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**Collaborative study of a candidate WHO reference reagent for Tumour
Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL), human.**

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Summary

A preparation of human sequence recombinant **T**umour Necrosis Factor-**R**elated **A**poptosis-**I**nducing **L**igand (**TRAIL**) synthesized in *E. coli* was formulated and lyophilized at NIBSC prior to evaluation in a collaborative study for its suitability to serve as a reference standard. The preparation was tested by three laboratories using *in vitro* bioassays. The preparation 04/166 was judged sufficiently active and stable to serve as a reference standard. On the basis of the results reported here, it is proposed that the preparation coded 04/166 be established as the WHO reference reagent for human TRAIL, with an assigned unitage of 10,000 international units per ampoule.

Introduction

Tumour Necrosis Factor-**R**elated **A**poptosis-**I**nducing **L**igand (**TRAIL**) is a cell membrane protein of the **T**umour **N**ecrosis **F**actor **S**uper **F**amily (TNFSF) ligands that are mediators of inflammation and immunoregulation (Locksley et al., 2001). TRAIL is thus alternatively named TNFSF10. It occurs naturally as a membrane-bound ligand, but the extracellular domains (ectodomains) may be cleaved to produce a soluble TRAIL molecule consisting of three non-covalently-bound ectodomains (Bodmer et al., 2000). Addition of TRAIL induces programmed cell death or apoptosis in many tumour-derived cell lines *in vitro* but, interestingly, has no apoptotic effect on most non-transformed cells (Almasan and Ashkenazi, 2003). In humans, four members of the TNF Receptor Super Family (TNFRSF) expressed at the cell surface can bind TRAIL: TRAIL-R1 (*TNFRSF10A*), TRAIL-R2 (*TNFRSF10B*), TRAIL-R3 (*TNFRSF10C*) and TRAIL-R4 (*TNFRSF10D*) (Ashkenazi and Dixit, 1999). TRAIL-R1 and -R2 possess an intracellular tail containing a conserved motif known as the “death domain (DD)”. The DD allows interaction with downstream adaptor proteins to initiate apoptotic signals in tumour cells. In contrast, TRAIL-R3 and -R4 do not have DDs or any other signalling motifs and appear to act as “decoy” receptors that either inhibit or modulate TRAIL’s apoptotic activity (Ashkenazi and Dixit, 1999; Ashkenazi, 2002).

Although the ability to induce apoptosis in transformed cells is well established, the role of TRAIL and its receptors in normal mammalian physiology is not understood. TRAIL has been shown to be expressed on the surface of natural killer (NK) cells and T cells, macrophages, dendritic cells and vascular endothelial cells (Kayagaki et al., 1999; Haalaas et al., 2000; Sato et al., 2001; Li et al., 2003; Almasan and Ashkenazi, 2003); however, its function remains unclear.

In a variety of rodent tumour models, including those involving human xenografted tumour cells, human TRAIL can selectively kill the tumour cells without appreciable host toxicity (Walzak et al., 1999; Hylander et al., 2005). Recombinant human TRAIL has been found to be well-tolerated *in vivo* in cynomolgus monkeys and chimpanzees (Ashkenazi et al., 1999; Kelley et al., 2001). Combination of TRAIL with chemotherapeutic drugs has been shown to enhance tumour cell killing and, with the exception of combination with cisplatin, to be non-toxic to normal cells (Shankar et al., 2005; Ganten et al., 2006; Meurette et al., 2006). This wide therapeutic window has raised hopes that TRAIL can be useful in the treatment of human cancers. Recombinant TRAIL

is currently under development as a biotherapeutic agent for cancer treatment (Yagita et al., 2004; Secchiero et al., 2004). In addition, monoclonal antibodies (mAbs) have been developed that bind to TRAIL-R1 or TRAIL-R2 and activate the apoptotic pathway, thus mimicking TRAIL activity but without interference from the two decoy receptors (Pukac et al., 2005; Georgakis et al., 2005; Zeng et al., 2006; Marini et al., 2006; Menoret et al., 2006; Shimada et al., 2007). Agonistic mAbs against TRAIL-R1 or TRAIL-R2 are currently being assessed in clinical trials against a range of tumour types (Marini, 2006; Fanale and Younes, 2007; Tolcher et al., 2007).

A reference standard for TRAIL would facilitate measurement of the potency and stability of therapeutic preparations of TRAIL, its antagonists and, in addition, measurement of TRAIL levels for diagnostic and research purposes. It would also provide a useful calibrant for bioassays designed to monitor/measure the activity of agonistic mAbs to TRAIL-R1 and TRAIL-R2. At its 54th meeting in Geneva in November 2004, the ECBS of the WHO noted that a reference reagent for TRAIL was required. The National Institute for Biological Standards and Control (NIBSC) therefore sought donation of and lyophilized in ampoules, a preparation of TRAIL and after assessment “in-house” organised a collaborative study to evaluate the preparation.

Aims of the study

The purpose of the study was to characterize a candidate reference standard and assign a unitage for TRAIL. To achieve this, the study sought to

1. Assess the suitability of the candidate preparation of human TRAIL to serve as a reference standard for the bioassay of human TRAIL by measuring its
 - a) biological activity in routine, 'in-house' bioassays,
 - b) assessing the relative activity in different bioassays in current use.
2. Compare the candidate preparation with characterised 'in-house' laboratory standards of TRAIL where available.
3. Assess the stability of the candidate preparation by comparing it with ampoules of the same preparation subjected to accelerated thermal degradation.

Participants

The following participants contributed data to the study:-

Participant 1.

Ms Melody Woods, R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN 55413, USA.

Participant 2.

Dr Irena Fonda, National Institute of Chemistry, Hajdrihova 19, SI-1000, Ljubljana, Slovenia.

Participant 3.

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Materials and Methods

A preparation of recombinant human sequence TRAIL (rhTRAIL), synthesized in *E. coli*, was donated to the WHO (see Acknowledgements). The rhTRAIL contains the extracellular domain – Val114-Gly281, 168 amino acids – of the membrane bound TRAIL molecule, and forms biologically active homotrimers. The preparation was supplied as a frozen 5.0 ml buffered salt solution of rhTRAIL at 0.97 mg rhTRAIL per ml. The rhTRAIL was >97% pure as determined by SDS-PAGE and visualized by silver stain. Endotoxin level was <0.1 ng per µg rhTRAIL as determined by the LAL method. A trial fill (02/128; processed on 31.05.02; Table 1) was conducted with this preparation and the biological activity of the lyophilized rhTRAIL preparation compared with the bulk material in a bioassay assessing the cytotoxicity in the KD4 clone 21 human rhabdomyosarcoma cell line. The KD4 clone 21 cell line has been developed at NIBSC for the sensitive measurement of the cytotoxicity of tumour necrosis factor alpha (TNFα) (Meager 1999), but, as it has similar responsiveness to TRAIL, it has also been used in routine cytotoxicity assays for measuring the activity of TRAIL preparations. As the trial lyophilized preparation of rhTRAIL performed appropriately in the bioassay, a definitive fill and lyophilization of rhTRAIL in glass ampoules was carried out at NIBSC (04/166; processed on 23.09.04; Table 1) in accordance with the procedures used for International Biological Standards (WHO Technical Report Series 927, 2005).

Formulation solution without rhTRAIL, final composition as shown in Table 1, was prepared using non pyrogenic, sterile 6-salt phosphate buffered saline, pH 7.0, and de-pyrogenated glassware. It was filtered using sterile non pyrogenic 0.2 µm cellulose acetate filters (Schleicher & Schuell Microscience GmbH, Dassel, Germany). The appropriate amount of the bulk rhTRAIL preparation was added to the formulation solution (250 ml for Trial fill 02/128; 4,000 ml for Definitive fill 04/166) on the day of the fills to a final rhTRAIL concentration of 1.0 µg/ml*.

Table 1. Composition of ampoule contents including excipients

| | | Supplier | Product Code |
|--|----------------------------|--------------|----------------------|
| 1) Fill code 02/128 (Trial fill: 200 ampoules) | | | |
| rhTRAIL | 1.0 µg/ml (Predicted mass) | | |
| HSA (Albutein 20%) | 10.0 mg/ml | Grifols | IAABOD3001 |
| Bovine casein (carbohydrate and fatty acid free) | 3.0 mg/ml | Calbiochem | CAS 21859 |
| 6-salt phosphate buffered saline, pH 7.0 | | NIBSC | |
| 2) Fill code 04/166 (Definitive fill: approximately 3,700 ampoules) | | | |
| rhTRAIL | 1.0 µg/ml (Predicted mass) | | |
| HSA (Zenalb 20%) | 10.0 mg/ml | Bio Products | ABCN6254 ABCN6526 |
| Bovine casein (carbohydrate and fatty acid free) | 1.6 mg/ml | Calbiochem | CAS 21859 |
| 6-salt phosphate buffered saline, pH 7.0 | | NIBSC | |

The HSA preparations were certified free of HIV-1/2 antibody, HepBsAg, HepC antibody and HCV RNA. Bovine casein was certified to be sourced from countries wherein BSE (Bovine Spongiform Encephalopathy) is not known to exist.

*The mass content of bulk rhTRAIL preparation was determined by the manufacturer. Since the protein content of the ampoules cannot be verified by direct measurement of absolute mass, the content of rhTRAIL per ampoule is assumed to be that calculated from the dilution of the bulk rhTRAIL preparation of the protein mass content assigned by the manufacturer, and the volume of formulated solution delivered to the ampoule. This value is given as “predicted µg”.

For each fill, an approximate of 1% of the filled ampoules was weighed. The mean fill weights are shown in Table 2. Each solution was lyophilized, and the ampoules were sealed under dry nitrogen by heat fusion of the glass and stored at -20°C in the dark. Residual moisture of each preparation, measured by the Karl-Fischer method, is shown in Table 2. At the same time, sets of 10 ampoules were assigned to the accelerated thermal degradation study and stored at -150°C, -70°C, -20°C, 4°C, 20°C, 37°C, 45°C and 56°C. The reconstituted contents of 10 ampoules of 04/166 were tested for microbial contamination, but none was detected.

All ampoules, except those assigned to the accelerated thermal degradation study, are stored -20°C in the dark at the NIBSC facility (NIBSC, South Mimms, Herts, EN6 3QG, UK).

Table 2. Fill weights and residual moisture

| Fill code | Fill weight | Moisture |
|-----------|---------------------------|-----------------------|
| 02/128 | n=6 mean 1.0046 cv 0.02% | n =6 0.2684% cv 6.4% |
| 04/166 | n=45 mean 1.0038 cv 0.07% | n =5 1.1470% cv 4.28% |

Design of the Study

Each participant received several ampoules of preparation 04/166. Since this was the only candidate preparation to be evaluated, no coding was applied. Participants were asked to titrate the reconstituted contents of two ampoules of 04/166, following their normal *in vitro* bioassay protocol, in at least two independent bioassays and, if available, to include their in-house rhTRAIL standard in all bioassays. It was requested that responses be measured at several dose levels with replicates for each sample. Thermal degradation samples (TDS) of 02/128 and 04/166 were tested by Laboratory 3 alone. These were retrieved following prolonged storage, 2 yrs 4mths (850 days), at -150°C, -70°C, -20°C, 4°C, 20°C, 37°C, 45°C and 56°C from the fill dates. Following reconstitution, contents of TDS ampoules were titrated in the specified in-house bioassays (Table 3).

Bioassays contributed to the study

TRAIL-induced cytotoxicity is the most common and reliable measured parameter of TRAIL activity. Relative activity of TRAIL preparations is evaluated by incubating mammalian cells *in vitro* with serial dilutions of TRAIL until maximal cell killing is achieved at the highest concentration applied. Viable cells that remain at lower concentrations are measured by stain up-take or release of coloured metabolites of added tetrazolium salts. In most cases, actinomycin D, a metabolic inhibitor, is added with TRAIL to augment cell killing and shorten the incubation period of the assay. A variety of murine and human cell lines have been used for cytotoxicity assays, both for TNF α and TRAIL (Meager, 2003 and 2006). The participants in the study used several distinct versions of the basic cytotoxicity assay as shown in Table 3.

Participant 1's cytotoxicity assay used murine L-929 cells and an in-house rhTRAIL standard, whereas Participant 2 used human KYM-1D4 rhabdomyosarcoma cells (Meager, 1991) but with the WHO 2nd International Standard of TNF α , 88/786, as in-house standard. The latter has qualitatively similar cytotoxic activity to TRAIL and its inclusion thus provides useful comparative data. Participant 3 used three different target human cell lines. The cytotoxicity assay based on human KD4 clone 21 rhabdomyosarcoma cells (Meager, 1999) was used for evaluating the activity of 04/166

candidate reference standard of TRAIL in relation to (i) non-lyophilised pre-fill and residue solution of 04/166 (taken at the beginning and end the filling process) as in-house standards and (ii) 02/128 trial fill preparation of TRAIL, and for measuring the relative activities of the TDS of 04/166. Cytotoxicity assays based on human A375 melanoma and human REH pre-B leukaemia cells were also carried out by Participant 3 to determine relative activities of the TDS ampoules of 04/166.

Table 3. Bioassay systems used in the study

| <i>Participant</i> | <i>Bioassay system</i> | <i>Readout</i> | <i>In-house standard</i> |
|--------------------|--|--|--|
| 1 | rhTRAIL-induced cytotoxicity in murine L-929 cells (with actinomycin D) | Absorbance: Crystal violet stain 540nm | rhTRAIL from <i>E. coli</i> |
| 2 | rhTRAIL-induced cytotoxicity in human KYM-1D4 rhabdomyosarcoma cells (with actinomycin D) | Absorbance: MTT* 570nm | 88/786: WHO 2 nd International Standard of TNF α |
| 3 | rhTRAIL-induced cytotoxicity in human KD4 clone 21 rhabdomyosarcoma cells (with actinomycin D) | Absorbance: MTS** 492nm | 02/128: Trial fill of rhTRAIL 04/166: Ampoule stored at -20°C for TDS study |
| | rhTRAIL-induced cytotoxicity in human A375 melanoma cells (with actinomycin D) | Absorbance: MTS** 492nm | 04/166: Ampoule stored at -20°C for TDS study |
| | rhTRAIL-induced cytotoxicity in human REH pre-B leukemia cells (without actinomycin D) | Absorbance: **MTS 492nm | 04/166: Ampoule stored at -20°C for TDS study |

*MTT: 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide.

**MTS: 3-[4,5-dimethylthiazol-2-yl]-5-[3-carboxymethoxyphenyl]-2-[4-sulphopenyl]-2H-tetrazolium, inner salt.

Statistical analysis

Preliminary graphical analysis was carried out to assess the overall form of the dose – response curves and to identify any markedly anomalous values (Gaines Das and Rice, 1985). Except for a consistent marked non-linearity occurring in the same place on two adjacently numbered plates in one assay by Participant 3, no responses were omitted as outliers. The majority of dose – response curves followed a broadly sigmoid shape and

were satisfactorily described by a four-parameter logistic function. Upper and lower limits were determined for each plate in each assay, and responses on each plate were transformed to proportions of these limits. For each plate in each assay, the proportions were transformed to logits and analyzed as multiple parallel line assays using an in house program (WRANL, Gaines Das and Tydeman, 1982). Potencies have been determined as the displacement of parallel log dose – logit response lines.

Estimates of potencies have been combined as geometric means and comparisons among estimates have been made using the logarithms of the potency estimates (Gaines Das and Tydeman, 1982). The geometric coefficient of variation (GCV) is given as a summary description of variability.

Results

Data contributed. A total of 106 plates from 20 independent assays carried out by three participants were contributed to this study. Details of these are shown in the Appendix.

Dose – response lines. These data do not show significant deviations from similarity between the preparations tested in these assays. The majority of these analyses did not show significant deviations from linearity or parallelism. The apparently statistically significant deviations from the linear parallel line model did not occur consistently for any one participant or assay, were not consistently related to any of the preparations included, and estimates from these assays were similar to estimates obtained from assays in the same laboratory which did not show such deviations. The highly structured designs used for many of these assay formats give responses which tend to be correlated and which do not satisfy the underlying assumptions for the classical statistical analysis. It is suggested that underestimation of the residual variability which is a consequence of this design is a likely cause of many of the apparently significant deviations. For this reason, the estimates obtained from all plates have been included in the analyses reported here.

Estimates of relative potency. Detailed estimates of potency for the various comparisons carried out are shown in the Appendix.

Comparisons of the duplicate ampoules of the candidate 04/166 reference standard with solutions (pre-fill and residue) retained from their preparation (Appendix Table 3b) suggest that there were no gross changes in measured activity during the preparation process.

Comparisons of the candidate 04/166 reference standard with the various preparations used as in-house standards are shown in Table 4.

Comparison of the definitive fill of the candidate 04/166 reference standard with the trial fill 02/128 (used as in-house standard by Participant 3) gave a mean estimate of 1.10 predicted nanogram (ng) / predicted ng, not significantly different from 1.0 (95% limits of 0.91 to 1.32 combined over assay mean estimates for duplicate ampoules). These data indicate that the trial and definitive fills prepared in a similar way, except for total pre-fill volume, retain the same activity level.

The activity of the candidate 04/166 reference standard in terms of the 88/786 TNF α standard used by Participant 2 indicated that 1.0 predicted ng of the candidate standard has activity in this assay system equivalent to 0.04 nominal ng of TNF α . Dose response lines generated by TRAIL and TNF α are parallel when TRAIL and TNF α are compared in this assay system and their respective activities are stably related (Table 4), but the ratio of their activities in other systems is known to vary (Meager, 2006). TNF α would therefore not be a suitable reference standard for TRAIL.

Comparisons of the duplicate ampoules of the candidate 04/166 reference standard indicate that, in general, the assays are consistent and that estimates are not biased based on these comparisons where the relative potency is known to be 1.0.

Table 4. Assay and laboratory means for estimates of relative potency in terms of the various in-house standards for the duplicate ampoules of the candidate 04/166 reference standard, denoted cs1 and cs2, and of duplicates of an in-house preparation (denoted it1 and it2). The assay geometric means of within plate comparisons of the ampoule of 04/166 denoted cs2 in terms of that denoted cs1 are given as csrat, and the ratio of assay means for estimates of cs2 in terms of cs1 are denoted csrm. (csrat and csrm are the same where all comparisons of the duplicate ampoules were within the same plates. However, in the assays of Participant 2, cs1 and cs2 were not included on the same plate in any assay, and only csrm is available.)

| parti | asdate | cs1 | cs2 | it1 | it2 | csrat | csrm |
|--|--------|--------|--------|-------|-------|------------|------------|
| Part1 | 050404 | 7.561 | 8.132 | . | . | 0.930 | 0.930 |
| | 050406 | 12.244 | 12.079 | . | . | 1.014 | 1.014 |
| | 050411 | 24.652 | 25.540 | . | . | 0.965 | 0.965 |
| Geometric mean | | 13.17 | 13.59 | | | 0.97 | 0.97 |
| GCV | | 81% | 79% | | | 4% | 4% |
| Part2 | A06 | 0.060 | 0.051 | 0.876 | 0.752 | . | 1.168 |
| | A07 | 0.052 | 0.039 | 0.969 | 0.912 | . | 1.346 |
| | A09 | 0.027 | 0.026 | 0.820 | 0.848 | . | 1.023 |
| | A10 | 0.029 | 0.027 | 0.805 | 1.030 | . | 1.098 |
| | A11 | 0.035 | 0.028 | 0.811 | 0.901 | . | 1.233 |
| | A13 | 0.040 | 0.026 | 0.936 | 1.020 | . | 1.514 |
| Geometric mean | | 0.039 | 0.032 | 0.867 | 0.905 | | 1.22 |
| GCV | | 38% | 32% | 8% | 13% | | 15% |
| Part3 | KD1310 | 1.326 | . | . | . | . | . |
| | KD4131 | 1.173 | 1.143 | . | . | 1.027 | 1.026 |
| | KD4191 | 0.928 | 0.959 | . | . | 0.881 | 0.968 |
| Geometric Mean | | 1.13 | 1.05 | | | 0.95 | 1.00 |
| GCV | | 20% | 13% | | | 11% | 4% |
| ----- | | | | | | | |
| Overall Geometric Mean of individual estimates | | | | | | 0.96 | 1.10 |
| GCV | | | | | | 7% | 17% |
| 95% limits | | | | | | 0.89, 1.04 | 1.00, 1.22 |

Stability

Assay of ampoules of the candidate 04/166 reference standard exposed to thermally accelerated degradation, and of a similar material (02/128) indicate that the candidate standard stored at the customary temperature is stable, with yearly losses of activity predicted to be less than 0.1% (Appendix tables 3c and 3d). The predicted upper 95% limit for the yearly loss at -20°C is 0.06%, based on the assumptions that the mean estimates and assumed weight of 500 are representative of the data. No significant losses of activity have been found for reconstituted 04/166 following storage at -20°C for at least one year.

Conclusions

The results of this study have shown that the candidate 04/166 reference standard of rhTRAIL, 04/166, retains full activity in a range of cytotoxicity bioassays following lyophilisation in glass ampoules and reconstitution in sterile distilled water. The lyophilisation process was shown to be reproducible since the activities of the reconstituted ampoule contents of the trial fill preparation and the candidate 04/166 reference standard were not significantly different from one another. It was also shown that the activity of the reconstituted ampoule contents of 04/166 was not diminished by the lyophilisation process. Participants in the study showed that the activity of rhTRAIL was stably related to that of rhTRAIL preparations chosen as in-house reference standards, or to that of the WHO IS of TNF α , 88/786. While TRAIL and TNF α are known to manifest similar cytotoxic activity, the ratio of their relative activity can vary depending on the target cells (Meager, 2006). This variation is probably largely accounted for by the differential expression of TRAIL and TNF α receptors among different cell types. A reference standard of TNF α would thus not be suitable for the calibration of assays for determining the potency of TRAIL preparations and *vice versa*.

Titration of TDS ampoules of 04/166 in cytotoxicity assays after 2ys 4mths (850 days) predicted that the activity of 04/166 stored at the recommended storage temperature of -20°C was very stable. The candidate 04/166 reference standard of rhTRAIL was therefore found to be suitable to serve as a reference standard for TRAIL in the calibration of bioassays to determine the potencies of other TRAIL preparations. However, the assays and comparator preparations of TRAIL available for this study are limited and it is therefore proposed that this preparation be established as the WHO reference reagent of TRAIL. Since 04/166 is the first reference standard/ reagent of TRAIL, its unitage may be arbitrarily assigned. On the basis of the results of this study, it is recommended that 04/166 be assigned 10,000 international units per ampoule. Its primary use should be for the calibration of cytotoxicity assays and the establishment of secondary working standards of TRAIL. Its suitability for the calibration of other types of bioassay or immunoassays has not been established.

Comments from participants

None received.

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Appendix

Set of full tables created and used during analysis.

The notation is that used for computation and is generally self-explanatory or otherwise clear from context.

Participants (parti) are coded 1 to 3 except for the degradation study coded Degst. Each assay is uniquely identified by a code denoted 'asdate'. In the case of the degradation study, these codes also indicate the type of assay used.

Preparations are assigned names as follow which reflect their composition and, in the case of the degradation studies, the temperatures or other conditions of storage.

| Study code | Assigned Name | Description |
|---------------------|----------------------|---|
| Collaborative Study | | |
| N/A | ih, it1, it2 | Various in-house standards, as described in Methods |
| I | cs1 | Ampoule of candidate reference reagent 04/166 |
| J | cs2 | Duplicate ampoule of 04/166 |
| K | csr | Residual from fill of 04/166 |
| L | csp | Pre-fill solution from 04/166 |

Degradation study carried out at NIBSC (temperatures °C)

| | | |
|----|------|------------------------------------|
| RS | ih | 02/128 stored continuously at -70 |
| A | ihf | frozen baseline samples of 02/128 |
| B | ih00 | 02/128 stored continuously at -150 |
| C | ih01 | 02/128 stored continuously at -20 |
| D | ih04 | 02/128 stored continuously at 4 |
| E | ih20 | 02/128 stored continuously at 20 |
| F | ih37 | 02/128 stored continuously at 37 |
| G | ih45 | 02/128 stored continuously at 45 |
| H | ih56 | 02/128 stored continuously at 56 |
| RS | cs | 04/166 stored continuously at -20 |
| A | cs00 | 04/166 stored continuously at -150 |
| B | cs01 | 04/166 stored continuously at -70 |
| C | cs04 | 04/166 stored continuously at 4 |
| D | cs20 | 04/166 stored continuously at 20 |
| E | cs37 | 04/166 stored continuously at 37 |
| F | cs45 | 04/166 stored continuously at 45 |
| G | cs56 | 04/166 stored continuously at 56 |
| H | csf | frozen baseline samples of 04/166 |

Table 1. Summary of all assays carried out, and preparations included in the assay.

Table 1a. Assays carried out for degradation study

Table 1b. Assays contributed for collaborative study

Table 2. Slopes of log dose - (logit) response lines.

Table 2a. Slopes of log dose response lines of the candidate reference standard and various in house standards for assays contributed to the collaborative study.

Table 2b. Slopes of log dose - response lines for the candidate reference standard and related residual and pre-filling solutions.

Table 2c. Slopes of log dose response lines of thermally accelerated degradation samples of the in house standard for assays contributed to the collaborative study by Participant 3.

Table 3. Estimates of relative potency determined as the displacement of parallel log dose - response lines for analysis of all samples on an assay plate treated as a multiple parallel line assay.

Table 3a. Estimates of the potency of the candidate reference standard in terms of the various in house standards, and of the duplicate ampoules of the candidate standard in terms of one another.

Table 3b. Estimates of the relative potency of the duplicate ampoules of the candidate reference standard and of the pre-filling and residual solutions used in the ampoule preparation in terms of the sample shown with potency '-1-'.

Table 3c. Estimates of the potency of the thermally accelerated degradation samples of 02/128 and the frozen baseline sample of 02/128 after storage for 2 years and 4 months at the raised temperatures. Potencies are expressed as equivalent ampoules of samples of 02/128 stored continuously at -70.

Table 3d. Estimates of the relative potency of the thermally accelerated degradation samples of the candidate reference reagent 04/166 and the frozen baseline sample of 04/166 after storage for 2 years and 4 months at the raised temperatures. Potencies are expressed as equivalent ampoules of samples of 04/166 stored continuously at -20.

Table 1. Summary of all assays carried out, and preparations included in the assay.

Table 1a. Assays carried out for degradation studies

| Obs | parti | asdate | plate | _NAME_ | COL1 | COL2 | COL3 | COL4 | COL5 |
|-----|-------|--------|-------|--------|------|------|------|------|------|
| 1 | Degst | A3751 | P1 | pname | cs | cs00 | cs01 | | |
| 2 | Degst | A3751 | P2 | pname | cs | cs04 | cs20 | | |
| 3 | Degst | A3751 | P3 | pname | cs | cs37 | cs45 | | |
| 4 | Degst | A3751 | P4 | pname | cs | cs56 | csf | | |
| 5 | Degst | KD41 | P1 | pname | cs | cs00 | cs01 | | |
| 6 | Degst | KD41 | P2 | pname | cs | cs04 | cs20 | | |
| 7 | Degst | KD41 | P3 | pname | cs | cs37 | cs45 | | |
| 8 | Degst | KD41 | P4 | pname | cs | cs56 | csf | | |
| 9 | Degst | KD41 | P5 | pname | cs | cs00 | cs01 | | |
| 10 | Degst | KD41 | P6 | pname | cs | cs04 | cs20 | | |
| 11 | Degst | KD41 | P7 | pname | cs | cs37 | cs45 | | |
| 12 | Degst | KD41 | P8 | pname | cs | cs56 | csf | | |
| 13 | Degst | KD42 | P1 | pname | cs | cs56 | csf | | |
| 14 | Degst | KD42 | P2 | pname | cs | cs37 | cs45 | | |
| 15 | Degst | KD42 | P3 | pname | cs | cs04 | cs20 | | |
| 16 | Degst | KD42 | P4 | pname | cs | cs00 | cs01 | | |
| 17 | Degst | KD42 | P5 | pname | cs | cs56 | csf | | |
| 18 | Degst | KD42 | P6 | pname | cs | cs37 | cs45 | | |
| 19 | Degst | KD42 | P7 | pname | cs | cs04 | cs20 | | |
| 20 | Degst | KD42 | P8 | pname | cs | cs00 | cs01 | | |
| 21 | Degst | KD43 | P1 | pname | cs | cs56 | csf | | |
| 22 | Degst | KD43 | P2 | pname | cs | cs37 | cs45 | | |
| 23 | Degst | KD43 | P3 | pname | cs | cs04 | cs20 | | |
| 24 | Degst | KD43 | P4 | pname | cs | cs00 | cs01 | | |
| 25 | Degst | KD43 | P5 | pname | cs | cs56 | csf | | |
| 26 | Degst | KD43 | P6 | pname | cs | cs00 | cs01 | | |
| 27 | Degst | KD43 | P7 | pname | cs | cs04 | cs20 | | |
| 28 | Degst | KD43 | P8 | pname | cs | cs37 | cs45 | | |
| 29 | Degst | KD43b | P1 | pname | cs | cs56 | csf | | |
| 30 | Degst | KD43b | P2 | pname | cs | cs37 | cs45 | | |
| 31 | Degst | KD43b | P3 | pname | cs | cs04 | cs20 | | |
| 32 | Degst | KD43b | P4 | pname | cs | cs00 | cs01 | | |
| 33 | Degst | KD43b | P5 | pname | cs | cs56 | csf | | |
| 34 | Degst | KD43b | P6 | pname | cs | cs00 | cs01 | | |
| 35 | Degst | KD43b | P7 | pname | cs | cs04 | cs20 | | |
| 36 | Degst | KD43b | P8 | pname | cs | cs37 | cs45 | | |
| 37 | Degst | REH0 | P1 | pname | cs | cs37 | cs45 | csf | |
| 38 | Degst | REH1 | P1 | pname | cs | cs00 | cs01 | cs04 | cs20 |
| 39 | Degst | REH1 | P2 | pname | cs | cs37 | cs45 | cs56 | csf |
| 40 | Degst | REH1 | P3 | pname | cs | cs00 | cs01 | cs04 | cs20 |
| 41 | Degst | REH1 | P4 | pname | cs | cs37 | cs45 | cs56 | csf |
| 42 | Degst | REH2 | P1 | pname | cs | cs00 | cs01 | cs04 | cs20 |
| 43 | Degst | REH2 | P2 | pname | cs | cs37 | cs45 | cs56 | csf |
| 44 | Degst | REH2 | P3 | pname | cs | cs00 | cs01 | | |

Table 1. Summary of all assays carried out, and preparations included in the assay.

Table 1b. Assays contributed for collaborative study

| Obs | parti | asdate | plate | _NAME_ | COL1 | COL2 | COL3 | COL4 | COL5 |
|-----|-------|--------|-------|--------|------|------|------|------|------|
| 45 | Part1 | 050404 | P1 | pname | cs1 | cs2 | ih | | |
| 46 | Part1 | 050404 | P2 | pname | cs1 | cs2 | ih | | |
| 47 | Part1 | 050406 | P1 | pname | cs1 | cs2 | ih | | |
| 48 | Part1 | 050406 | P2 | pname | cs1 | cs2 | ih | | |
| 49 | Part1 | 050411 | P1 | pname | cs1 | cs2 | ih | | |
| 50 | Part1 | 050411 | P2 | pname | cs1 | cs2 | ih | | |
| 51 | Part2 | A06 | P1 | pname | cs1 | ih | it1 | | |
| 52 | Part2 | A06 | P2 | pname | cs1 | ih | it1 | | |
| 53 | Part2 | A06 | P3 | pname | cs1 | ih | it1 | | |
| 54 | Part2 | A06 | P4 | pname | cs2 | ih | it2 | | |
| 55 | Part2 | A06 | P5 | pname | cs2 | ih | it2 | | |
| 56 | Part2 | A06 | P6 | pname | cs2 | ih | it2 | | |
| 57 | Part2 | A07 | P1 | pname | cs1 | ih | it1 | | |
| 58 | Part2 | A07 | P2 | pname | cs1 | ih | it1 | | |
| 59 | Part2 | A07 | P3 | pname | cs1 | ih | it1 | | |
| 60 | Part2 | A07 | P4 | pname | cs2 | ih | it2 | | |
| 61 | Part2 | A07 | P5 | pname | cs2 | ih | it2 | | |
| 62 | Part2 | A07 | P6 | pname | cs2 | ih | it2 | | |
| 63 | Part2 | A09 | P1 | pname | cs1 | ih | it1 | | |
| 64 | Part2 | A09 | P2 | pname | cs1 | ih | it1 | | |
| 65 | Part2 | A09 | P3 | pname | cs1 | ih | it1 | | |
| 66 | Part2 | A09 | P4 | pname | cs2 | ih | it2 | | |
| 67 | Part2 | A09 | P5 | pname | cs2 | ih | it2 | | |
| 68 | Part2 | A09 | P6 | pname | cs2 | ih | it2 | | |
| 69 | Part2 | A10 | P1 | pname | cs1 | ih | it1 | | |
| 70 | Part2 | A10 | P2 | pname | cs1 | ih | it1 | | |
| 71 | Part2 | A10 | P3 | pname | cs1 | ih | it1 | | |
| 72 | Part2 | A10 | P4 | pname | cs2 | ih | it2 | | |
| 73 | Part2 | A10 | P5 | pname | cs2 | ih | it2 | | |
| 74 | Part2 | A10 | P6 | pname | cs2 | ih | it2 | | |
| 75 | Part2 | A11 | P1 | pname | cs1 | ih | it1 | | |
| 76 | Part2 | A11 | P2 | pname | cs1 | ih | it1 | | |
| 77 | Part2 | A11 | P3 | pname | cs1 | ih | it1 | | |
| 78 | Part2 | A11 | P4 | pname | cs2 | ih | it2 | | |
| 79 | Part2 | A11 | P5 | pname | cs2 | ih | it2 | | |
| 80 | Part2 | A11 | P6 | pname | cs2 | ih | it2 | | |
| 81 | Part2 | A13 | P1 | pname | cs1 | ih | it1 | | |
| 82 | Part2 | A13 | P2 | pname | cs1 | ih | it1 | | |
| 83 | Part2 | A13 | P3 | pname | cs1 | ih | it1 | | |
| 84 | Part2 | A13 | P4 | pname | cs2 | ih | it2 | | |
| 85 | Part2 | A13 | P5 | pname | cs2 | ih | it2 | | |
| 86 | Part2 | A13 | P6 | pname | cs2 | ih | it2 | | |

Table 1. Summary of all assays carried out, and preparations included in the assay.

Table 1b (Continued). Assays contributed for collaborative study

| Obs | parti | asdate | plate | _NAME_ | COL1 | COL2 | COL3 | COL4 | COL5 |
|-----|-------|--------|-------|--------|------|------|------|------|------|
| 87 | Part3 | KD1310 | P1 | pname | ih | ih00 | ih01 | ihf | |
| 88 | Part3 | KD1310 | P2 | pname | ih04 | ih20 | ih37 | ih45 | |
| 89 | Part3 | KD1310 | P3 | pname | cs1 | cs2 | csp | ih56 | |
| 90 | Part3 | KD1310 | P4 | pname | cs1 | csr | ih01 | ihf | |
| 91 | Part3 | KD4131 | P1 | pname | ih | ih00 | ih01 | ihf | |
| 92 | Part3 | KD4131 | P2 | pname | ih | ih04 | ih20 | ih37 | |
| 93 | Part3 | KD4131 | P3 | pname | cs1 | ih | ih45 | ih56 | |
| 94 | Part3 | KD4131 | P4 | pname | cs2 | csp | csr | ih | |
| 95 | Part3 | KD4131 | P5 | pname | ih | ih00 | ih01 | ihf | |
| 96 | Part3 | KD4131 | P6 | pname | ih | ih04 | ih20 | ih56 | |
| 97 | Part3 | KD4131 | P7 | pname | csp | csr | ih | ih37 | |
| 98 | Part3 | KD4131 | P8 | pname | cs1 | cs2 | ih | ih45 | |
| 99 | Part3 | KD4191 | P1 | pname | ih | ih00 | ih01 | ihf | |
| 100 | Part3 | KD4191 | P2 | pname | ih | ih04 | ih20 | ih37 | |
| 101 | Part3 | KD4191 | P3 | pname | cs1 | ih | ih45 | ih56 | |
| 102 | Part3 | KD4191 | P4 | pname | cs2 | csp | csr | ih | |
| 103 | Part3 | KD4191 | P5 | pname | ih | ih00 | ih01 | ihf | |
| 104 | Part3 | KD4191 | P6 | pname | ih | ih04 | ih20 | ih56 | |
| 105 | Part3 | KD4191 | P7 | pname | csp | csr | ih | ih37 | |
| 106 | Part3 | KD4191 | P8 | pname | cs1 | cs2 | ih | ih45 | |

Table 2. Slopes of log dose - (logit) response lines.

Table 2a. Slopes of log dose response lines of the candidate reference reagent and various in house standards for assays contributed to the collaborative study.

| parti | asdate | plate | cs1 | cs2 | ih | it1 | it2 |
|-------|--------|-------|-------|-------|-------|-------|-------|
| Part1 | 050404 | P1 | -2.03 | -1.91 | -1.93 | . | . |
| | | P2 | -2.00 | -1.86 | -1.94 | . | . |
| Part1 | 050406 | P1 | -1.92 | -2.29 | -1.96 | . | . |
| | | P2 | -1.89 | -2.23 | -1.85 | . | . |
| Part1 | 050411 | P1 | -1.90 | -1.95 | -1.47 | . | . |
| | | P2 | -1.89 | -1.83 | -1.54 | . | . |
| Part2 | A06 | P1 | -1.78 | . | -1.81 | -2.28 | . |
| | | P2 | -1.54 | . | -2.28 | -2.24 | . |
| | | P3 | -2.63 | . | -2.11 | -1.90 | . |
| | | P4 | . | -1.98 | -1.92 | . | -1.53 |
| | | P5 | . | -1.69 | -2.12 | . | -1.78 |
| | | P6 | . | -2.63 | -2.23 | . | -2.04 |
| Part2 | A07 | P1 | -2.32 | . | -1.76 | -1.59 | . |
| | | P2 | -1.83 | . | -1.76 | -1.95 | . |
| | | P3 | -2.27 | . | -1.56 | -1.80 | . |
| | | P4 | . | -3.40 | -1.94 | . | -1.66 |
| | | P5 | . | -2.88 | -2.00 | . | -2.07 |
| | | P6 | . | -2.01 | -1.61 | . | -1.80 |
| Part2 | A09 | P1 | -2.68 | . | -2.30 | -3.38 | . |
| | | P2 | -2.48 | . | -2.38 | -2.68 | . |
| | | P3 | -3.07 | . | -2.47 | -2.53 | . |
| | | P4 | . | -3.17 | -2.66 | . | -2.89 |
| | | P5 | . | -2.59 | -1.81 | . | -1.30 |
| | | P6 | . | -3.22 | -2.77 | . | -2.01 |
| Part2 | A10 | P1 | -3.05 | . | -3.93 | -2.88 | . |
| | | P2 | -2.57 | . | -2.88 | -4.15 | . |
| | | P3 | -2.81 | . | -2.85 | -4.64 | . |
| | | P4 | . | -1.78 | -1.51 | . | -1.37 |
| | | P5 | . | -2.54 | -3.32 | . | -3.76 |
| | | P6 | . | -2.52 | -6.17 | . | -2.10 |
| Part2 | A11 | P1 | -2.09 | . | -2.06 | -2.12 | . |
| | | P2 | -1.86 | . | -2.06 | -2.18 | . |
| | | P3 | -1.66 | . | -2.04 | -1.80 | . |
| | | P4 | . | -2.18 | -2.08 | . | -1.97 |
| | | P5 | . | -1.83 | -2.48 | . | -2.16 |
| | | P6 | . | -1.78 | -2.12 | . | -1.87 |
| Part2 | A13 | P1 | -2.18 | . | -2.22 | -1.72 | . |
| | | P2 | -1.79 | . | -1.52 | -1.64 | . |
| | | P3 | -2.24 | . | -2.20 | -1.83 | . |
| | | P4 | . | -1.76 | -1.57 | . | -1.60 |
| | | P5 | . | -1.66 | -1.15 | . | -1.30 |
| | | P6 | . | -1.61 | -1.21 | . | -1.27 |

Table 2. Slopes of log dose - (logit) response lines.

Table 2a (Continued). Slopes of log dose response lines of the candidate reference standard and various in house standards for assays contributed to the collaborative study.

| parti | asdate | plate | cs1 | cs2 | ih | it1 | it2 |
|-------|--------|-------|-------|-------|-------|-----|-----|
| Part3 | KD1310 | P3 | -2.29 | -2.36 | . | . | . |
| | | P4 | -2.48 | . | . | . | . |
| Part3 | KD4131 | P3 | -2.15 | . | -2.34 | . | . |
| | | P4 | . | -2.35 | -2.88 | . | . |
| | | P8 | -2.19 | -1.97 | -1.71 | . | . |
| Part3 | KD4191 | P3 | -2.66 | . | -2.41 | . | . |
| | | P4 | . | -3.07 | -2.76 | . | . |
| | | P8 | -3.01 | -3.35 | -3.13 | . | . |

Table 2b. Slopes of log dose - response lines for the candidate reference standard and related residual and pre-filling solutions.

| parti | asdate | plate | cs1 | cs2 | csp | csr |
|-------|--------|-------|-------|-------|-------|-------|
| Part3 | KD1310 | P3 | -2.29 | -2.36 | -2.42 | . |
| | | P4 | -2.48 | . | . | -2.43 |
| Part3 | KD4131 | P3 | -2.15 | . | . | . |
| | | P4 | . | -2.35 | -2.69 | -2.49 |
| | | P7 | . | . | -1.87 | -1.97 |
| | | P8 | -2.19 | -1.97 | . | . |
| Part3 | KD4191 | P3 | -2.66 | . | . | . |
| | | P4 | . | -3.07 | -2.96 | -2.65 |
| | | P7 | . | . | -2.88 | -2.94 |
| | | P8 | -3.01 | -3.35 | . | . |

Table 2. Slopes of log dose - (logit) response lines.

Table 2c. Slopes of log dose response lines of thermally accelerated degradation samples of the in house standard for assays contributed to the collaborative study by Participant 3.

| parti | asdate | plate | ih | ih00 | ih01 | ih04 | ih20 | ih37 | ih45 | ih56 | ihf |
|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Part3 | KD1310 | P1 | -2.55 | -2.50 | -2.44 | . | . | . | . | . | -2.16 |
| | | P2 | . | . | . | -2.40 | -2.55 | -2.54 | -2.31 | . | . |
| | | P4 | . | . | -2.72 | . | . | . | . | . | -2.70 |
| Part3 | KD4131 | P1 | -2.04 | -2.39 | -2.41 | . | . | . | . | . | -2.31 |
| | | P2 | -2.73 | . | . | -2.26 | -2.57 | -2.21 | . | . | . |
| | | P3 | -2.34 | . | . | . | . | . | -2.18 | -2.40 | . |
| | | P4 | -2.88 | . | . | . | . | . | . | . | . |
| | | P5 | -1.88 | -2.14 | -1.74 | . | . | . | . | . | -1.97 |
| | | P6 | -1.63 | . | . | -1.41 | -2.01 | . | . | -1.89 | . |
| | | P7 | -1.62 | . | . | . | . | -1.78 | . | . | . |
| | | P8 | -1.71 | . | . | . | . | . | -1.74 | . | . |
| Part3 | KD4191 | P1 | -2.51 | -2.80 | -2.03 | . | . | . | . | . | -2.63 |
| | | P2 | -2.12 | . | . | -2.12 | -2.59 | -2.50 | . | . | . |
| | | P3 | -2.41 | . | . | . | . | . | -2.31 | -2.45 | . |
| | | P4 | -2.76 | . | . | . | . | . | . | . | . |
| | | P5 | -2.36 | -2.72 | -2.49 | . | . | . | . | . | -2.51 |
| | | P6 | -2.23 | . | . | -2.93 | -2.38 | . | . | -2.29 | . |
| | | P7 | -2.82 | . | . | . | . | -2.97 | . | . | . |
| | | P8 | -3.13 | . | . | . | . | . | -2.77 | . | . |

Table 2. Slopes of log dose - (logit) response lines.

Table 2d. Slopes of log dose response lines of thermally accelerated degradation samples of the candidate.

| part | asdate | plate | cs | cs00 | cs01 | cs04 | cs20 | cs37 | cs45 | cs56 | csf |
|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Degst | A3751 | P1 | -0.96 | -1.20 | -0.99 | . | . | . | . | . | . |
| | | P2 | -1.54 | . | . | -1.39 | -1.74 | . | . | . | . |
| | | P3 | -1.49 | . | . | . | . | -1.17 | -1.30 | . | . |
| | | P4 | -1.20 | . | . | . | . | . | . | -0.95 | -1.51 |
| Degst | KD41 | P1 | -1.67 | -1.43 | -1.13 | . | . | . | . | . | . |
| | | P2 | -2.34 | . | . | -2.48 | -1.32 | . | . | . | . |
| | | P3 | -1.91 | . | . | . | . | -1.24 | -1.34 | . | . |
| | | P4 | -2.57 | . | . | . | . | . | . | -3.27 | -2.91 |
| | | P5 | -3.44 | -2.50 | -3.50 | . | . | . | . | . | . |
| | | P6 | -2.43 | . | . | -1.58 | -1.71 | . | . | . | . |
| | | P7 | -2.41 | . | . | . | . | -3.19 | -2.11 | . | . |
| | | P8 | -3.56 | . | . | . | . | . | . | -3.10 | -3.23 |
| Degst | KD42 | P1 | -2.58 | . | . | . | . | . | . | -7.60 | -3.51 |
| | | P2 | -1.95 | . | . | . | . | -1.52 | -2.59 | . | . |
| | | P3 | -1.72 | . | . | -1.70 | -2.09 | . | . | . | . |
| | | P4 | -2.76 | -2.47 | -2.85 | . | . | . | . | . | . |
| | | P5 | -3.77 | . | . | . | . | . | . | -2.82 | -3.88 |
| | | P6 | -2.46 | . | . | . | . | -2.55 | -2.27 | . | . |
| | | P7 | -2.33 | . | . | -2.78 | -2.24 | . | . | . | . |
| | | P8 | -1.26 | -1.21 | -1.99 | . | . | . | . | . | . |
| Degst | KD43 | P1 | -1.91 | . | . | . | . | . | . | -2.33 | -1.62 |
| | | P2 | -2.08 | . | . | . | . | -2.09 | -2.17 | . | . |
| | | P3 | -2.69 | . | . | -2.55 | -2.52 | . | . | . | . |
| | | P4 | -2.10 | -2.89 | -2.88 | . | . | . | . | . | . |
| | | P5 | -2.74 | . | . | . | . | . | . | -3.36 | -3.53 |
| | | P6 | -3.40 | -3.56 | -3.60 | . | . | . | . | . | . |
| | | P7 | -2.74 | . | . | -2.66 | -3.39 | . | . | . | . |
| | | P8 | -2.67 | . | . | . | . | -3.49 | -3.07 | . | . |
| Degst | KD43b | P1 | -2.06 | . | . | . | . | . | . | -1.61 | -2.16 |
| | | P2 | -1.43 | . | . | . | . | -0.92 | -1.06 | . | . |
| | | P3 | -1.39 | . | . | -1.31 | -1.69 | . | . | . | . |
| | | P4 | -1.20 | -1.10 | -1.32 | . | . | . | . | . | . |
| | | P5 | -1.33 | . | . | . | . | . | . | -1.15 | -1.25 |
| | | P6 | -1.72 | -1.71 | -1.68 | . | . | . | . | . | . |
| | | P7 | -1.72 | . | . | -1.89 | -2.35 | . | . | . | . |
| | | P8 | -1.46 | . | . | . | . | -1.51 | -1.24 | . | . |
| Degst | REH0 | P1 | -2.24 | . | . | . | . | -2.46 | -1.95 | . | -2.16 |
| Degst | REH1 | P1 | -2.72 | -2.54 | -2.56 | -2.59 | -2.54 | . | . | . | . |
| | | P2 | -2.51 | . | . | . | . | -2.72 | -2.69 | -3.33 | -2.48 |
| | | P3 | -2.43 | -2.62 | -2.57 | -2.56 | -2.55 | . | . | . | . |
| | | P4 | -2.48 | . | . | . | . | -2.72 | -2.81 | -2.25 | -2.53 |
| Degst | REH2 | P1 | -2.98 | -2.60 | -2.82 | -2.92 | -2.45 | . | . | . | . |
| | | P2 | -1.90 | . | . | . | . | -2.01 | -2.09 | -2.12 | -2.32 |
| | | P3 | -2.37 | -2.17 | -2.31 | . | . | . | . | . | . |

Table 3a. Estimates of the potency of the candidate standard in terms of the various in house standards, and of the duplicate ampoules of the candidate standard in terms of one another.

| parti | asdate | plate | cs1 | cs2 | it1 | it2 | csrat (cs1/cs2) |
|-------|--------|-------|----------------|--------|-------|-------|--------------------|
| | | | Relative to ih | | | | |
| Part1 | 050404 | P1 | 7.483 | 7.797 | . | . | 0.960 |
| | | P2 | 7.639 | 8.482 | . | . | 0.901 |
| Part1 | 050406 | P1 | 13.063 | 11.370 | . | . | 1.149 |
| | | P2 | 11.475 | 12.833 | . | . | 0.894 |
| Part1 | 050411 | P1 | 22.556 | 24.352 | . | . | 0.926 |
| | | P2 | 26.943 | 26.786 | . | . | 1.006 |
| Part2 | A06 | P1 | 0.044 | . | 0.758 | . | . |
| | | P2 | 0.076 | . | 0.972 | . | . |
| | | P3 | 0.065 | . | 0.912 | . | . |
| | | P4 | . | 0.041 | . | 0.799 | . |
| | | P5 | . | 0.068 | . | 0.794 | . |
| | | P6 | . | 0.049 | . | 0.670 | . |
| Part2 | A07 | P1 | 0.049 | . | 0.863 | . | . |
| | | P2 | 0.056 | . | 1.098 | . | . |
| | | P3 | 0.051 | . | 0.960 | . | . |
| | | P4 | . | 0.036 | . | 0.854 | . |
| | | P5 | . | 0.038 | . | 1.058 | . |
| | | P6 | . | 0.043 | . | 0.840 | . |
| Part2 | A09 | P1 | 0.030 | . | 0.996 | . | . |
| | | P2 | 0.025 | . | 0.745 | . | . |
| | | P3 | 0.025 | . | 0.744 | . | . |
| | | P4 | . | 0.021 | . | 0.750 | . |
| | | P5 | . | 0.027 | . | 0.942 | . |
| | | P6 | . | 0.031 | . | 0.861 | . |
| Part2 | A10 | P1 | 0.026 | . | 0.792 | . | . |
| | | P2 | 0.031 | . | 0.732 | . | . |
| | | P3 | 0.031 | . | 0.902 | . | . |
| | | P4 | . | 0.021 | . | 1.002 | . |
| | | P5 | . | 0.031 | . | 0.954 | . |
| | | P6 | . | 0.029 | . | 1.142 | . |
| Part2 | A11 | P1 | 0.035 | . | 0.849 | . | . |
| | | P2 | 0.033 | . | 0.778 | . | . |
| | | P3 | 0.037 | . | 0.807 | . | . |
| | | P4 | . | 0.026 | . | 0.846 | . |
| | | P5 | . | 0.029 | . | 0.906 | . |
| | | P6 | . | 0.031 | . | 0.954 | . |
| Part2 | A13 | P1 | 0.047 | . | 0.857 | . | . |
| | | P2 | 0.042 | . | 1.180 | . | . |
| | | P3 | 0.032 | . | 0.811 | . | . |
| | | P4 | . | 0.025 | . | 0.938 | . |
| | | P5 | . | 0.026 | . | 1.023 | . |
| | | P6 | . | 0.028 | . | 1.105 | . |

Table 3a (Continued). Estimates of the potency of the candidate reference standard in terms of the various in house standards, and of the duplicate ampoules of the candidate reference standard in terms of one another.

| parti | asdate | plate | cs1 | cs2 | it1 | it2 | csrat (cs1/cs2) |
|-------|--------|-------|----------------|-------|-----|-----|--------------------|
| | | | Relative to ih | | | | |
| Part3 | KD1310 | P3 | . | . | . | . | 0.852* |
| | | P4 | 1.326 | . | . | . | . |
| Part3 | KD4131 | P3 | 1.115 | . | . | . | . |
| | | P4 | . | 1.088 | . | . | . |
| | | P8 | 1.233 | 1.201 | . | . | 1.027 |
| Part3 | KD4191 | P3 | 1.102 | . | . | . | . |
| | | P4 | . | 1.037 | . | . | . |
| | | P8 | 0.781 | 0.887 | . | . | 0.881 |

*Although no ih on P3 both cs1 and cs2 were (see Table 1b), and the ratio of their potencies is as given.

Table 3b. Estimates of the relative potency of the duplicate ampoules of the candidate reference standard and of the pre-filling and residual solutions used in the ampoule preparation in terms of the sample shown with potency '-1-'.

| parti | asdate | plate | cs1 | cs2 | csp | csr |
|-------|--------|-------|-----|------|------|------|
| Part3 | KD1310 | P3 | -1- | 1.17 | 1.18 | . |
| | | P4 | -1- | . | . | 1.08 |
| Part3 | KD4131 | P3 | -1- | . | . | . |
| | | P4 | . | -1- | 0.70 | 0.96 |
| | | P7 | . | . | -1- | 1.12 |
| | | P8 | -1- | 0.97 | . | . |
| Part3 | KD4191 | P3 | -1- | . | . | . |
| | | P4 | . | -1- | 0.99 | 1.15 |
| | | P7 | . | . | -1- | 1.04 |
| | | P8 | -1- | 1.14 | . | . |

Comment: Although means have not been determined, estimates for csp and csr do not differ significantly from 1.

Table 3c. Estimates of the potency of the thermally accelerated degradation samples of 02/128 and the frozen baseline sample of 02/128 after storage for 2 years and 4 months at the raised temperatures. Potencies are expressed as equivalent ampoules of samples of 02/128 stored continuously at -70.

| parti | asdate | plate | ih00 | ih01 | ih04 | ih20 | ih37 | ih45 | ih56 | ihf |
|-----------------|--------|-------|------|------|------|------|------|------|------|------|
| Part3 | KD1310 | P1 | 0.98 | 0.85 | . | . | . | . | . | 1.04 |
| | | P2*i | . | . | 1.00 | 0.98 | 0.80 | 0.64 | . | . |
| | | P4*c | . | 0.75 | . | . | . | . | . | 0.96 |
| Part3 | KD4131 | P1 | 0.94 | 0.80 | . | . | . | . | . | 0.97 |
| | | P2 | . | . | 1.08 | 0.80 | 0.65 | . | . | . |
| | | P3 | . | . | . | . | . | 0.94 | 0.05 | . |
| | | P4 | . | . | . | . | . | . | . | . |
| | | P5 | 0.94 | 1.15 | . | . | . | . | . | 1.15 |
| | | P6 | . | . | 1.23 | 0.80 | . | . | 0.28 | . |
| | | P7 | . | . | . | . | 0.71 | . | . | . |
| | | P8 | . | . | . | . | . | 0.66 | . | . |
| Part3 | KD4191 | P1 | 0.64 | 0.82 | . | . | . | . | . | 1.88 |
| | | P2 | . | . | 0.99 | 0.68 | 0.74 | . | . | . |
| | | P3 | . | . | . | . | . | 0.40 | 0.24 | . |
| | | P4 | . | . | . | . | . | . | . | . |
| | | P5 | 0.76 | 0.94 | . | . | . | . | . | 1.68 |
| | | P6 | . | . | 0.77 | 0.81 | . | . | 0.31 | . |
| | | P7 | . | . | . | . | 0.53 | . | . | . |
| | | P8 | . | . | . | . | . | 0.35 | . | . |
| Geometric mean* | | | 0.84 | 0.90 | 1.00 | 0.77 | 0.65 | 0.54 | 0.18 | 1.30 |
| Number, n | | | 5 | 5 | 4 | 4 | 4 | 4 | 4 | 5 |
| GCV | | | 20 | 16 | 22 | 8 | 16 | 58 | 133 | 35 |
| Lower 95% limit | | | 0.67 | 0.75 | 0.73 | 0.68 | 0.52 | 0.26 | 0.05 | 0.90 |
| Upper 95% limit | | | 1.06 | 1.09 | 1.38 | 0.88 | 0.82 | 1.13 | 0.70 | 1.88 |

*In all cases, the between assay variation is not significantly larger than the within assay variation ($p > 0.05$), and all individual estimates have therefore been combined, with the GCV and 95% limits based on the variance of the individual estimates combined.

Using combined estimates for temperatures +20 to +56 with constant weight of 500 (typical for such assays implying 95% limits from ~80-125% of estimate), storage for 850 days gives yearly predicted losses of activity of 0.05%, 0.8%, 3.7% and 15% for -20, 4, 20, 37 respectively.

Table 3d. Estimates of the relative potency of the thermally accelerated degradation samples of the candidate standard 04/166 and the frozen baseline sample of 04/166 after storage for 2 years and 4 months at the raised temperatures. Potencies are expressed as equivalent ampoules of samples of 04/166 stored continuously at -20.

| parti | asdate | plate | cs00 | cs01 | cs04 | cs20 | cs37 | cs45 | cs56 | csf |
|-------------|--------|-------|------|------|------|------|------|------|------|------|
| Degst A3751 | P1 | | 0.76 | 0.66 | . | . | . | . | . | . |
| | P2 | | . | . | 1.09 | 1.01 | . | . | . | . |
| | P3 | | . | . | . | . | 0.59 | 0.49 | . | . |
| | P4 | | . | . | . | . | . | . | 0.06 | 0.79 |
| Degst KD41 | P1 | | 1.50 | 2.19 | . | . | . | . | . | . |
| | P2 | | . | . | 0.87 | 1.66 | . | . | . | . |
| | P3 | | . | . | . | . | 0.44 | 0.27 | . | . |
| | P4 | | . | . | . | . | . | . | 0.14 | 1.13 |
| | P5 | | 1.15 | 1.08 | . | . | . | . | . | . |
| | P6 | | . | . | 1.45 | 1.01 | . | . | . | . |
| | P7 | | . | . | . | . | 0.67 | 0.68 | . | . |
| | P8 | | . | . | . | . | . | . | 0.20 | 1.29 |
| Degst KD42 | P1 | | . | . | . | . | . | . | 0.40 | 0.94 |
| | P2 | | . | . | . | . | 0.92 | 0.45 | . | . |
| | P3 | | . | . | 1.23 | 0.87 | . | . | . | . |
| | P4 | | 1.07 | 1.03 | . | . | . | . | . | . |
| | P5 | | . | . | . | . | . | . | 0.19 | 1.12 |
| | P6 | | . | . | . | . | 0.66 | 0.64 | . | . |
| | P7 | | . | . | 0.77 | 0.73 | . | . | . | . |
| | P8 | | 0.98 | 0.57 | . | . | . | . | . | . |
| Degst KD43 | P1 | | . | . | . | . | . | . | 0.10 | 1.47 |
| | P2 | | . | . | . | . | 0.58 | 0.36 | . | . |
| | P3 | | . | . | 0.97 | 0.80 | . | . | . | . |
| | P4 | | 0.91 | 0.91 | . | . | . | . | . | . |
| | P5 | | . | . | . | . | . | . | 0.09 | 1.03 |
| | P6 | | 1.00 | 0.97 | . | . | . | . | . | . |
| | P7 | | . | . | 1.30 | 1.01 | . | . | . | . |
| | P8 | | . | . | . | . | 0.83 | 0.70 | . | . |
| Degst KD43b | P1 | | . | . | . | . | . | . | 0.14 | 1.03 |
| | P2 | | . | . | . | . | 0.67 | 0.46 | . | . |
| | P3 | | . | . | 0.88 | 0.69 | . | . | . | . |
| | P4 | | 1.03 | 0.86 | . | . | . | . | . | . |
| | P5 | | . | . | . | . | . | . | 0.11 | 1.15 |
| | P6 | | 0.95 | 0.92 | . | . | . | . | . | . |
| | P7 | | . | . | 1.10 | 0.86 | . | . | . | . |
| | P8 | | . | . | . | . | 0.66 | 0.63 | . | . |
| Degst REH0 | P1 | | . | . | . | . | 0.73 | 0.65 | . | 0.97 |
| Degst REH1 | P1 | | 0.99 | 1.08 | 1.19 | 1.10 | . | . | . | . |
| | P2 | | . | . | . | . | 0.74 | 0.66 | 0.34 | 1.03 |
| | P3 | | 0.96 | 0.95 | 0.95 | 0.91 | . | . | . | . |
| | P4 | | . | . | . | . | 0.76 | 0.69 | 0.41 | 1.15 |
| Degst REH2 | P1 | | 0.93 | 0.92 | 0.92 | 0.80 | . | . | . | . |
| | P2 | | . | . | . | . | 0.75 | 0.62 | 0.27 | 0.92 |
| | P3 | | 1.03 | 0.98 | . | . | . | . | . | . |

Table 3d (continued). Estimates of the relative potency of the thermally accelerated degradation samples of the candidate standard 04/166 and the frozen baseline sample of 04/166 after storage for 2 years and 4 months at the raised temperatures. Potencies are expressed as equivalent ampoules of samples of 04/166 stored continuously at -20.

| part | asdate | plate | cs00 | cs01 | cs04 | cs20 | cs37 | cs45 | cs56 | csf |
|-----------------|--------|-------|------|------|------|------|------|------|-------|------|
| Geometric Mean | | | 1.01 | 0.96 | 1.04 | 0.93 | 0.68 | 0.54 | 0.17§ | 1.07 |
| Number, n | | | 13 | 13 | 12 | 12 | 13 | 13 | 12 | 13 |
| GCV | | | 17 | 36 | 21 | 26 | 20 | 34 | 85 | 17 |
| Lower 95% limit | | | 0.92 | 0.80 | 0.92 | 0.80 | 0.61 | 0.45 | 0.12 | 0.97 |
| Upper 95% limit | | | 1.11 | 1.16 | 1.17 | 1.07 | 0.76 | 0.65 | 0.26 | 1.17 |

§ Estimates show significant differences between different assays ($p \sim 0.02$). Data suggest that the REH assays detect less degradation than the A or KD assays for this sample, although this difference is only marginally significant ($p \sim 0.1$) given the inter-assay variability for the KD assays.

For all except cs56, between assay variability is not significantly larger than within assay variability ($p > 0.05$).

Using combined estimates for temperatures +4 to +56 with constant weight of 500, storage for 850 days gives yearly predicted losses of activity of 0.02%, 0.4%, 2.5% and 13% for -20, 4, 20, 37 respectively. The predicted upper 95% limit for the yearly loss at -20 is 0.06% based on the assumptions that the mean estimates and assumed weight of 500 are representative of the data.

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