change over the course of the assay was related to initial weight except in laboratories 8 and

9, where the rats which were larger also tended to show larger weight gains. However, in all assays there was a significant correlation between final weights and weight change over the course of the assay, with rats showing larger weight changes having larger final weights (to some extent, reflecting the uniform distribution of initial weights and the less uniform change in weight).

Analysis of variance showed no significant difference among treatment groups for any of the three measures of weight in the majority of assays in any laboratory. Exceptions were laboratories 8 and 9, in each of which the weight changes over the course of the assay differed significantly in the differently treated groups in most assays. The overall regression of each of the three measures of weight on log dose was also assessed. In the majority of assays there was no significant regression (exceptions are shown in Table 1). However, mean slopes combined over all assays, both for the common slope within an assay and the individual slopes for samples with 10 or more assays, were significantly greater than 0 for weight change over the course of the assay, and were also greater than 0 for final weights (although with less extreme probabilities than for weight change). These data suggest the possibility that weight change may be related to dose of LH, although this effect is not consistent between laboratories, and was not apparent in the laboratories (7 and 5) in which rats showed the smallest weight gains.

The correlations among overall assay means of initial and final weights, weight change, organ weight were also assessed. Assay means of initial and final weights were significantly correlated, r~0.95. Both the assay mean organ weight and the weight change over the course of the assay were significantly correlated with the final body weight (r~0.85 for organ weight and r~0.89 for weight change), and to a lesser extent were also significantly correlated with initial body weight (r~0.55 for organ weight and 0.70 for weight change).

Caging information was available in laboratories 1, 4, 5 and 7. In laboratory 1, cages were a block factor in the assay design, with each treatment group replicated once in each cage. Final weights did not differ significantly between cages in the first assay, and initial and final weights differed marginally ($p \sim 0.05$) between cages in the second assay. In laboratory 4, cages and preparations were confounded, with mice treated with a single preparation in a single cage, although a few preparations were replicated. Final weights differed marginally ($p \sim 0.1$) between replicated cages. In laboratories 5 and 7 treatments were completely randomized. Analysis indicated that there were significant differences between cages in some assays for one or more of the weight measures, but the differences were not consistent between assays. Analysis excluding cage effects in laboratories 1, 5 and 7 will give an increased residual variance in those assays where there are significant differences between cages, but estimates of potency will not be biased. In laboratory 4 classical analysis ignoring cage effects incorporates any differences between cages into the estimated differences between preparations, and the effect of this cannot be predicted. In the absence of design information in the other laboratories, the effects of ignoring cage information cannot be predicted.

Organ weight, the ratio of organ weight to final body weight (hereafter referred to as ratio) and log transformations of each of these were assessed. Log transformations of both organ weight and ratio were homogeneous in the majority of

assays, in contrast to organ weight and ratio, each of which showed significant heterogeneity in a number of assays. Log transformation has therefore been used for all subsequent analysis.

In laboratory 1 assay 2, an organ weight of 63 was reported for the middle dose of one preparation. This value was detected as a significant outlier in a group with variance which contributed significantly to the heterogeneity chi-square. This was also the largest organ weight in the assay, with the second largest weight being 61 at the largest dose of a different preparation. In laboratory 9 assay 3, an organ weight of 7.5 was reported for the middle dose of LHD, for which all other organ weights were in the range 14.1 to 17. The variance of this group contributed significantly to the heterogeneity chi-square and the organ weights for the saline controls in this assay ranged from 6.3 to 10.3. These two organ weights have been omitted from subsequent analysis.

Analysis of all data for each assay showed similar assay precision and statistical validity whether log of organ weight or log of ratio was used as the response, although the variance of the potency estimates tended to be slightly larger when log ratio was used as the response. Estimates of potency were also similar with either response. It is likely that the similarity in results reflects, at least in part, the narrow weight range of initial body weights, and the relatively short assay period. In the absence of any clear advantage, for precision or assay validity in these assays, to either log organ weight or log ratio of organ weight to body weight, the measure used routinely by the participant has been used for the reported analysis. Thus for laboratories 5, 7 and 9 log ratio has been used and for the remainder log organ weight has been used. Individual assay analysis is nevertheless given in the Appendix for both measures as response, except for laboratory 3 where only log of organ weight was available. It was noted that the range of initial body weights in this laboratory was less than half that observed in most other laboratories, and if it had been available, use of ratio would be expected to give very similar results to those obtained with organ weight.

The assay precision within laboratories differed significantly in the different laboratories. The reported weights for log potency estimates (reciprocal of variance of log10 of potency estimates) were larger than about 2500 in laboratories 3, 5 and 6, and generally less than about 1000 in the remaining laboratories.

Dose response relationships

Slopes of log dose – log response lines for the existing and candidate standards are shown in Appendix Tables 1a and 1b, and for the thermally accelerated degradation samples are shown in Appendix Tables 2a and 2b. The majority of assays were carried out using three doses of each preparation. Exceptions were laboratory 6 where two doses per preparation were used, and assays 15 and 16 in laboratory 7, where the design was based on two doses for all except one preparation. In several other assays the dose – response lines were non-linear, with either the smallest doses giving responses which did not differ significantly from control responses, or the largest doses giving responses which suggested that a maximal response had been achieved. These doses giving 'flat' non-linear responses were omitted from analysis. Slopes based on fewer than three doses are indicated in Appendix Tables 1 and 2.

All assays (after deletion of doses giving non-linear responses) were statistically valid. That is, there were no significant deviations from linearity or parallelism ($p > 0.05$), with the exception of assay 2 in laboratory 1, where the slope for IS 98/704 was significantly steeper than the slopes for IS 80/552 or the proposed IS, which did not differ significantly from one another.

Although slope differences among the existing and candidate standards were not detected as significant in the individual assays except in one assay, comparison of slopes over all assays (paired t-test for slopes in the same assay, separately for log organ weight or log ratio as response) showed that slopes for IS 98/704 were significantly ($p < 0.05$) larger than those for IS 80/552, and also that slopes for IS 98/704 were significantly ($p < 0.01$) larger than those for the proposed IS. In contrast, slopes for IS 80/552 and the proposed IS did not differ significantly from one another ($p \sim 0.5$). Comparisons of slopes for IS 71/223 with those for IS 80/552 and the proposed IS in one laboratory gave results consistent with the over all comparison with IS 98/704; that is slopes for IS 71/223 were larger than those for either IS 80/552 or the proposed IS. LH 96/816 and LH 96/820 were included in a limited number of assays; slopes obtained for these preparations did not differ significantly from slopes obtained for IS 80/552 and the proposed IS.

Comparisons among slopes for the thermally accelerated degradation samples showed no consistent differences among slopes for the samples stored continuously at -20°C and those for samples stored at elevated temperatures, and in particular, no consistent tendency for slopes to change with change in temperature.

In summary, these results show a likely difference in dose – response lines between the urinary LH of the IS 98/704 (and of the IS 71/223) and the other LH preparations, namely pituitary LH of the IS 80/552 and the recombinant LH preparations, but no detectable difference among the dose response lines for the pituitary LH of the IS 80/552 and the recombinant LH preparations. Calculation of potency estimates for IS 80/552 in terms of IS 98/704 and of the proposed IS in terms of IS 98/704 has been based on direct analysis of the available data for these pairs of preparations so that the common estimate of slope used to determine the potency will be determined only by the relevant pair of preparations. However, estimates of the potency of the proposed IS and the other recombinant LH preparations in terms of IS 80/552 and one another have been based on analysis of data for these preparations as multiple assays, so that a more precise estimate of slope than that based on pairwise analysis can be used.

Calibration of the proposed IS in terms of IS 98/704 (and IS 71/223)

Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 (and IS 71/223 in one laboratory) using the participant's routinely used response are shown in Table 2 and Figure 1, and for the two responses separately are shown in Appendix Tables 3a and 3b.

Based on the within assay variance, the estimates of potency are not homogeneous in the majority of laboratories, and the individual estimates are not homogeneous overall. The laboratory geometric means appear to be homogeneous, but this reflects the relatively very small weights (a result of the within laboratory

heterogeneity) for the laboratory means showing differences from the overall mean. However, unweighted analysis of variance shows no significant difference between estimates from the different laboratories based on the pooled between assay variance within laboratories.

The unweighted geometric mean estimate (with 95% fiducial limits) of all individual assay estimates was 189 (175-204) IU/ampoule (Table 2); and the weighted geometric mean estimate (with 95% fiducial limits) of laboratory mean estimates was 191 (186-196) IU/ampoule. The unweighted geometric mean of unweighted laboratory geometric means was 188 (172-205) IU per ampoule.

Calibration of proposed IS in terms of IS 80/552

Estimates of the LH content of the proposed IS by bioassay in terms of IS 80/552 using participants' routinely used response are shown in Table 3 and Figure 2, and for the two responses separately in Appendix Tables 4a and 4b.

Based on the within assay variance, the estimates of potency are not homogeneous in the majority of laboratories, and are not homogeneous overall. However, unweighted analysis of variance shows no significant difference between estimates from the different laboratories based on the pooled between assay variance within laboratories.

The unweighted geometric mean (95% fiducial limits) for all individual estimates was 247 (220-278) IU per ampoule, and the unweighted geometric mean of laboratory geometric means was 248 (213-289) IU per ampoule.

Comparison of IS 98/704 and IS 80/552

Estimates by bioassay of the potency of the IS 80/552 in terms of IS 98/704 expressed as IU of IS 98/704 equivalent to 1 IU of IS 80/552 using participants' routinely used response are shown in Table 4 and Figure 3, and estimates for the two responses separately are given in Appendix Tables 5a and 5b.

Based on the within assay variance, the estimates of potency are not homogeneous in a number of laboratories, and are not homogeneous overall. Unweighted analysis of variance shows a significant difference ($p \sim 0.03$) between estimates from the different laboratories based on the pooled between assay variance within laboratories. This difference results largely from the estimate obtained by laboratory 1, and if estimates from laboratory 1 are excluded there is no significant difference between laboratories ($p > 0.25$).

The unweighted geometric mean of all individual estimates (95% fiducial limits) was 0.80 (0.70-0.92) IU of IS 98/704 equivalent to 1 IU of IS 80/552. The unweighted geometric mean of laboratory geometric means was 0.79 (0.64-0.97) IU of IS 98/704 equivalent to 1 IU of IS 80/552.

Comparison of proposed IS, LH 96/816 and LH 96/820

Estimates of the LH content of the proposed IS, LH 96/816 and LH 96/820, where each sample is calibrated in terms of IS 80/552, are shown in Table 5, and separately for each response measure in Appendix Tables 4a and 4b. In Table 5 unweighted geometric means relative to the unweighted geometric mean for the proposed IS in the same laboratory are also given.

Based on the within assay variance, the estimates of potency are not homogeneous in the majority of laboratories, and are not homogeneous overall. However, based on the pooled between assay within laboratory variance, there is no significant difference among the estimates for these three preparations

The unweighted geometric mean estimate (with 95% fiducial limits) of the laboratory mean LH content of LH 96/816 was 96 (86-107) as % of that of the proposed IS. The unweighted geometric mean estimate (with 95% fiducial limits) of the laboratory mean LH content of LH 96/820 was 103 (94-112) as % of that of the proposed IS.

Accelerated thermal degradation studies of the proposed IS, LH 96/816 and LH 96/820

Estimates of the LH content of the thermally accelerated degradation samples of the LH preparations, relative to the same preparation stored at -20°C are shown in Table 6. Estimates for samples stored at 20°C for over five years do not show a significant loss of activity, and geometric mean estimates over all assays for these samples do not differ significantly from 1.0. In the absence of detectable loss of activity at 20°C, no rate of degradation can be predicted for LH 96/816 or LH 96/820. Using all data for the proposed IS and assuming that the degradation rate is relative to temperature by an Arrhenius equation, the predicted yearly loss of activity for samples stored at -20°C is less than 0.01%. These data indicate that the proposed IS is sufficiently stable to serve as an international standard. Consistency of results for the samples stored at 20°C of LH 96/816 or LH 96/820 with those for the proposed IS suggests that these preparations are similarly stable.

Comparison of the LH preparations under study in one immunoassay system

Four independent assays were carried out by laboratory 1, each comprising four parts (presumed to be separate microtitre plates). Each apparent plate included an in-house reference preparation, and has therefore been analysed separately to give estimates in terms of the in house reference. Estimates for the ampouled LH preparations in terms of one another have been determined as ratios of the mean estimates in terms of the in-house reference. Preliminary analysis of data suggested the occurrence of significant positional effects in the assay, although this could not be confirmed since details of assay plate layout were not available. Nevertheless, responses to nominally the same doses of the same preparations showed significant differences related to the order and grouping of the response data. Separate analyses for each assay plate were statistically valid (i.e. there were no significant deviations from linearity or parallelism) for the majority of plates. The limited number of exceptions may reflect artefacts of a non-random assay design. There were no

consistent differences among the slopes of the different preparations. Estimates for each ampouled LH preparation in terms of the in-house reference preparation were broadly consistent over all plates and assays, with geometric coefficient of variation of some 10-15%. The combined estimate for the LH content of the proposed IS in terms of IS 98/704 was 825 IU per ampoule, significantly higher than the estimates by bioassay. The combined estimate for the LH content of the proposed IS in terms of IS 80/552 was 71.8 IU per ampoule, significantly lower than the estimates by bioassay. Comparison of IS 98/704 and IS 80/552 gave 11.5 IU of IS 98/704 equivalent to 1 IU of IS 80/552, significantly higher than the estimates by bioassay. These differences between the relative potencies of these three types of LH preparation by bioassay and immunoassay are probably a reflection of differences in their isoform compositions, as well as of their differing degrees of purity. Thus, whereas IS 80/552 and the proposed IS consist of highly purified preparations of LH, IS 98/704 consists of a relatively impure preparation of LH. Comparisons of the proposed IS, LH 96/816 and LH 96/820 showed no significant differences among them, estimates for LH 96/816 and LH 96/820 relative to the proposed IS being 97% and 91%, respectively. Degradation samples of the proposed IS kept for 2067 days at +4°C and +20°C did not differ significantly from estimates for the proposed IS. However, estimates for the proposed IS kept for 2067 days at +37°C were significantly smaller than those for the proposed IS, and for those of samples of the proposed IS kept for 2067 days at +4°C and at +20°C. Relative to the proposed IS, estimates for samples of the proposed IS kept for 2067 days at +4°C, +20°C and at +37°C were 100%, 96% and 75%, respectively.

Differences between pituitary, urinary and recombinant LH

This study showed significant differences in the bioassay between the slopes of the log dose-response lines for the urinary LH of IS 98/704 and for those of each of the pituitary LH of IS 80/552 and the recombinant LH of the proposed IS, which were not significantly different from one another. These differences were not apparent in the data from individual assays, but became apparent from an analysis of the data over all the assays. Another feature of the data of this study was the finding that estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 and in terms of IS 80/552 were not homogeneous in the majority of laboratories, and were not homogeneous overall. This between assay heterogeneity of estimates is unusual for estimates from this type of assay and was not found in previous collaborative assays where preparations of pituitary LH were compared with one another, and where preparations of urinary LH were compared with one another [2;5;9;16]. The between laboratory heterogeneity is also greater than expected for this type of assay. The assays of laboratory 6 appeared to be more sensitive to IS 80/552 and the proposed IS and to see IS 98/704 as relatively less potent, whereas laboratory 1, and to a lesser extent laboratories 7 and 8, tended to see IS 98/704 as relatively more potent than IS 80/552 or the proposed IS in the context of all assays in this study. There were some differences between the strains of rats used by different laboratories, but these did not clearly explain the apparent differences in specificities between their assays. These data taken together suggest differences between the biological properties of the recombinant LH of the proposed IS, the urinary LH of IS 98/704 and the pituitary LH of IS 80/552. This is consistent with the reported differences between the isoform

compositions of pituitary and urinary LH [17], and those between recombinant, serum and urinary forms of other glycoprotein hormones such as EPO [18].

However, the differences found in this study between the magnitude of the IU of pituitary LH and that of urinary LH, namely that 1 IU of pituitary LH is equivalent to approximately 0.80 IU of urinary LH, appear to be due mainly to the differences between the procedures used to calibrate the standards which define these units (Table 7). The current international units of pituitary and urinary LH can both be traced back to a common standard, the 2nd International Reference Preparation of Human Menopausal Gonadotrophins (FSH and ICSH) (2nd IRP HMG)[19] (Table 7). The values assigned to successive international standards and reference preparations defining the international unit of pituitary LH, namely the 1st IRP of Human Pituitary Gonadotrophins (FSH and LH (ICSH)) for Bioassay (ampoule code 69/104) [20;21], the 2nd IRP of Human Pituitary Gonadotrophins (FSH and LH (ICSH) for Bioassay (ampoule code 78/549)[22], the 1st IRP of Human Pituitary LH for Immunoassay (ampoule code 68/40)[16], and IS 80/552[5], in all cases approximated closely to the estimates of LH content by OAAD assay. These estimates by OAAD assay tended to be higher than the estimates of LH content by SVW and related male accessory organ weight gain assays. The differences were particularly significant in the transition from the 1st IRP of Human Pituitary Gonadotrophins (FSH and LH (ICSH)) for Bioassay to the 1st IRP of Human Pituitary LH for Immunoassay, where the mean estimate of LH content in the collaborative assay by OAAD bioassays, was more than 50 % higher than that by SVW bioassays. In contrast, the values assigned to successive international standards defining the international unit of urinary LH, namely 1st IS for Human Urinary FSH and for Human Urinary LH (ICSH) for Bioassay (ampoule code 70/45) [9], 2nd IS for Human Urinary FSH and LH for Bioassay (ampoule code 71/223)[10], 3rd IS for Urinary FSH and LH for Bioassay (ampoule code 71/264)[3] and IS 98/704[2], in all cases approximated closely to the estimates of LH content by SVW and related male accessory organ weight gain assays. Taken together these differences in calibration data between the two series of reference materials could readily account for the differences found in this study between the magnitude of the IU of pituitary LH and that of urinary LH.

Suitability of the proposed IS for the standardization of LH

The proposed IS appears to be suitable to serve as the international standard for recombinant LH for bioassay since: (a) it contains a preparation of highly purified recombinant LH with a specific LH bioactivity of more than 20,000 IU/mg, as estimated, in terms of IS 98/704 or IS 80/552, by the in-vivo seminal vesicle weight gain bioassay method which is specific for LH, and which is specified for the estimation of LH in pharmacopoeial monographs; and (b) accelerated thermal degradation studies indicated that the LH biological activity of the proposed IS would be adequately stable when the proposed IS is stored under normal conditions, at – 20°C in the dark.

Although the LH of the proposed IS is of recombinant origin, it is known that recombinant glycoprotein hormone products, such as erythropoietin [23], may differ between manufacturers according to the cells and culture conditions used for their synthesis, and the selectivity of purification procedures used to isolate them. However, most glycoprotein hormone products have to-date been synthesized in CHO

cells. Furthermore the LH of the proposed IS was produced by the sole manufacturer with, currently, a licensed product on market.

Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 or IS 80/552 were not homogeneous in the majority of laboratories, and were not homogeneous overall. However, unweighted analysis of variance indicated that there were no significant differences between estimates from the different laboratories. Significant differences were found between the slopes of log dose-response lines in the bioassay for the urinary LH preparation IS 98/704 and for those of each of the pituitary (IS 80/552) and recombinant (proposed IS) LH preparations, which were not significantly different from one another. These differences between the biological properties of these three types of LH preparation are consistent with differences in their LH isoform compositions, and indicate the need for separate international standards for these three types of LH.

Assignment of unitage

Although the recombinant LH of the proposed IS appeared to be more similar in its biological properties to those of the pituitary LH of the IS 80/552 than to those of the urinary LH of IS 98/704 (and of IS 71/223), there is a need to maintain, if possible, a continuity with the international units of urinary LH, which have been, and still are being, used to calibrate LH-containing products currently used in medical treatment. It is therefore recommended that the contents of each ampoule of the proposed IS be assigned an activity of 189 International Units of recombinant LH on the basis of their calibration in terms of IS 98/704.

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REFERENCES

1. The European Recombinant Human LH Study Group. Recombinant human luteinizing hormone (LH) to support recombinant human follicle-stimulating hormone (FSH)-induced follicular development in LH- and FSH-deficient anovulatory women: a dose-finding study. *J.Clin.Endocrinol.Metab* 1998;83:1507-14.

2. Storring PL,.Gaines Das RE. The fourth International Standard for Human Urinary FSH and LH: specificities of LH seminal vesicle weight gain assays in the collaborative study differ between laboratories. *J.Endocrinol.* 2001;171:119-29.

3. WHO Expert Committee on Biological Standardization. Forty-fourth Report. 1994. WHO Technical Report Series No 848.

4. WHO Expert Committee on Biological Standardization. Forty-fifth Report. 1995. WHO Technical Report Series No 858.

5. Storring PL,.Gaines Das RE. The Second International Standard for Human Pituitary LH: its collaborative study by bioassays and immunoassays. *J.Endocrinol.* 1993;138:345-9.

6. Van Hell, H., Matthijsen, R., and Overbeek, G. A. Effects of human menopausal gonadotrophin preparations in different bioassay methods. Acta Endocrinologica 47, 409-418. 1964.

7. Campbell PJ. International biological standards and reference preparations. II. Procedures used for the production of biological standards and reference preparations. *J.Biol.Stand.* 1974;2:259-67.

8. WHO Expert Committee on Biological Standardization. Twenty-ninth Report. 1978. WHO Technical Report Series No 626.

9. Storring PL, Dixon H, Bangham DR. The first international standard for human urinary FSH and for human urinary LH (ICSH), for bioassay. *Acta Endocrinol.(Copenh)* 1976;83:700-10.

10. WHO Expert Committee on Biological Standardization. Thirty-ninth Report. 1989. WHO Technical Report Series No. 786.

11. Parlow AF. Bioassay of pituitary luteinizing hormone by depletion of ovarian ascorbic acid. In Albert A, ed. *Human Pituitary Gonadotropins*, pp 300-10. Springfield, Illinois: Charles C. Thomas, 1961.

12. Gaines Das RE,.Rice LR. SCAN, an exploratory program for preliminary analysis of bioassay and immunoassay data. *Comput.Methods Programs Biomed.* 1985;21:25-33.

13. Gaines Das RE. Role of ancillary variables in the design, analysis, and interpretation of animal experiments. *ILAR.J.* 2002;43:214-22.

14. Finney DJ. Statistical Method in Biological Assay. London: Griffin, 1978.

15. Gaines-Das, R. E. and Tydeman, S. Iterative weighted regression analysis of logit responses; a computer program for analysis of bioassays and immunoassays. Computer Programs in Biomedicine 15, 13-22. 1982.

16. Storring PL, Bangham DR, Cotes PM, Gaines Das RE, Jeffcoate SL. The international reference preparation of human pituitary luteinizing hormone, for immunoassay. *Acta Endocrinol.(Copenh)* 1978;88:250-9.

17. Van Damme MP, Robertson DM, Diczfalusy E. Biological and immunological characterization of human luteinizing hormone: III. Biological and immunological profiles of urine preparations after electrofocusing. *Mol.Cell Endocrinol.* 1977;9:69-79.
18. Tam RC, Coleman SL, Tiplady RJ, Storring PL, Cotes PM. Comparisons of human, rat and mouse erythropoietins by isoelectric focusing: differences between serum and urinary erythropoietins. *Br.J.Haematol.* 1991;79:504-11.

19. WHO Expert Committee on Biological Standardization. Seventeenth Report. 1964. WHO Technical Report Series No 293.

20. Bangham DR, Berryman I, Burger H, Cotes PM, Furnival BE, Hunter WM *et al.* An international collaborative study of 69-104, a reference preparation of human pituitary FSH and LH. *J.Clin.Endocrinol.Metab* 1973;36:647-60.

21. WHO Expert Committee on Biological Standardization. Twenty-sixth Report. 1975. WHO Technical Report Series No 565.

22. WHO Expert Committee on Biological Standardization. Thirty-first Report. 1981. WHO Technical Report Series No 658.

23. Storring PL,.Gaines Das RE. The International Standard for Recombinant DNA-derived Erythropoietin: collaborative study of four recombinant DNA-derived erythropoietins and two highly purified human urinary erythropoietins. *J.Endocrinol.* 1992;134:459-84.

Figure legends

Figure 1. Frequency distribution of estimates of the LH content of the proposed international standard for recombinant human luteinizing hormone in terms of the fourth International Standard for Human Urinary FSH and LH by seminal vesicle weight gain assays in different laboratories. Each box denotes the estimate from an individual assay, and the number in the box identifies the laboratory.

Figure 2. Frequency distribution of estimates of the LH content of the proposed international standard for recombinant human luteinizing hormone in terms of the second International Standard for Human Pituitary LH by seminal vesicle weight gain assays in different laboratories. Each box denotes the estimate from an individual assay, and the number in the box identifies the laboratory.

Figure 3. Frequency distribution of estimates of the LH potency of the second International Standard for Human Pituitary LH in terms of the fourth International Standard for Human Urinary FSH and LH by seminal vesicle weight gain assays in different laboratories. Each box denotes the estimate from an individual assay, and the number in the box identifies the laboratory.

Table 1. Summary ancillary information.

Lab	Rat strain (age in days)	Assay	Initial body weight (g)			Final body weight (g)		Weight change (g)		
			Mean	Range	%CV	Mean	%CV	Mean	Range	%CV
1	Lewis (21-28)	1				67	6.5			
		2	45	13	7.4	64	*9.4*	*19.0*	*36.0*	*27*
							8.1	19.6	22.0	18
2	Wistar (24-30)	1	111	28	5.3	140	5.9	29.3	22.0	14
		2	99	13	3.6	130	4.2	31.1*	21.0	12
		3	64	12	5.5	90	5.9	25.8	15.0	12
		4	68	13	5.8	95	5.4	26.7*	16.0	11
		5	68	12	4.9	95	5.3	26.9*	14.0	11
		6	65*	15	6.3	91**	7.0	26.1*	12.0	11
3	Sprague-Dawley (20)	1&2	51	3	1.6					
4	Sprague-Dawley (22)	1	53	11	6.4	76	7.5	22.6	37.0	29
5	Wistar (19-22)	1	39	6	4.4	49	6.2	10.3	9.3	20
		2	36	8	6.0	49	6.9	12.8	11.1	16
6	Wistar (21)	1	52	11	5.6	84	5.7	32.1	13.5	10
		2	53	10	5.0	83	5.3	30.5	18.5	10
7	Wistar (21-24)	1	41	9	5.3	43	7.0	1.5	9.1	138
		2	43*	13	6.9	45	8.9	2.7	12.1	101
		3	42	5	3.9	46**	5.7	4.2**	9.3	53
		4	42*	6	4.1	45	8.0	2.5	10.5	101
		5	42	7	4.0	45	5.8	2.8	10.8	79
		6	42	7	4.7	44	5.7	2.1*	9.7	98
		7	42	8	5.2	47	7.9	5.1	11.5	52
		8	40*	8	5.1	44	9.5	3.6	11.7	92
		9	41	8	4.9	45	5.9	3.6	12.2	69
		10	41	8	5.1	44	6.3	3.1	9.5	68
		11	50	9	5.2	60	5.9	9.5	9.7	23
		12	50	10	4.7	59	5.1	9.1	9.0	22
		13	49	10	4.7	61	6.0	12.1	14.2	18
		14	51	9	4.4	62	4.9	11.6	12.3	17
		15	52	9	4.5	63	5.9	10.9	9.1	19
		16	48	9	4.8	61	4.9	12.6	8.5	12
8	CH-BB Thom (20-23)	1	66	8	3.8	95	5.7	29.1	17.3	12
		2	66	7	2.6	97*	5.5	30.9**	16.3	13
		3	64	7	2.6	92	4.9	28.7**	15.6	11
		4	62	5	2.7	90	5.0	27.5*	14.6	12
		5	63	7	3.2	92	4.7	28.7**	13.9	10
9	Wistar (20-23)	1	51*	26	12.0	69**	13.6	17.7**	19.0	22
		2	47	17	10.2	74	9.5	27.6	15.0	12
		3	38	16	11.2	53*	11.4	14.9**	14.0	22

Table 1 continued:

Assays in which the measure of weight showed a significant regression, combined over all preparations in the assay, on dose are indicated * ($0.05 > p > 0.01$) or ** ($p < 0.01$). Except in laboratory 7, assay 2, where the slopes were negative, all statistically significant regressions gave positive slopes. For assay 2 in laboratory 1, the numbers in italics show the values based on all data, while the numbers in the second line show the values after replacement of the single outlying final weight.

Table 2. Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 (except assays marked + where IS 71/223 was used as standard).

Laboratory	Assay	LH content as IU/ampoule		Statistical weight
		Geometric mean	95% fiducial limits	
1	1.0	134		950
	2.0	172		1888
	All	152*–unweighted	31-735	
2	1.0	258		1107
	2.0	276		845
	3.0	150		1352
	6.1	205		696
	All	216*–unweighted	140-335	
3	1.0	188		5106
	2.0	189		7769
	All	189–weighted	181-197	
		189–unweighted	179-198	
4	1.1	208		782
	1.5	183		884
	1.6	211		1975
	1.7	169		711
	All	197–§weighted	184-211	
		192–unweighted	162-227	
5	1.0	193		2409
	2.0	187		2186
	All	190–weighted	177-203	
		190–unweighted	158-227	
6	1.0	237		1957
	2.0	207		5163
	All	221*–unweighted	94-521	
7	1.0+	191		990
	2.0+	243		576
	13.0	135		210
	14.0	157		746
	All	177*–unweighted	118-266	
8	1.0	222		319
	2.0	137		219
	3.0	182		867
	All	182–§weighted	161-206	
		177–unweighted	97-323	
9	1.0	233		1262
	2.0	151		1011
	All	187*–unweighted	12-3004	
All individual estimates		189*–unweighted	175-204	
All laboratory mean estimates		191–weighted	186-196	
		188–unweighted	172-205	
Laboratory	Assay	LH content as IU/ampoule		Statistical weight
		Geometric mean	95% fiducial limits	
All laboratory mean estimates except laboratory 1		192–weighted	186-197	
		193–unweighted	180-207	

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$).

Table 3. Estimates of the LH content of the proposed IS by bioassay in terms of IS 80/552.

Laboratory	Assay	LH content as IU/ampoule		Statistical weight
		Geometric mean	95% fiducial limits	
1	1.0	368		473
	2.0	375		491
	All	372–weighted	320-431	
		371–unweighted	331-417	
2	1.0	262		950
	2.0	329		666
	3.0	184		805
	All	251*–unweighted	121-520	
3	1.0	229		4692
	2.0	219		5307
	All	224–weighted	214-234	
		224–unweighted	169-297	
4	1.1	191		770
	1.5	300		761
	1.6	208		1730
	1.7	204		934
	All	222*–unweighted	161-307	
5	1.0	220		2563
	2.0	218		2448
	All	219–weighted	206-234	
		219–unweighted	205-234	
6	1.0	191		4181
	2.0	207		4101
	All	199§–weighted	189-209	
		199–unweighted	119-334	
7	1.0	246		685
	2.0	339		300
	All	289*unweighted	38-2213	
8	1.0	366		135
	2.0	215		216
	5.0	288		546
	All	278§–weighted	239-324	
		283–unweighted	146-549	
9	1.0	317		665
	2.0	147		1020
	All	216*unweighted	2-27800	
All individual estimates		247*–unweighted	220-278	
All laboratory mean estimates		248*-unweighted	213-288	
All laboratory mean estimates except laboratory 1		226§–weighted	218-234	
		242–unweighted	216-272	

Table 3 continued:

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$).

Table 4. Estimates by bioassay of the potency of the IS 80/552 in terms of IS 98/704 expressed as IU of IS 98/704 equivalent to 1 IU of IS 80/552, and calculated in a direct comparison of these two preparations only.

Laboratory	Assay	IU of IS 98/704 equivalent to 1 IU of IS 80/552		Statistical weight
		Geometric mean	95% fiducial limits	
1	1.0	0.40		516
	2.0	0.49		778
	All	0.45§–weighted	0.40-0.52	
		0.44–unweighted	0.12-1.69	
2	1.0	1.03		705
	2.0	0.88		678
	3.0	0.84		1570
	All	0.89–weighted	0.82-0.97	
		0.91–unweighted	0.70-1.19	
3	1.0	0.82		4375
	2.0	0.86		4943
	All	0.84–weighted	0.80-0.88	
		0.84–unweighted	0.62-1.14	
4	1.1	1.10		707
	1.5	0.63		1165
	1.6	1.02		1323
	1.7	0.84		1267
	All	0.88*–unweighted	0.59-1.30	
5	1.0	0.88		2296
	2.0	0.86		2155
	All	0.87–weighted	0.81-0.93	
		0.87–unweighted	0.76-0.99	
6	1.0	1.22		2533
	2.0	1.00		3548
	All	1.10*–unweighted	0.31-3.88	
7	1.0	0.77		829
	2.0	0.76		473
	All	0.76–weighted	0.67-0.87	
		0.76–unweighted	0.73-0.80	
8	1.0	0.81		342
	2.0	0.41		59
	All	0.58*–unweighted	0.01-43.6	
9	1.0	0.76		850
	2.0	1.05		857
	All	0.90*–unweighted	0.12-6.81	
All individual estimates		0.80*–unweighted	0.70-0.92	
All laboratory mean estimates		0.79*–unweighted	0.64-0.96	
All laboratory mean estimates except laboratory 1		0.86§–weighted	0.83-0.89	
		0.84–unweighted	0.72-0.98	

Table 4 continued:

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$).

Table 5. Estimates of the LH content of LH 96/816 and LH 96/820 by bioassay in terms of IS 80/552, and in terms of that of the proposed IS.

Laboratory	Assay	LH content as IU/ampoule in terms of IS 80/552		LH content as % of that of the proposed IS	
		LH 96/816	LH 96/820	LH 96/816	LH 96/820
		Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)
2	1.0	293			
	2.0	.	242		
	3.0	209	.		
	All	247*-unweighted	242	0.99-unweighted	0.96
4	1.1	177	183		
	1.5	302	298		
	All	231*-unweighted	233*-unweighted	1.04-unweighted	1.05-unweighted
7	3.0		319		
	4.0		278		
	13.0	232			
	14.0	319			
	15.1		415		
	16.1		272		
	All	300§-weighted 272-unweighted	316*-unweighted	0.94-unweighted	1.09-unweighted
8	3.0	250		0.88	
	5.0		284		1.00
All individual estimates		250*(205-305) -unweighted	280*(230-340) -unweighted	0.97*(0.80-1.18) -unweighted	1.06*(0.90-1.23) -unweighted
All laboratory mean estimates		270(244-299) -weighted	273(246-303) -weighted	0.96(0.97-1.07) -weighted	1.02(0.92-1.13) -weighted
		250(225-278) -unweighted	267(213-334) -unweighted	0.96(0.86-1.07) -unweighted	1.03(0.94-1.12) -unweighted

Table 5 continued:

Estimates of the LH content in terms of that of the proposed IS were calculated using an assumed potency for the proposed IS based on its calibration in terms of the IS 80/552 in the same laboratory, namely 251 IU/ampoule in laboratory 2, 222 IU/ampoule in laboratory 4, 289 IU/ampoule in laboratory 7 and 283 IU/ampoule in laboratory 8. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$).

Table 6. Estimates by bioassay of the LH content of ampoules of the proposed IS, LH 96/816 and LH 96/820 kept at elevated temperatures in terms of that of ampoules kept at -20°C.

LH preparation (time in days at elevated temperature)	Laboratory	Assay	LH content as % of that of the same preparation kept at -20°C			
			Kept at 4°C	Kept at 20°C	Kept at 37°C	Kept at 45°C
			Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)
Proposed IS (367 days)	7	5.0 6.0 All		108 92 95(85-105) –weighted 100(37-271) -unweighted		79 82 82(73-91) -weighted 81(62-105) -unweighted
Proposed IS (1619 days)	7	11.0 12.0 All		96 104 99(83-118) -weighted 100(61-163) -unweighted	94 75 85(71-103) -weighted 84(21-343) -unweighted	
Proposed IS (2067 days)	2	4.0 5.0 6.2	92	*38* 109	*27* 67 64	*31* 53
	4	1.2	112	94		
	8	4.0		82	57	
	9	3.0		107	76	
		All	105§(94-117) -weighted 102(28-366) -unweighted	100(92-108) -weighted 97(79-120) -unweighted	68(62-74) -weighted 66(54-80) -unweighted	53

Table 6 continued:

LH preparation (time in days at elevated temperature)	Laboratory	Assay	LH content as % of that of the same preparation kept at -20°C			
			Kept at 4°C	Kept at 20°C	Kept at 37°C	Kept at 45°C
			Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)	Geometric mean (95% fiducial limits)
LH 96/816 (228 days)	7	7.0		145		106
		8.0		67		72
		All		99(1-12,900)* -unweighted		87(7-1,020)* -unweighted
LH 96/816 (1928 days)	4	1.3		101		
	7	15.3		110		
		16.3		121		
		All		107(98-117) -weighted 110(89-137) -unweighted		
LH 96/820 (220 days)	7	9.0		73		49
		10.0		86		49
		All		83(71-99) -weighted 79(28-224) -unweighted		49(39-60) -weighted 49(47-50) -unweighted
LH 96/820 (1920 days)	4	1.4		1.04		
	7	15.2		85		
		16.2		112		
		All		102(90-115) -weighted 100(71-141) -unweighted		

Table 6 continued:

Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Estimates from assay 4 in Laboratory 2 contributed significantly to heterogeneity both within the laboratory when considered with other estimates in the same laboratory, and between all laboratories; these estimates are shown in italics but have been omitted from calculation of mean estimates.

Table 7. Successive international reference preparations (IRPs) and international standards (ISs) for pituitary and urinary LH

Standards for pituitary LH	Standards for urinary LH
1st IRP of Human Pituitary Gonadotrophins (FSH and LH (ICSH)) for Bioassay (ampoule code 69/104) [1] - Calibrated in terms of 2nd IRP of Human Menopausal Gonadotrophins (FSH and ICSH)[2] - Estimated LH content as IU/ampoule: 25 by ovarian ascorbate depletion (OAAD) bioassays[3] - Value assigned: 25 IU of pituitary LH/ampoule	1st IS for Human Urinary FSH and for Human Urinary LH (ICSH) for Bioassay (ampoule code 70/45)[4] 2nd IS for Human Urinary FSH and LH for Bioassay (71/223) [5] 3rd IS for Urinary FSH and LH for Bioassay (71/264) [6]
2nd IRP of Human Pituitary Gonadotrophins (FSH and LH (ICSH) for Bioassay (78/549)[7] - prepared from same batch of master ampoules of bulk, and by the same method as the 1st IRP (69/104) - Value assigned: 25 IU of pituitary LH/ampoule	- All prepared from same batch of master ampoules of bulk, and by the same method[4] - Each calibrated in terms of 2nd IRP of Human Menopausal Gonadotrophins (FSH and ICSH) - Estimated LH content as IU/ampoule: 37.5 by OAAD bioassays and 47.5 by seminal vesicle weight gain (SVW) and related bioassays[4] - Values assigned: 46 IU of urinary LH/ampoule
1st IRP of Human Pituitary LH for Immunoassay (68/40) [8] - Calibrated in terms of 1st IRP (69/104) - Estimated LH content as IU/ampoule: 80.2 by OAAD bioassays and 52.1 by SVW and related bioassays - Value assigned: 77 IU of pituitary LH/ampoule	4th IS for Human Urinary FSH and LH for Bioassay (98/704) [9] - Calibrated in terms of 3rd IS (71/264) - Estimated LH content as IU/ampoule: 70 by SVW bioassays - Value assigned: 70 IU of urinary LH/ampoule
2nd IS for Human Pituitary LH (80/552) [10] - Calibrated in terms of 1st IRP (68/40) - Estimated LH content as IU/ampoule: 37.1 by OAAD bioassays and 26.4 by SVW bioassays - Value assigned: 35 IU of pituitary LH/ampoule	

Table 7 continued:

1. WHO Expert Committee on Biological Standardization. Twenty-sixth Report. 1975. WHO Technical Report Series No 565.

2. WHO Expert Committee on Biological Standardization. Seventeenth Report. 1964. WHO Technical Report Series No 293.

3. Bangham DR, Berryman I, Burger H, Cotes PM, Furnival BE, Hunter WM *et al.* An international collaborative study of 69-104, a reference preparation of human pituitary FSH and LH. *J.Clin.Endocrinol.Metab* 1973;**36**:647-60.

4. Storring PL, Dixon H, Bangham DR. The first international standard for human urinary FSH and for human urinary LH (ICSH), for bioassay. *Acta Endocrinol.(Copenh)* 1976;**83**:700-10.

5. WHO Expert Committee on Biological Standardization. Thirty-ninth Report. 1989. WHO Technical Report Series No. 786.

6. WHO Expert Committee on Biological Standardization. Forty-fourth Report. 1994. WHO Technical Report Series No 848.

7. WHO Expert Committee on Biological Standardization. Thirty-first Report. 1981. WHO Technical Report Series No 658.

8. Storring PL, Bangham DR, Cotes PM, Gaines Das RE, Jeffcoate SL. The international reference preparation of human pituitary luteinizing hormone, for immunoassay. *Acta Endocrinol.(Copenh)* 1978;**88**:250-9.

9. Storring PL,.Gaines Das RE. The fourth International Standard for Human Urinary FSH and LH: specificities of LH seminal vesicle weight gain assays in the collaborative study differ between laboratories. *J.Endocrinol.* 2001;**171**:119-29.

10. Storring PL,.Gaines Das RE. The Second International Standard for Human Pituitary LH: its collaborative study by bioassays and immunoassays. *J.Endocrinol.* 1993;**138**:345-9.

Appendix

Appendix-Table 1a.

Slopes of log dose – log seminal vesicle weight response lines for different LH preparations.

Laboratory	Assay	IS 98/704	IS 71/223	IS 80/552	Proposed IS	LH 96/816	LH 96/820
1	1.0	1.03		0.69	0.75		
	2.0	1.02		0.55	0.72		
2	1.0	1.18		0.87	1.22	0.96	
	2.0	1.23		0.97	1.17		1.04
	3.0	1.70+		1.38+	*	1.43+	
	6.1	1.30			0.91		
3	1.0	2.21+		2.51+	2.26+		
	2.0	2.30+		2.13+	2.29+		
4	1.1	0.90		0.90	0.99	1.14	1.14
	1.5	1.16		0.97	0.84	1.19	0.95
	1.6	1.20		1.04	1.00		
	1.7	1.08		1.01	0.91		
5	1.0	0.73		0.82	0.77		
	2.0	0.71		0.76	0.71		
6	1.0	1.95+		1.84+	1.53+		
	2.0	1.82+		1.95+	1.72+		
7	1.0		1.25	0.89	0.93		
	2.0		1.06	0.95	0.82		
	3.0		1.00			0.81	0.80
	4.0		1.06			0.96	0.92
	13.0	0.74			0.62	0.62	
	14.0	0.98			1.06	0.87	
	15.1					0.89	1.15+
	16.1					0.71	0.89+
8	1.0	0.98		0.56	0.84+		
	2.0	0.97+		0.51	0.94		
	3.0	1.47+			1.12+	1.39+	
	5.0			0.93	1.39		1.21
9	1.0	1.34		1.06	1.33		
	2.0	1.06		1.45+	0.96		

Slopes marked + have been estimated using two doses only. * indicates inclusion of the preparation at a single dose. Where no value for slope is shown this denotes that the preparation was not included in the assay.

Appendix-Table 1b.

Slopes of log dose – log ratio of seminal vesicle weight : final body weight response lines for different LH preparations.

Laboratory	Assay	IS 98/704	IS 71/223	IS 80/552	Proposed IS	LH 96/816	LH 96/820
1	1.0	0.93		0.72	0.71		
	2.0	1.09		0.65	0.73		
2	1.0	1.17		0.85	1.23	0.85	
	2.0	1.12		0.94	1.14		1.06
	3.0	1.51+		1.48+	*	1.27+	
	6.1	1.20			0.78		
4	1.1	0.92		0.92	0.80	1.01	0.99
	1.5	1.10		0.97	0.76	1.24	0.98
	1.6	1.23		1.11	1.05		
	1.7	1.10		0.97	0.93		
5	1.0	0.76		0.83	0.73		
	2.0	0.69		0.72	0.69		
6	1.0	1.89+		1.75+	1.57+		
	2.0	1.90+		1.98+	1.68+		
7	1.0		1.25	0.79	0.92		
	2.0		1.07	0.75	0.76		
	3.0		1.01			0.93	0.97
	4.0		0.96			0.89	0.83
	13.0	0.69			0.66	0.57	
	14.0	0.99			1.07	0.85	
	15.1					0.89	1.43+
	16.1					0.72	0.86+
8	1.0	1.06		0.58	0.65+		
	2.0	0.83+		0.47	0.92		
	3.0	1.37+			1.08+	1.29+	
	5.0			0.90	1.36		1.17
9	1.0	1.10		0.74	1.07		
	2.0	1.05		1.25+	0.95		

Slopes marked + have been estimated using two doses only. * indicates inclusion of the preparation at a single dose. Where no value for slope is shown this denotes that the preparation was not included in the assay.

Appendix-Table 2a.
Slopes of log dose – log seminal vesicle weight response lines for ampoules of the proposed IS, LH 96/816 and LH 96/820 kept at elevated temperatures.

LH preparation (time at elevated temperature)	Laboratory	Assay	Storage temperature in °C				
			-20°C	4°C	20°C	37°C	45°C
Proposed IS (367 days)	7	5.0	0.79		0.62		0.74
		6.0	1.42+		1.78+		1.15+
Proposed IS (1619 days)	7	11.0	0.78		0.76	0.69	
		12.0	0.82		0.49	0.65	
Proposed IS (2067 days)	2	4.0	0.57		0.62	0.41	0.26
		5.0	1.02	0.89	0.79	0.73	
		6.2	1.50+			1.57+	1.88+
	4	1.2	0.99	1.13	0.96		
	8	4.0	0.88		0.73	0.59	
	9	3.0	1.17		0.99	1.06	
LH 96/816 (228 days)	7	7.0	1.07		0.64		1.12
		8.0	1.82+		0.93+		0.92+
LH 96/816 (1928 days)	4	1.3	1.14		1.04		
	7	15.3	0.89		1.40+		
		16.3	0.71		0.87+		
LH 96/820 (220 days)	7	9.0	1.00+		0.71+		1.02+
		10.0	1.79+		1.20+		*
LH 96/820 (1920 days)	4	1.4	1.14		0.78		
	7	15.2	1.15+		1.68+		
		16.2	0.89+		1.08+		

Slopes marked + have been estimated using two doses only. * indicates inclusion of the preparation at a single dose. Where no value for slope is shown this denotes that the preparation was not included in the assay.

Appendix-Table 2b.

Slopes of log dose – log ratio of seminal vesicle weight: body weight response lines for ampoules of the proposed IS, LH 96/816 and LH 96/820 kept at elevated temperatures.

LH preparation (time at elevated temperature)	Laboratory	Assay	Storage temperature in °C				
			-20°C	4°C	20°C	37°C	45°C
Proposed IS (367 days)	7	5.0	0.71		0.63		0.63
		6.0	1.27+		1.77+		1.19+
Proposed IS (1619 days)	7	11.0	0.84		0.76	0.67	
		12.0	0.74		0.60	0.65	
Proposed IS (2067 days)	2	4.0	0.44		0.67	0.43	0.26
		5.0	1.09	0.82	0.76	0.74	
		6.2	1.31+			1.38+	1.82+
	4	1.2	0.80	1.08	0.76		
	8	4.0	0.79		0.77	0.53	
	9	3.0	0.92		0.89	1.01	
LH 96/816 (228 days)	7	7.0	1.02		0.63		1.04
		8.0	1.79+		0.88+		0.78+
LH 96/816 (1928 days)	4	1.3	1.01		1.13		
	7	15.3	0.89		1.41+		
		16.3	0.72		0.95+		
LH 96/820 (220 days)	7	9.0	1.04+		0.73+		0.93+
		10.0	1.87+		1.09+		*
LH 96/820 (1920 days)	4	1.4	0.99		0.83		
	7	15.2	1.43+		1.71+		
		16.2	0.86+		1.02+		

Slopes marked + have been estimated using two doses only. * indicates inclusion of the preparation at a single dose. Where no value for slope is shown this denotes that the preparation was not included in the assay.

Appendix-Table 3a.
Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 (except in two assays marked + where IS 71/223 was used as standard). Estimates of the LH content were calculated using log seminal vesicle weight as response in a direct comparison of these two preparations only.

Laboratory	Assay	Geometric mean estimate of LH content as IU/ampoule	Statistical weight	Unweighted laboratory geometric mean estimate of LH content as IU/ampoule
1	1.0	134	950	
	2.0	172	1888	152*
2	1.0	258	1107	
	2.0	276	845	
	3.0	150	1352	
	6.1	205	696	216*
3	1.0	188	5106	
	2.0	189	7769	189
4	1.1	208	782	
	1.5	183	884	
	1.6	211	1975	*(197)*
	1.7	169	711	192§
5	1.0	195	1665	
	2.0	191	1585	193
6	1.0	237	1957	
	2.0	207	5163	221*
7	1.0+	204	899	
	2.0+	217	839	
	13.0	137	186	
	14.0	160	972	176*
8	1.0	222	319	
	2.0	137	219	*(182)*
	3.0	182	867	181§
9	1.0	205	2032	
	2.0	160	755	181*
Overall geometric mean		189*		*(191)*
				188
95% fiducial limits		175-204		172-205

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not more than marginally heterogeneous ($0.1 > p > 0.05$), the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Appendix-Table 3b.
Estimates of the LH content of the proposed IS by bioassay in terms of IS 98/704 (except in two assays marked + where IS 71/223 was used as standard). Estimates of the LH content were calculated using log ratio of seminal vesicle weight: body weight as response in a direct comparison of these two preparations only.

Laboratory	Assay	Geometric mean estimate of LH content as IU/ampoule	Statistical weight	Unweighted laboratory geometric mean estimate of LH content as IU/ampoule
1	1.0	159	861	*(167)*
	2.0	173	1278	166
2	1.0	260	1037	
	2.0	284	666	
	3.0	144	981	
	6.1	203	608	215*
3	1.0	Ratio of seminal vesicle weight: body weight not available		
	2.0			
4	1.1	260	471	
	1.5	186	651	
	1.6	198	1592	
	1.7	174	484	202*
5	1.0	193	2409	
	2.0	187	2186	190
6	1.0	240	1576	
	2.0	207	4465	223*
7	1.0+	191	990	
	2.0+	243	576	
	13.0	135	210	
	14.0	157	746	177*
8	1.0	215	362	
	2.0	139	230	*(186)*
	3.0	188	936	178§
9	1.0	233	1262	
	2.0	151	1011	187*
Overall geometric mean		192*		*(188)*
				191
95% fiducial limits		176-211		176-208

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not more than marginally heterogeneous ($0.1 > p > 0.05$), the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Appendix-Table 4a.

Estimates of the LH content of the proposed IS, LH 96/816 and LH 96/820 by bioassay in terms of IS 80/552 except as marked + where the preparation indicated in the column headed 'Standard' has been used as standard with assumed potency as shown in parentheses based on calibration in terms of the IS 80/552 in the same laboratory. Estimates of the LH content were calculated using log seminal vesicle weight as response.

Lab	Assay	Standard	Proposed IS			LH 96/816			LH 96/820		
			Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)
1	1.0	IS 80/552	368	473	*(372)*						
	2.0	IS 80/552	375	491	371						
2	1.0	IS 80/552	262	950		293	916				
	2.0	IS 80/552	329	666					242	738	
	3.0	IS 80/552	184	805	251*	209	1318	247*			242
3	1.0	IS 80/552	229	4692							
	2.0	IS 80/552	219	5307	224						
4	1.1	IS 80/552	191	770		177	766		183	768	
	1.5	IS 80/552	300	761		302	760		298	763	
	1.6	IS 80/552	208	1730							
	1.7	IS 80/552	204	934	222*			231*			233*
5	1.0	IS 80/552	226	2302							
	2.0	IS 80/552	221	1267	224						
6	1.0	IS 80/552	191	4181							
	2.0	IS 80/552	207	4101	199§						

Appendix-Table 4a continued:

Lab	Assay	Standard	Proposed IS			LH 96/816			LH 96/820		
			Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)
7	1.0	IS 80/552	254	917							
	2.0	IS 80/552	277	523							
	3.0	LH96/816 (278)							304+	1027	
	4.0	LH96/816							278+	2332	
	13.0	Proposed IS (262)				240+	146				
	14.0	Proposed IS				286+	781				
	15.1	LH96/816			*(262)*			*(277)*	364+	415	*(289)*
	16.1	LH96/816			265			278	260+	453	299§
8	1.0	IS 80/552	366	135							
	2.0	IS 80/552	215	216							
	3.0	Proposed IS (278)			*(278)*	250+	873				
	5.0	IS 80/552	288	546	283§			250	284	549	284
9	1.0	IS 80/552	271	769							
	2.0	IS 80/552	147	575	200*						
Overall geometric mean			244*		244*	247*		*(261)* 247	272*		*(281)* 263
95% fiducial limits			219-272		210-284						

Appendix-Table 4a continued:

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not more than marginally heterogeneous ($0.1 > p > 0.05$), the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Appendix-Table 4b.
Estimates of the LH content of the proposed IS, LH 96/816 and LH 96/820 by bioassay in terms of IS 80/552 except as marked + where the preparation indicated in the column headed 'Standard' has been used as standard with assumed potency as shown in parentheses based on calibration in terms of the IS 80/552 in the same laboratory. Estimates of the LH content were calculated using log ratio of seminal vesicle weight: body weight as response.

Lab	Assay	Standard	Proposed IS			LH 96/816			LH 96/820		
			Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean estimate of LH content (IU/ampoule)
1	1.0	IS 80/552	396	503							
	2.0	IS 80/552	401	528	399						
2	1.0	IS 80/552	280	787		285	783				
	2.0	IS 80/552	331	695					247	770	
	3.0	IS 80/552	168	1129	249*	206	1758	242*			247
3	1.0	IS 80/552	Ratio of seminal vesicle weight: body weight not available								
	2.0	IS 80/552									
4	1.1	IS 80/552	221	550		185	550		183	550	
	1.5	IS 80/552	333	647		315	660		302	668	
	1.6	IS 80/552	193	1054							
	1.7	IS 80/552	232	481	239*			241*			235*
5	1.0	IS 80/552	220	2563							
	2.0	IS 80/552	218	2448	219						
6	1.0	IS 80/552	196	3105							
	2.0	IS 80/552	205	3840	200						

Appendix-Table 4b continued:

Lab	Assay	Standard	Proposed IS			LH 96/816			LH 96/820		
			Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)	Geometric mean estimate of LH content (IU/ampoule)	Stat. wt	Unweighted laboratory geometric mean of LH content (IU/ampoule)
7	1.0	IS 80/552	246	685							
	2.0	IS 80/552	339	300					319+	1361	
	3.0	LH96/816 (300)							278+	1278	
	4.0	LH 96/816				232+	162				
	13.0	Proposed IS (289)				319+	701				
	14.0	Proposed IS						*(300)*	415+	368	
	15.1	LH96/816						272§	272+	492	316*
	16.1	LH 96/816			289*						
8	1.0	IS 80/552	390	141							
	2.0	IS 80/552	206	223							
	3.0	Proposed IS (285)				258+	750				
	5.0	IS 80/552	289	496	285*			258	283	500	283
9	1.0	IS 80/552	317	665							
	2.0	IS 80/552	147	1020	216*						
Overall geometric mean			256*		256*	252*		*(274)* 253	281*		*(274)* 268
95% fiducial limits			233-294		213-308						

Appendix-Table 4b continued:

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not more than marginally heterogeneous ($0.1 > p > 0.05$), the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Appendix-Table 5a.
Estimates by bioassay of the potency of the IS 80/552 in terms of IS 98/704 expressed as IU of IS 98/704 equivalent to 1 IU of IS 80/552. Estimates were calculated using log seminal vesicle weight as response in a direct comparison of these two preparations only.

Laboratory	Assay	Geometric mean estimate of IU of IS 98/704 equivalent to 1 IU of IS 80/552	Statistical weight	Unweighted laboratory geometric mean estimate of IU of IS 98/704 equivalent to 1 IU of IS 80/552
1	1.0	0.40	516	*(0.45)*
	2.0	0.49	778	0.44§
2	1.0	1.03	705	
	2.0	0.88	678	*(0.89)*
	3.0	0.84	1570	0.91
3	1.0	0.82	4375	
	2.0	0.86	4943	0.84
4	1.1	1.10	707	
	1.5	0.63	1165	
	1.6	1.02	1323	
	1.7	0.84	1267	0.88*
5	1.0	0.86	1991	
	2.0	0.87	1640	0.86
6	1.0	1.22	2533	
	2.0	1.00	3548	1.10*
7	1.0+	0.81	822	
	2.0+	0.80	705	0.81
8	1.0	0.81	342	
	2.0	0.41	59	0.58*
9	1.0	0.76	861	
	2.0	1.12	526	0.92*
Overall geometric mean		0.81*		0.79*
95% fiducial limits		0.70-0.92		0.64-0.98

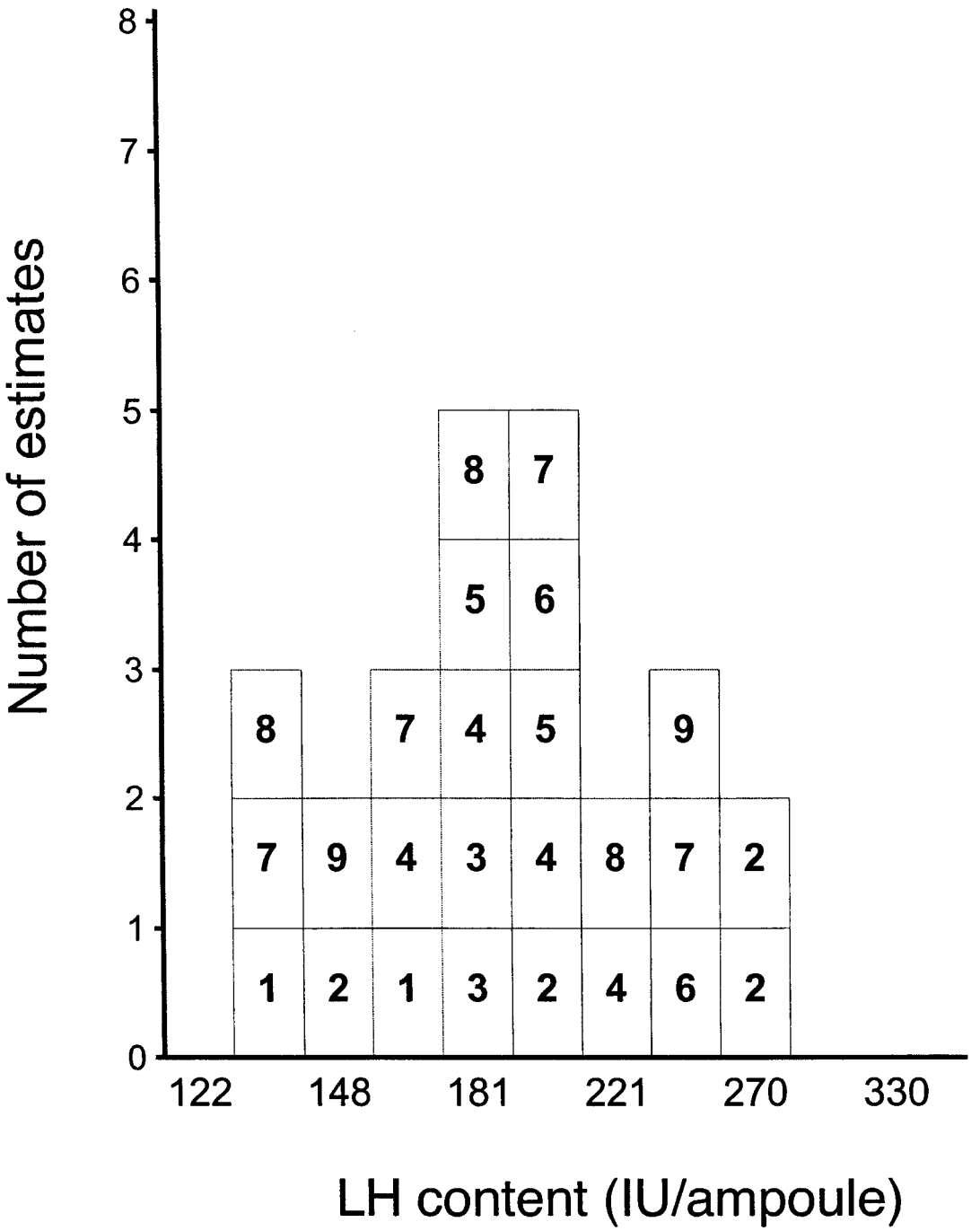
Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. In two assays marked + IS 71/223 has been used as standard. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not heterogeneous, the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Appendix-Table 5b.
Estimates by bioassay of the potency of the IS 80/552 in terms of IS 98/704 expressed as IU of IS 98/704 equivalent to 1 IU of IS 80/552. Estimates were calculated using log ratio of seminal vesicle weight:body weight as response in a direct comparison of these two preparations only.

Laboratory	Assay	Geometric mean estimate of IU of IS 98/704 equivalent to 1 IU of IS 80/552	Statistical weight	Unweighted laboratory geometric mean estimate of IU of IS 98/704 equivalent to 1 IU of IS 80/552
1	1.0	0.44	525	
	2.0	0.47	636	0.46
2	1.0	0.99	651	
	2.0	0.88	507	*(0.90)*
	3.0	0.87	1189	0.91
3	1.0	Ratio of seminal vesicle weight:body weight not available		
	2.0			
4	1.1	1.16	618	
	1.5	0.58	898	
	1.6	1.02	936	
	1.7	0.76	887	0.85*
5	1.0	0.88	2296	
	2.0	0.86	2155	0.87
6	1.0	1.21	2029	
	2.0	1.01	3603	1.11*
7	1.0+	0.77	829	
	2.0+	0.76	473	0.76
8	1.0	0.79	441	*(0.74)*
	2.0	0.42	51	0.58§
9	1.0	0.76	850	
	2.0	1.05	857	0.90*
Overall geometric mean (95% fiducial limits)		0.79* (0.68-0.92)		*(0.80* (0.65-0.99))* 0.78 (0.61-0.99)

Statistical weight determined as reciprocal of the variance of $\log_{10}$potency. In two assays marked + IS 71/223 has been used as standard. Estimates marked * indicate that the individual estimates are heterogeneous ($p < 0.05$) and estimates marked § indicate that the individual estimates are marginally heterogeneous ($0.1 > p > 0.05$). Where the estimates were not heterogeneous, the weighted geometric mean is shown in italics if it differed from the unweighted mean.

Figure1


Number of estimates
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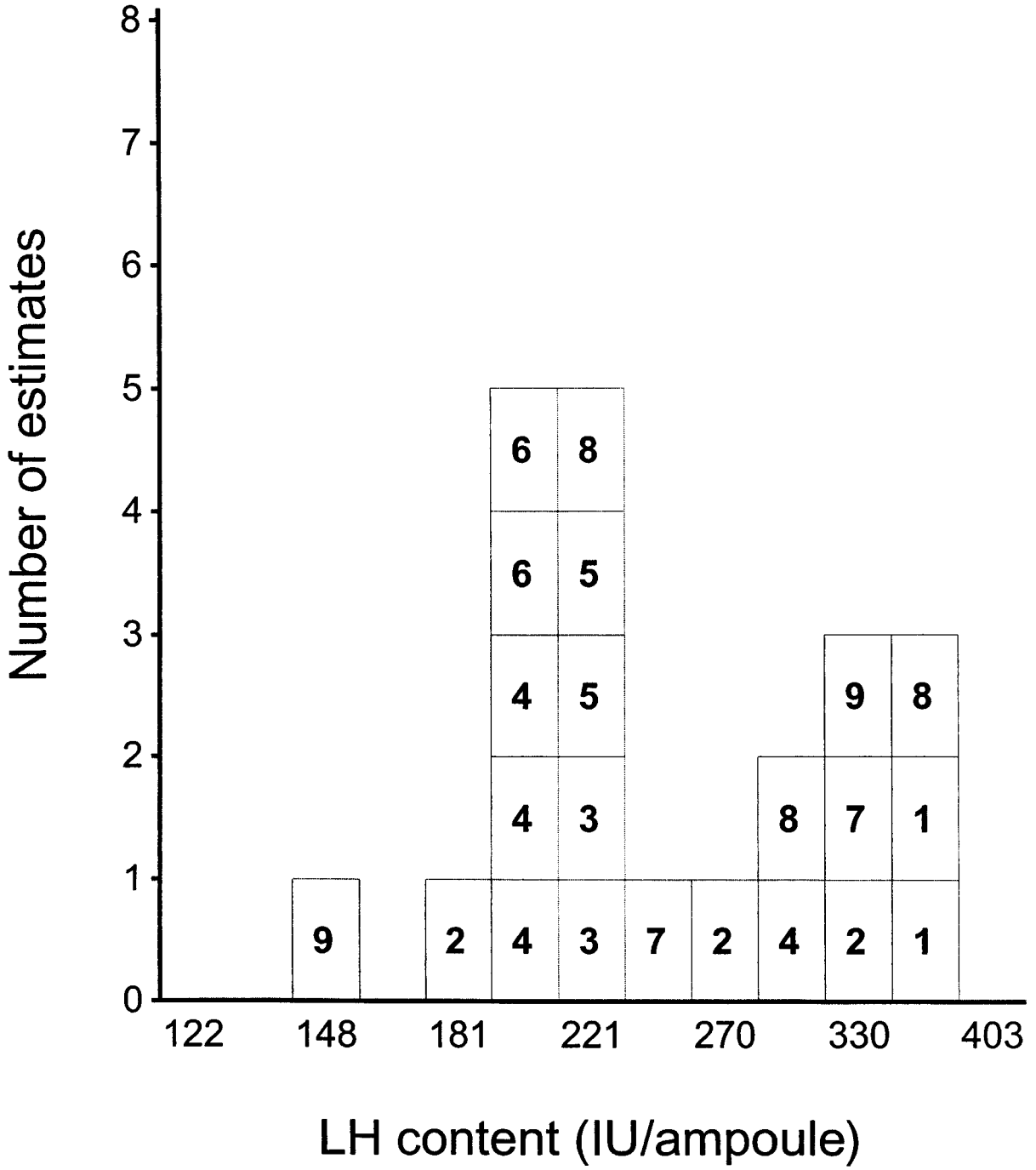
1
2
3
4
5
6
7
8
122
148
181
221
270
330
LH content (IU/ampoule)
1 2 1 3 2 4 6 2
7 9 4 3 4 8 7 2
8 7 4 5 9
5 6
8 7

Figure 2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4
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2
4
3
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2
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2
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3
8
7
1
6
5
4
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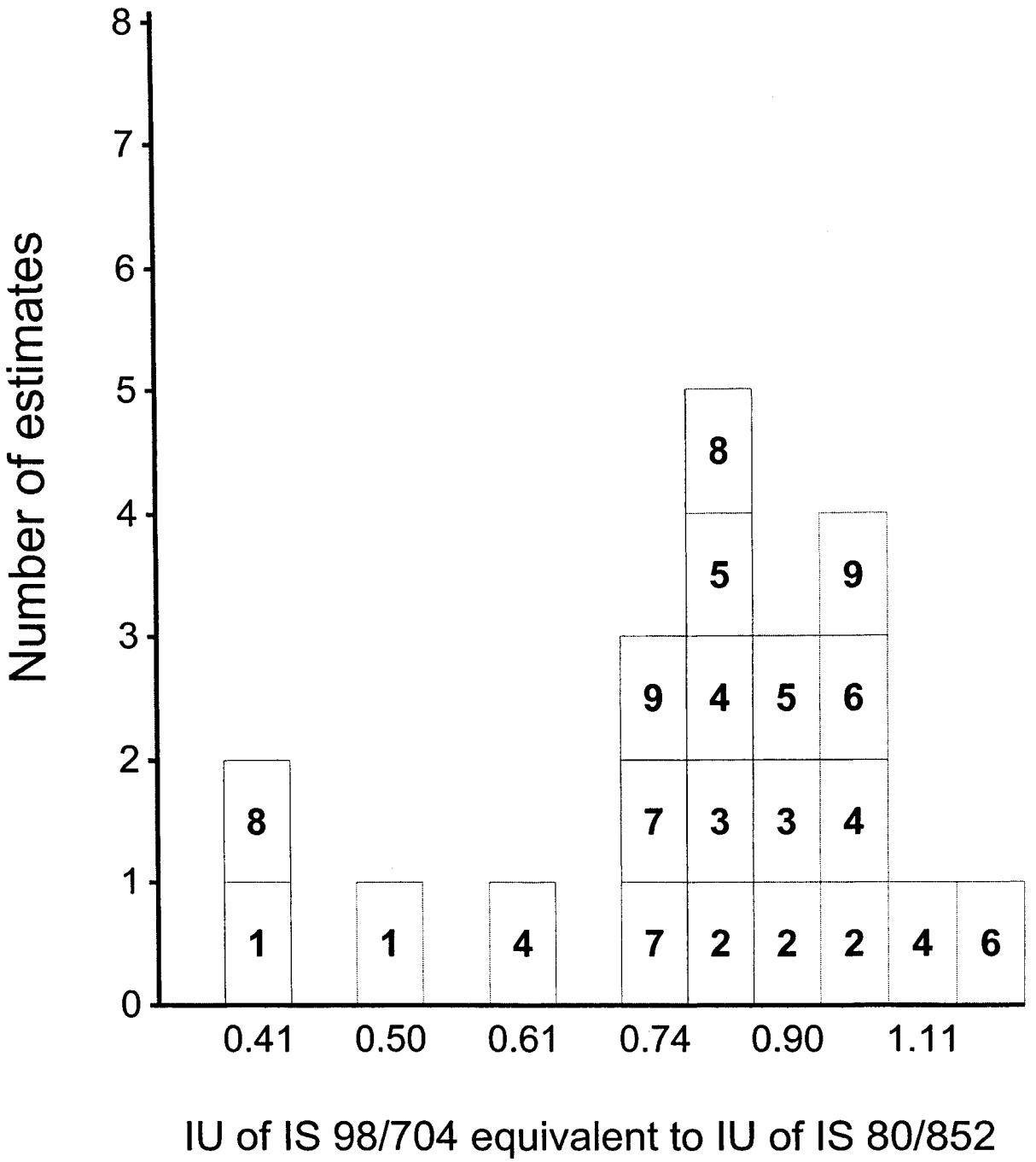
9
8
6
8
122
148
181
221
270
330
403
LH content (IU/ampoule)

Figure 3


Number of estimates
0
1
2
3
4
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1
8
1
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2
4
5
3
8
2
9
6
4
2
4
6
0.41
0.50
0.61
0.74
0.90
1.11
IU of IS 98/704 equivalent to IU of IS 80/852