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**Collaborative Study for the Establishment of the Third International
Standard for Dihydrostreptomycin**

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Summary

An international collaborative study was organised to establish the third World Health Organization (WHO) International Standard (IS) for dihydrostreptomycin. The report presents this study in which 11 laboratories from different countries participated. Potencies of the candidate material were estimated by microbiological assays with sensitive micro-organisms. To ensure continuity between consecutive batches, the second IS for dihydrostreptomycin was used as standard.

This report provides details about the material donated by a manufacturer, the processing involved to establish a candidate batch and the analytical controls to assess its quality. It describes the statistical analysis of the results, the conclusions made thereof and the recommendation to the WHO Expert Committee for Biological Standardization (ECBS).

It is proposed that the *third WHO International Standard for dihydrostreptomycin* (EDQM internal code ISA_42688) be assigned an antimicrobiological activity of 19 425 IU per vial.

Introduction

Dihydrostreptomycin is an antibiotic consisting of a hydrogenated form of streptomycin. It is used in the prophylaxis of tuberculosis and tularemia and the treatment of infections caused by Gram-negative bacteria. It prevents the initiation of protein synthesis by binding to the 30S subunit of the bacterial ribosome and thus leads to death of microbial cells. Humans and mammals have structurally different ribosomes from bacteria, thus explaining the selectivity of this antibiotic for bacteria.

The second IS for dihydrostreptomycin was established by the WHO in 1964 on the basis of an international collaborative study [1]. It was assigned with a potency of 820 International Units per mg (IU/mg), each ampoule containing approximately 200 mg (potency 164 000 IU per vial).

As stocks of the second IS for dihydrostreptomycin were becoming exhausted, the European Directorate for the Quality of Medicines & HealthCare (EDQM), the WHO custodian laboratory for antibiotics, was requested by the ECBS to undertake appropriate steps for its replacement by the establishment of a new batch.

Bulk material, processing and stability

Candidate bulk material was kindly donated by Leshan Longmarch CO. Ltd, Sichuan, China. Eight bottles each containing 250 g of dihydrostreptomycin sulfate of a pharmaceutical grade appropriate for therapeutic use was received by the EDQM in May 2006. Upon receipt, the bulk material was stored at -20°C before processing. The candidate material was claimed by the manufacturer to comply with the quality standards of the European Pharmacopoeia (Ph. Eur.) monograph “Dihydrostreptomycin Sulfate for Veterinary Use, 0485”. A certificate of analysis was provided in the batch documentation.

Production of the third WHO IS for Dihydrostreptomycin candidate batch

Due to the potential hygroscopic character of the dihydrostreptomycin powder, it was decided to distribute the standard as a freeze-dried preparation rather than a powder fill as was the case with the previous IS. The parameters of the freeze-drying process were derived from information obtained from a company manufacturing freeze-dried dosage forms.

All powder weighing was performed in a glove box under controlled atmosphere by use of argon gas. Several vials containing precisely weighed amounts were prepared concomitantly to enable further testing of the bulk powder and to prepare the solution to be freeze dried.

The bottle containing dihydrostreptomycin bulk was allowed to equilibrate at room temperature and subsequently submitted to homogenisation in a Turbula mixer. Formulation: 92.55 g of dihydrostreptomycin bulk were dissolved in 3 550.3 g of purified water and stirred until complete dissolution. The final concentration of the solution was 26.07 mg dihydrostreptomycin bulk per g of solution.

Filling: The solution was filled into 9.0 ml amber glass vials by using automated equipment. The theoretical filling weight was 1.0 g.

Control of filling weight: 36 vials were randomly sampled across the lot. Results were as follows: mean filling weight: 1.0064 g; RSD: 0.05 %. The filling was considered homogeneous.

Lyophilisation: The vials were placed onto 12 trays and underwent lyophilisation. At the end of the process, vacuum was broken by filling of the chamber with nitrogen. Vials were closed with teflon coated rubber stopper and subsequently sealed by an aluminium cap. The final product was registered under the batch number Fab 10/11-15. A total of 3 411 vials were produced.

Selection of a batch suitable as “reference standard” for monitoring purposes

WHO IS are primary reference materials and as such they cannot be tested against higher order reference standards. As a consequence, real time stability studies are not usual practice and in many cases, stability of WHO IS is assessed by means of accelerated degradation studies.

Nevertheless, it was decided to store some of the vials of the second WHO IS for dihydrostreptomycin at -80°C and to use them, at regular intervals in the future, to assess the potency of vials stored at -20°C , the customary storage temperature of the WHO IS batch for dihydrostreptomycin. Vials stored at -80°C were registered under EDQM internal number 28294.

Quality control on bulk and final batch

Conformity of the bulk

As described above, precisely weighed samples generated during a single weighing session were submitted to physico-chemical analysis according to the Ph. Eur. monograph, “Dihydrostreptomycin Sulfate for Veterinary Use, 0485” to confirm compliance. The results obtained using the analytical methods described under “Identification B. Thin-layer chromatography”, “Tests. Loss on drying and Sulfated Ash” were in agreement with those of the certificate of analysis provided by the manufacturer. The bulk was therefore considered suitable for further processing.

Visual appearance of final vials

Vials were randomly sampled from the freeze-dried batch and inspected visually. The appearance of the cakes was judged satisfactory.

Residual water content

Vials of the candidate batch contain about 26 mg of freeze-dried dihydrostreptomycin per vial. It was decided to estimate the residual water content in 6 vials randomly sampled from the batch. The determination of water content was performed as described in the Ph. Eur. general chapter "2.5.32. Water: Micro determination". The individual water content estimates of the vials were all below the lower limit of the validated range of the method namely 200 µg to 500 µg. The residual water content was therefore considered to be below the quantification limit of 200 µg representing less than 1 per cent of the 26 mg target fill.

Homogeneity of dihydrostreptomycin content in final vials

The homogeneity of the dihydrostreptomycin content in 12 vials randomly sampled across the batch was assessed by using the liquid chromatography (LC) method described in the Ph. Eur. monograph under "Test. Related substances". The content of each vial was dissolved with purified water to a final volume of 5.0 mL after thorough rinsing to ensure quantitative transfer. Twenty microliters of each solution were injected in duplicate. Almost identical impurity profiles were recorded with all samples and inter-vial variability did not appear to be visually significant. The dihydrostreptomycin main peak areas were used to calculate the mean and the RSD. A value of 0.78 % was obtained for the latter and it was considered that the homogeneity of the batch was satisfactory.

By comparing chromatograms with those recorded with the dihydrostreptomycin solution distributed into the vials before the freeze-drying, no differences could be detected and it was concluded that the process did not adversely affect the impurity profile of the substance.

Stability studies on the product in the final container

An accelerated degradation study was carried out at the EDQM by storing freeze-dried vials of the candidate batch of the third WHO IS for dihydrostreptomycin at +20°C, +37°C and +45°C in climatic chambers (Binder, KBF 720 model) for 1, 3 and 6 months respectively.

Accelerated Degradation Assessed by Microbiological Assay

The potencies of these vials were estimated as the relative potencies against vials of the same batch kept at -80°C. Two vials were analysed by two independent assays for each temperature using the diffusion method. All assays fulfilled the validity criteria prescribed in the European Pharmacopoeia for microbiological assays. The data are presented in Annex 1 in tabular or graphic format after one, three and six months of storage respectively. In addition, potencies of vials stored for six months at -20°C were also estimated against vials stored at -80°C to generate some baseline data for future monitoring purposes. No decrease in potency was observed (data not shown).

Assuming that the expected recovery should be 100% in the absence of any degradation, all except one potency estimate, were within the ± 5% acceptance criterion set to account for the variability of the analytical method based on the long history of monitoring data collected at the EDQM. The relative potency of 90 per cent observed for one vial after one month of incubation at 37°C was considered doubtful. Moreover such a reduction was not observed

with the second vial and longer incubation (3 and 6 months) at 37°C showed only marginal reduction if any.

Extrapolation by means of a model based on the Arrhenius equation shows no predictable loss of potency for a period of at least 10 years (<0.01% per year).

From these data it is anticipated that the stability of the third WHO IS for dihydrostreptomycin is satisfactory at the customary storage temperature of -20°C.

Accelerated Degradation Assessed by Liquid Chromatography

EDQM has a long record of experience in monitoring the stability of official European Pharmacopoeia (Ph.Eur) reference standards for antibiotics. Due to the inherent variability of the microbiological assay methods, it was decided some years ago to replace them by stability indicating methods such as reversed phase liquid chromatography (rp-LC) for monitoring the stability of the Ph. Eur reference standards. It was therefore believed to be of benefit to estimate the degradation at elevated temperature by rp-LC in addition to microbiological assays.

Two vials of each of the three elevated storage temperatures and two vials of those kept at the customary storage temperature of -20°C were analysed using the liquid chromatography analytical method described under “Test. Related substances” of the Ph. Eur monograph “Dihydrostreptomycin sulphate for veterinary use, 0485”.

Individual peaks were identified on each chromatogram and their contents were expressed as the mean areas by normalisation calculated from triplicate injections for each vial. The data are presented in Annex 2 in tabular or graphic format after one, three and six months of storage respectively.

Due to a technical problem with the liquid chromatography equipment, no data was collected for vial 2 after six months incubation at 45°C; fields in the corresponding table were not completed. Consequently mean values were not calculated and the corresponding graph (see +45°C*) was produced with data generated by only one vial.

A total of 28 peaks were detected on the chromatograms. From these, apart from impurity A, none of them seemed to exhibit a dramatic change in peak areas as a result of storage at elevated temperature. At a storage temperature of either -20°C or +20°C, the peak areas corresponding to impurity A remained unchanged, but a slight steady state increase in peak areas paralleled both temperature and time increases. Mean peak areas from the two vials were 0.57 %, 0.62 % and 0.73 % at 37°C and 0.66 %, 0.74 % and 0.88 % at 45°C; keeping in mind that the latter values are only from one vial.

These results are in good agreement with the results generated by the microbiological assay method and it is believed that the moderate increase in impurity A content does not adversely impact the potency of the third International Standard for dihydrostreptomycin when stored at elevated temperature for up to six months.

Conclusions from Accelerated Degradation

Vials of the batch proposed to become the third International Standard for dihydrostreptomycin were submitted to an accelerated degradation study to predict the stability at the customary storage temperature of -20°C. The results obtained with two

orthogonal analytical methods demonstrated that the vials did not exhibit any reduction in potency nor any significant change in the impurity profile. It is therefore concluded that the stability of the batch at -20°C is satisfactory.

Considering that the precision of the liquid chromatography method is much better than the precision of the microbiological assay, it is believed that with respect to the variability of these methods, any significant change in the impurity profile will be detected ahead of any significant loss of potency. It is therefore proposed to monitor in the future the stability of the third WHO International Standard for dihydrostreptomycin on an annual basis by means of liquid chromatography and to assess the impact of any significant modification (decrease in percentage of the principal peak / increase in the level of impurity or appearance of new impurity peak) on the potency by the microbiological assay.

Upon receipt, the third WHO International Standard for dihydrostreptomycin should be stored at -20°C unless used immediately. It is advised that the user dissolves the freeze-dried powder contained in the vial to generate a solution of appropriate concentration and that the solution be used within three days. No attempt to weigh out the freeze-dried cake should be made. Solutions should always be made fresh and never stored frozen prior to use. However, in case of repeat testing due to invalid test results, it has been demonstrated that the freshly made dihydrostreptomycin solution is stable for up to one week when stored at 4°C .

Collaborative study

Participants

A total of 11 laboratories from different countries around the world volunteered to participate in the study. Each participant is referred to in this report by an arbitrarily assigned number, not necessarily reflecting the order of listing in the Appendix.

Samples

Each laboratory was provided with:

- 3 vials of the second WHO IS for dihydrostreptomycin (62/103), 820 IU per mg containing approximately 200 mg of powder per ampoule (EDQM internal code: 32044)
- 7 vials of the third WHO IS for dihydrostreptomycin candidate batch, activity about 18 000 IU per vial (EDQM internal code: 42688)

Assay method and study design

The participants were instructed to exercise extreme care while dissolving the freeze-dried cake of the candidate batch.

Participants were asked to estimate the potency of the third WHO IS for dihydrostreptomycin candidate batch by the microbiological activity on target micro-organisms. The current second WHO IS for dihydrostreptomycin was used as reference standard.

It was requested that any analytical method used be in compliance with requirements set in regional compendia in particular with respect to method validity criteria. A total of six independent assays were to be carried out by each participant.

Prior to carrying out the study an enquiry was carried out which demonstrated that participants were going to use very similar testing procedures. Based on this enquiry, a pilot assay was performed in the EDQM laboratory in order to develop and provide details for the study protocol, taking the Ph. Eur. as the example.

Participating laboratories were requested to follow the study protocol design as far as possible and according to the prescription given in the pharmacopoeia which is their usual reference.

Results and statistical analysis

Statistical methods

The experimental data obtained in this study were analysed as parallel line assays [2], using the SAS-System [3] (GLM procedure) and CombiStats [4]. Both programs give identical outcomes but the output is somewhat easier to transform to tables with the SAS-system, whereas CombiStats provides a more streamlined output for individual assays.

All assays were submitted to visual inspection of the plots to check for unusual features. Validity of the assays was assessed according to the flow chart in Figure 1. In routine situations where decisions are based on only one assay or only a few assays, the level of significance is usually taken to be $P=0.05$. In collaborative studies with many participants, however, a more conservative level of significance is often used. This is because the level of $P=0.05$ leads to about 10 per cent errors of the first kind (incorrect rejection of assays), whereas errors of the second kind (incorrect acceptance of assays) will not influence the global outcome of the study much because of the large amount of data available. Hence, the level of significance in this study is taken to be $P=0.01$ which would imply an expectation of about 2 per cent incorrect rejections. A slight but significant curvature was not considered reason for rejection if the mean square for quadratic regression was less than 1/100 of the mean square for linear regression and the difference between preparations was small [5,6].

Whenever a laboratory performed several assays based on the same weighings, yielding several non-independent estimates of potency, a weighted mean potency of the valid sub-assays was calculated using weights proportional to the reciprocal of the variance. The valid assays per laboratory were combined using the same method of weighted combination, but a semi-weighted combination was used whenever the confidence intervals of the independent potency estimates did not satisfactorily overlap each other by means of a χ^2 test for homogeneity ($P<0.10$). The estimates (one for each of the participants) were then combined into one single estimate with a 95 per cent confidence interval using the same method of semi-weighted combination.

Results

Eleven (11) laboratories reported results from assays. One laboratory submitted 2 sets of assays performed by 2 different operators and are treated in this report as if they are from 2 different laboratories. Laboratories are referred to by their randomly assigned code-numbers (1 to 12), not necessarily corresponding with the order of listing in the list of participants. All participants carried out at least 6 assays as requested. Only the method by diffusion was performed although the protocol also allowed the use of the turbidimetric method. Laboratory 2 used a design in which the standard was tested at several dose levels, but the test preparation at only 1 dose. It was therefore not possible to check for parallelism. Laboratories 5 and 6 carried out duplicate assays for 2 vials, resulting in 8 (sub-)assays each. Laboratory 11 carried

out 6 replicates per assay resulting in a total of 36 sub-assays. For the calculations, all sub-assays were analysed as individual assays after which they were combined into one potency estimate per vial. If all sub-assays are counted as individual assays a total of 106 assays were reported or 4924 zone-diameter readings.

The complete computer output of the parallel line analyses as performed at the EDQM is available in PDF format to participants of the study (212 pages generated by CombiStats). A summary of the results, as generated by the SAS-System is given in Tables 1.1 and 1.2 (See Annex 3 for the essential SAS-scripts used). Shown are the potency estimates and associated 95 per cent confidence intervals, together with the relevant P-values. P-values below the significance level of 0.01 are printed on a grey background. The confidence intervals based on calculations by the participants are also listed. Laboratory 10 did not report confidence intervals.

A graphical representation of the confidence intervals of each individual (sub)-assay is shown in Figure 2 (EDQM calculations) and in Figure 3 (Participants' calculations). Potency estimates from valid assays ranged from 15455 IU/vial (Lab 10) to 21931 IU/vial (Lab 9). Combined potency estimates are shown in Table 2.

Laboratory 1

The 6 assays were statistically valid and the potency estimates were homogeneous (P=0.945). The weighted combined estimate is 19601 IU/vial ($\pm 1.2\%$).

Laboratory 2

Assay 1 had to be rejected due to significant deviations from linearity. The other 5 assays were statistically valid and the potency estimates were homogeneous (P=0.566). The weighted combined estimate is 19871 IU/vial ($\pm 1.7\%$).

Laboratory 3

The 6 assays were statistically valid and the potency estimates were homogeneous (P=0.794). The weighted combined estimate is 19270 IU/vial ($\pm 1.5\%$).

Laboratory 4

The 6 assays were statistically valid and the potency estimates were homogeneous (P=0.691). The weighted combined estimate is 19554 IU/vial ($\pm 2.2\%$).

Laboratory 5

The 8 (sub)-assays were statistically valid. The sub-assays for assay 2 and assay 5 were first combined to obtain 6 potency estimates (1 for each vial). The potency estimates were heterogeneous (P=0.099). The semi-weighted combined estimate is 19609 IU/vial ($\pm 1.5\%$).

Laboratory 6

The 8 (sub)-assays were statistically valid. The sub-assays for assay 1 and assay 4 were first combined to obtain 6 potency estimates (1 for each vial). The potency estimates were heterogeneous (P<0.001). The semi-weighted combined estimate is 19356 IU/vial ($\pm 2.0\%$).

Laboratory 7

Assays 1 to 5 had to be rejected due to significant deviations from linearity and/or parallelism. Assay 6 was statistically valid but of very low precision with confidence limits of about $\pm 15\%$. In addition, the potency estimate of 21427 IU/vial was rather high being the 2nd highest overall value obtained in this study. Given the multiple issues with this laboratory it was decided to also reject assay 6.

Laboratory 8

The 6 assays were statistically valid and the potency estimates were homogeneous ($P=0.975$). The weighted combined estimate is 18663 IU/vial ($\pm 1.8\%$).

Laboratory 9

The 6 assays were statistically valid and the potency estimates were heterogeneous ($P=0.019$). The semi-weighted combined estimate is 20752 IU/vial ($\pm 2.1\%$).

Laboratory 10

The 6 assays were statistically valid but assay 5 had to be rejected because the responses for the test sample were not in the same range as the responses for the standard which is a basic requirement for potency assays. The potency estimates were heterogeneous ($P<0.001$). The semi-weighted combined estimate is 17843 IU/vial ($\pm 5.6\%$).

Laboratory 11

The 36 sub-assays were statistically valid. The 6 sub-assays for each of the 6 assays were first combined to obtain 6 potency estimates (1 for each vial). The resulting 6 potency estimates were heterogeneous ($P=0.001$). The semi-weighted combined estimate is 18948 IU/vial ($\pm 0.3\%$).

Laboratory 12

The 6 assays were statistically valid and the potency estimates were homogeneous ($P=0.201$). The weighted combined estimate is 19573 IU/vial ($\pm 3.1\%$).

A histogram of all potency estimates per assay is shown in Figure 4 and a histogram of the mean results per laboratory is shown in Figure 5. The final confidence intervals per laboratory are summarised in Table 2 and a graphical representation is given in Figure 6. The χ^2 value for between-laboratory homogeneity is highly significant ($P<0.001$) so a semi-weighted combination was made which yields 19425 IU/vial with 95% confidence limits of 19251 to 19602 IU/vial (which is $\pm 0.9\%$).

Comments from Participants

None of the participants of the study opposed the conclusions of the report. Laboratories 5 and 6 submitted corrections with regards to the data reported which have been taken into consideration. The subsequent recalculations have resulted in a minor correction of the proposed antimicrobiological activity.

Recommendation

The proposed candidate batch is suitable for its intended purpose. It is proposed that the third WHO International Standard for dihydrostreptomycin (EDQM internal code ISA_42688) be assigned an antimicrobiological activity of **19 425 IU per vial**.

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Table 1.1
Overview of assay results generated by the SAS System

Lab	Assay	Calculated by participants (IU/vial)		Calculated at EDQM (IU/vial)		p-Values Analysis of variance				Quad. regr./ Lin. regr.
		Estimated Potency	95% Confidence Limits	Estimated Potency	95% Confidence Limits	Non- parallelism	Non- linearity	Lack of quadratic fit	Qua-dra-tic regression	
1	1	19512	(96.7% - 103.4%)	19510	(96.7% - 103.4%)	1.000	0.684	1.000	0.389	0.000
	2	19547	(97.4% - 102.7%)	19544	(97.4% - 102.7%)	0.358	0.318	0.376	0.220	0.000
	3	19591	(97.7% - 102.4%)	19593	(97.7% - 102.4%)	0.709	1.000	1.000	1.000	0.000
	4	19719	(96.5% - 103.6%)	19723	(96.5% - 103.6%)	0.802	0.592	0.316	0.885	0.001
	5	19470	(96.7% - 103.4%)	19469	(96.7% - 103.4%)	0.812	0.473	0.583	0.278	0.000
	6	19931	(96.0% - 104.2%)	19937	(96.0% - 104.2%)	0.147	0.599	0.806	0.332	0.000
2	1	19854	(95.7% - 104.5%)	20223	(96.6% - 103.6%)	n.a.	0.000	0.000	0.001	0.016
	2	19836	(95.4% - 104.9%)	19712	(95.9% - 104.4%)	n.a.	0.649	0.745	0.465	0.000
	3	19908	(95.0% - 105.3%)	19829	(95.3% - 105.0%)	n.a.	0.977	0.928	0.908	0.000
	4	19890	(94.4% - 106.0%)	19482	(95.7% - 104.6%)	n.a.	0.043	0.375	0.025	0.001
	5	20106	(96.1% - 104.2%)	19699	(96.4% - 103.8%)	n.a.	0.027	0.071	0.049	0.004
	6	20538	(94.6% - 105.6%)	20293	(97.0% - 103.2%)	n.a.	0.328	0.209	0.392	0.001
3	1	18999	(96.7% - 103.4%)	18999	(96.7% - 103.4%)	0.095	0.234	0.332	0.161	0.001
	2	19455	(95.8% - 104.3%)	19455	(95.8% - 104.3%)	0.092	0.886	0.877	0.642	0.000
	3	19004	(96.4% - 103.7%)	19004	(96.4% - 103.7%)	0.278	0.268	0.660	0.119	0.000
	4	19417	(96.5% - 103.6%)	19417	(96.5% - 103.6%)	0.132	0.534	0.266	0.928	0.001
	5	19301	(96.5% - 103.6%)	19301	(96.5% - 103.6%)	0.323	0.912	0.849	0.703	0.000
	6	19598	(96.1% - 104.1%)	19598	(96.1% - 104.1%)	0.143	0.266	0.294	0.212	0.001
4	1	19022	(93.8% - 106.6%)	19024	(93.8% - 106.6%)	0.435	0.379	0.409	0.263	0.001
	2	19446	(94.5% - 105.8%)	19448	(94.5% - 105.8%)	0.888	0.180	0.070	0.745	0.002
	3	18864	(93.1% - 107.4%)	18866	(93.1% - 107.4%)	0.691	0.089	0.080	0.167	0.003
	4	19630	(95.4% - 104.9%)	19632	(95.4% - 104.9%)	0.055	0.206	0.869	0.080	0.000
	5	20033	(93.2% - 107.2%)	20034	(93.2% - 107.2%)	0.270	0.250	0.120	0.566	0.002
	6	19896	(95.6% - 104.6%)	19898	(95.6% - 104.6%)	0.474	0.175	0.071	0.679	0.001
5	1	19153	(95.2% - 105.1%)	19153	(95.2% - 105.1%)	0.337	0.331	0.246	0.352	0.002
	2.1	19968	(97.0% - 103.1%)	19968	(97.0% - 103.1%)	0.571	0.909	0.885	0.684	0.000
	2.2	19870	(97.3% - 102.8%)	19870	(97.3% - 102.8%)	0.746	0.799	0.867	0.522	0.000
	3	19669	(95.9% - 104.3%)	19184	(94.5% - 106.0%)	0.227	0.446	0.650	0.240	0.000
	4	19103	(95.0% - 105.2%)	19035	(95.4% - 104.9%)	0.191	0.172	0.068	0.724	0.004
	5.1	20599	(95.2% - 105.3%)	20599	(95.2% - 105.3%)	0.057	0.091	0.112	0.118	0.004
5.2	19191	(97.2% - 102.9%)	19191	(97.2% - 102.9%)	0.066	0.114	0.040	0.859	0.002	
6	19941	(95.7% - 104.6%)	19759	(95.4% - 104.9%)	0.154	0.177	0.067	0.862	0.004	
6	1	19496	(96.5% - 103.6%)	19387	(96.6% - 103.6%)	0.805	0.635	0.438	0.588	0.000
	2.1	18932	(97.1% - 103.0%)	18932	(97.1% - 103.0%)	0.942	0.334	0.148	0.827	0.001
	2.2	18682	(96.8% - 103.4%)	18697	(96.9% - 103.2%)	0.593	0.858	0.589	0.931	0.000
	3	18218	(97.0% - 103.0%)	18218	(97.0% - 103.0%)	0.588	0.973	0.818	0.976	0.000
	4.1	19343	(96.6% - 103.5%)	19381	(96.7% - 103.5%)	0.281	0.637	0.379	0.741	0.000
	4.2	18440	(97.4% - 102.7%)	18440	(97.4% - 102.7%)	0.487	0.139	0.052	0.744	0.002
5	20734	(95.0% - 105.4%)	20734	(95.0% - 105.4%)	0.928	0.314	0.135	0.875	0.003	
6	20593	(96.7% - 103.5%)	20593	(96.7% - 103.5%)	0.800	0.823	0.623	0.708	0.000	
7	1	18388	(92.0% - 108.0%)	13368	(88.1% - 112.9%)	0.000	0.000	0.000	0.076	0.063
	2	18327	(91.0% - 109.0%)	12009	(86.7% - 114.3%)	0.000	0.500	0.854	0.385	0.000
	3	19304	(92.4% - 107.6%)	19545	(82.9% - 120.4%)	0.703	0.000	0.000	0.450	0.270
	4	19744	(95.4% - 104.6%)	18555	(83.3% - 119.7%)	0.886	0.000	0.113	0.000	0.010
	5	20017	(98.4% - 101.6%)	20433	(87.8% - 113.9%)	0.712	0.020	0.002	0.294	0.021
	6	20290	(99.1% - 100.9%)	21427	(86.3% - 116.0%)	0.929	0.220	0.259	0.219	0.003
8	1	18506	(95.4% - 104.9%)	18506	(95.4% - 104.9%)	1.000	0.921	0.687	1.000	0.000
	2	18403	(95.4% - 104.9%)	18403	(95.4% - 104.9%)	0.696	0.950	0.821	0.821	0.000
	3	18613	(95.4% - 104.8%)	18613	(95.4% - 104.8%)	1.000	0.348	0.328	0.282	0.000
	4	18741	(95.4% - 104.8%)	18741	(95.4% - 104.8%)	0.798	0.837	0.589	0.806	0.000
	5	18848	(95.4% - 104.9%)	18848	(95.4% - 104.9%)	0.626	0.422	0.193	0.925	0.001
	6	18778	(96.5% - 103.6%)	18778	(96.5% - 103.6%)	1.000	0.142	0.104	0.256	0.001

P-values below the significance level of 0.01 are printed on a grey background. n.r. = not reported. n.a. = not applicable.

Table 1.2
Overview of assay results generated by the SAS System

Lab	Assay	Calculated by participants (U/vial)		Calculated at EDQM (U/vial)		p-Values Analysis of variance				Quad. regr./ Lin. regr.
		Estimated Potency	95% Confidence Limits	Estimated Potency	95% Confidence Limits	Non- parallelism	Non- linearity	Lack of quadratic fit	Qua-dra-tic regression	
9	1	21285	(95.2% - 105.1%)	21285	(95.2% - 105.1%)	0.346	0.488	0.439	0.362	0.001
	2	20631	(96.3% - 103.8%)	20631	(96.3% - 103.8%)	0.199	0.390	0.178	0.830	0.001
	3	20880	(96.1% - 104.1%)	20880	(96.1% - 104.1%)	0.692	0.387	0.177	0.819	0.002
	4	20395	(96.3% - 103.8%)	20395	(96.3% - 103.8%)	0.947	0.079	0.029	0.611	0.004
	5	19643	(95.4% - 104.8%)	19643	(95.4% - 104.8%)	0.131	0.646	0.821	0.368	0.000
	6	21931	(95.4% - 104.9%)	21931	(95.4% - 104.9%)	0.458	0.092	0.033	0.676	0.006
10	1	15455	n.r.	15455	(91.2% - 108.7%)	0.017	0.084	0.029	0.832	0.019
	2	18382	n.r.	18382	(93.8% - 106.3%)	0.758	0.456	0.334	0.428	0.002
	3	20739	n.r.	20739	(89.6% - 111.3%)	0.644	0.055	0.018	0.789	0.040
	4	17566	n.r.	17566	(89.9% - 110.2%)	0.459	0.022	0.012	0.230	0.038
	5*	12634	n.r.	12634	(89.9% - 109.6%)	0.696	0.158	0.334	0.095	0.003
	6	17799	n.r.	17799	(93.2% - 107.0%)	0.081	0.860	0.881	0.601	0.000
11	1.1	18914	(98.6% - 101.4%)	18918	(98.7% - 101.3%)	0.532	0.051	0.079	0.079	0.000
	1.2	18859	(98.1% - 101.9%)	18808	(97.9% - 102.1%)	0.866	0.076	0.151	0.072	0.000
	1.3	18970	(98.3% - 101.8%)	18921	(98.4% - 101.6%)	0.642	0.162	0.069	0.591	0.000
	1.4	18950	(97.6% - 102.5%)	18925	(97.8% - 102.3%)	0.095	0.041	0.084	0.056	0.000
	1.5	18967	(98.7% - 101.3%)	18910	(98.6% - 101.4%)	0.780	0.974	0.872	0.872	0.000
	1.6	18812	(98.6% - 101.5%)	18785	(98.8% - 101.3%)	0.036	0.050	0.055	0.112	0.000
	2.1	19197	(98.1% - 101.9%)	19199	(98.2% - 101.8%)	0.183	0.071	0.077	0.127	0.000
	2.2	19165	(98.5% - 101.5%)	18983	(98.0% - 102.0%)	0.078	0.419	0.207	0.727	0.000
	2.3	19249	(98.1% - 101.9%)	19252	(98.1% - 101.9%)	0.127	0.255	0.103	0.897	0.000
	2.4	18796	(96.8% - 103.3%)	18798	(96.6% - 103.5%)	0.450	0.359	0.311	0.311	0.000
	2.5	19098	(97.5% - 102.5%)	18842	(97.3% - 102.8%)	0.868	0.222	0.111	0.502	0.000
	2.6	19039	(97.5% - 102.6%)	19069	(97.5% - 102.6%)	0.741	0.069	0.029	0.448	0.001
	3.1	18718	(98.6% - 101.4%)	18716	(98.7% - 101.3%)	0.050	0.243	0.150	0.382	0.000
	3.2	18810	(97.7% - 102.4%)	18620	(97.8% - 102.2%)	0.768	0.889	0.733	0.733	0.000
	3.3	18747	(98.1% - 101.9%)	18745	(98.2% - 101.8%)	0.472	0.802	0.533	0.835	0.000
	3.4	18574	(98.2% - 101.9%)	18573	(98.3% - 101.7%)	0.261	0.228	0.694	0.096	0.000
	3.5	18808	(97.9% - 102.1%)	18806	(98.1% - 101.9%)	0.692	0.046	0.048	0.118	0.000
	3.6	18954	(97.2% - 102.8%)	18893	(97.4% - 102.6%)	0.876	0.141	0.135	0.185	0.000
	4.1	19019	(99.1% - 100.9%)	19022	(99.2% - 100.9%)	0.475	0.465	0.221	1.000	0.000
	4.2	19066	(97.3% - 102.8%)	19070	(97.5% - 102.5%)	0.541	0.074	0.361	0.036	0.000
	4.3	19057	(98.2% - 101.9%)	19060	(98.2% - 101.8%)	0.317	0.166	0.249	0.128	0.000
	4.4	19013	(98.4% - 101.6%)	19017	(98.4% - 101.6%)	0.236	0.777	0.490	0.890	0.000
	4.5	19001	(98.8% - 101.2%)	19172	(98.3% - 101.8%)	0.403	0.403	0.021	0.021	0.000
	4.6	18994	(98.6% - 101.5%)	18998	(98.6% - 101.4%)	0.359	0.629	0.595	0.426	0.000
	5.1	18995	(98.5% - 101.5%)	19050	(98.8% - 101.2%)	0.611	0.135	0.048	1.000	0.000
	5.2	18949	(98.9% - 101.1%)	18952	(98.9% - 101.1%)	0.082	0.741	0.492	0.730	0.000
	5.3	18999	(98.7% - 101.3%)	19003	(98.8% - 101.2%)	0.794	0.813	0.652	0.652	0.000
	5.4	19129	(98.9% - 101.1%)	19034	(98.7% - 101.3%)	0.358	0.565	1.000	0.290	0.000
	5.5	19024	(97.8% - 102.3%)	19028	(98.0% - 102.1%)	0.081	0.023	0.046	0.046	0.000
	5.6	18952	(99.2% - 100.8%)	18956	(99.2% - 100.8%)	0.691	0.076	0.257	0.047	0.000
	6.1	18999	(99.1% - 100.9%)	18995	(99.2% - 100.8%)	0.073	0.328	0.291	0.291	0.000
	6.2	19031	(98.8% - 101.2%)	19028	(99.0% - 101.1%)	0.244	0.246	0.180	0.311	0.000
	6.3	19026	(98.7% - 101.3%)	19022	(98.8% - 101.3%)	1.000	0.595	0.573	0.400	0.000
	6.4	18911	(99.0% - 101.0%)	18931	(99.0% - 101.0%)	0.363	0.050	0.081	0.075	0.000
	6.5	19001	(98.9% - 101.1%)	18996	(99.1% - 100.9%)	0.737	0.055	0.182	0.041	0.000
	6.6	18868	(98.8% - 101.2%)	18864	(98.9% - 101.1%)	1.000	0.759	0.741	0.510	0.000
12	1	19411	(94.3% - 106.0%)	19411	(94.3% - 106.0%)	0.887	0.160	0.568	0.069	0.001
	2	18403	(92.3% - 108.3%)	18403	(92.3% - 108.3%)	0.944	0.140	0.823	0.051	0.000
	3	19917	(89.9% - 111.9%)	19917	(89.9% - 111.9%)	0.447	0.462	0.546	0.280	0.002
	4	20847	(90.9% - 110.5%)	20847	(90.9% - 110.5%)	0.789	0.721	0.719	0.473	0.001
	5	20780	(92.3% - 108.8%)	20780	(92.3% - 108.8%)	0.696	0.555	0.540	0.374	0.001
	6	19047	(92.8% - 107.8%)	19047	(92.8% - 107.8%)	0.253	0.149	0.924	0.054	0.000

* Assay 5 from Laboratory 10 was rejected because responses of test sample were not in the same range as the standard
P-values below the significance level of 0.01 are printed on a grey background. n.r. = not reported. n.a. = not applicable.

Table 2
Combined potency estimates per laboratory

Laboratory	Final Potency Estimates (IU/vial)	
	Estimated Potency	95% Confidence Limits
1	19601	(98.8% - 101.2%)
2	19871	(98.3% - 101.7%)
3	19270	(98.5% - 101.5%)
4	19554	(97.8% - 102.2%)
5	19609	(98.5% - 101.5%)
6	19356	(98% - 102%)
7	n.c.	
8	18663	(98.2% - 101.8%)
9	20752	(97.9% - 102.1%)
10	17843	(94.4% - 105.6%)
11	18948	(99.7 - 100.3%)
12	19573	(96.9% - 103.1%)
Combined	19425	(99.1 - 100.9%)

n.c. = not calculated

Figure 1
Flow chart for assay validity check

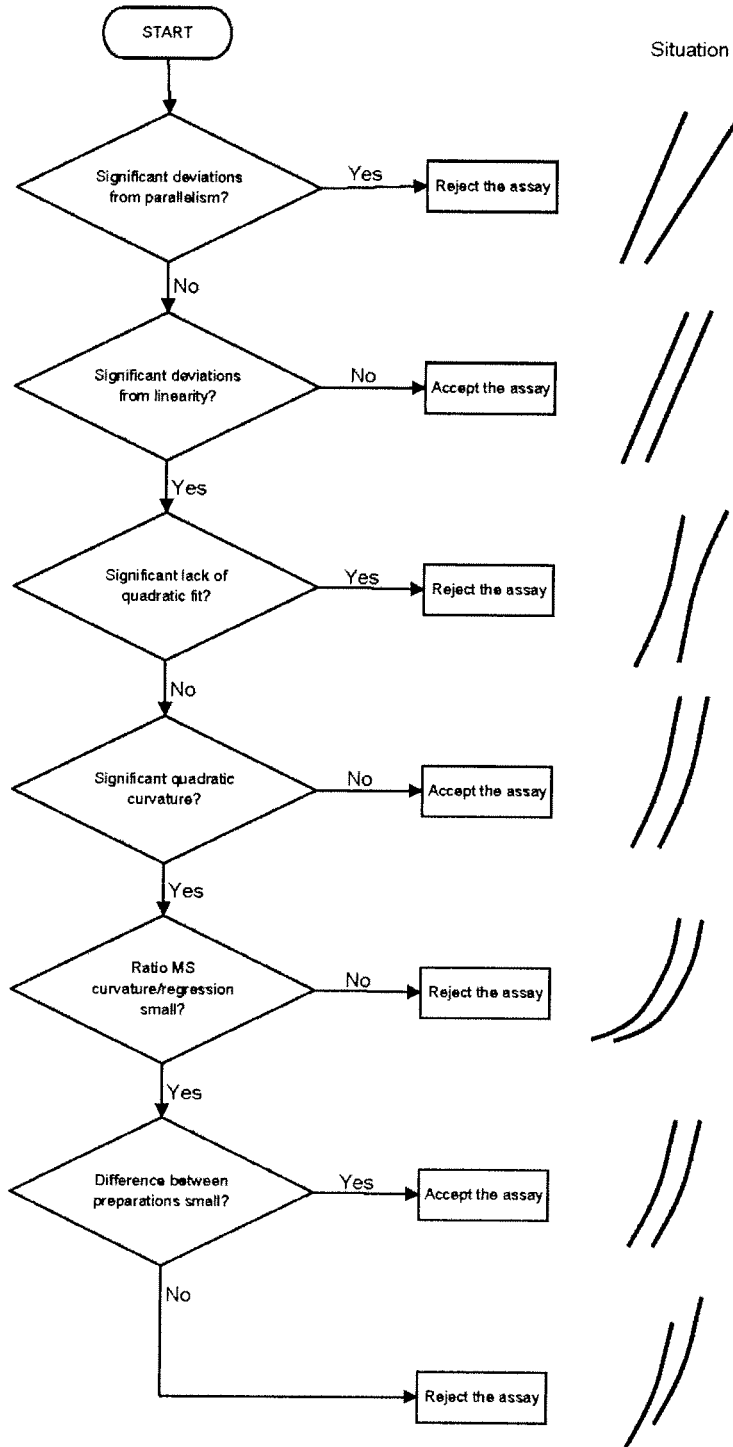
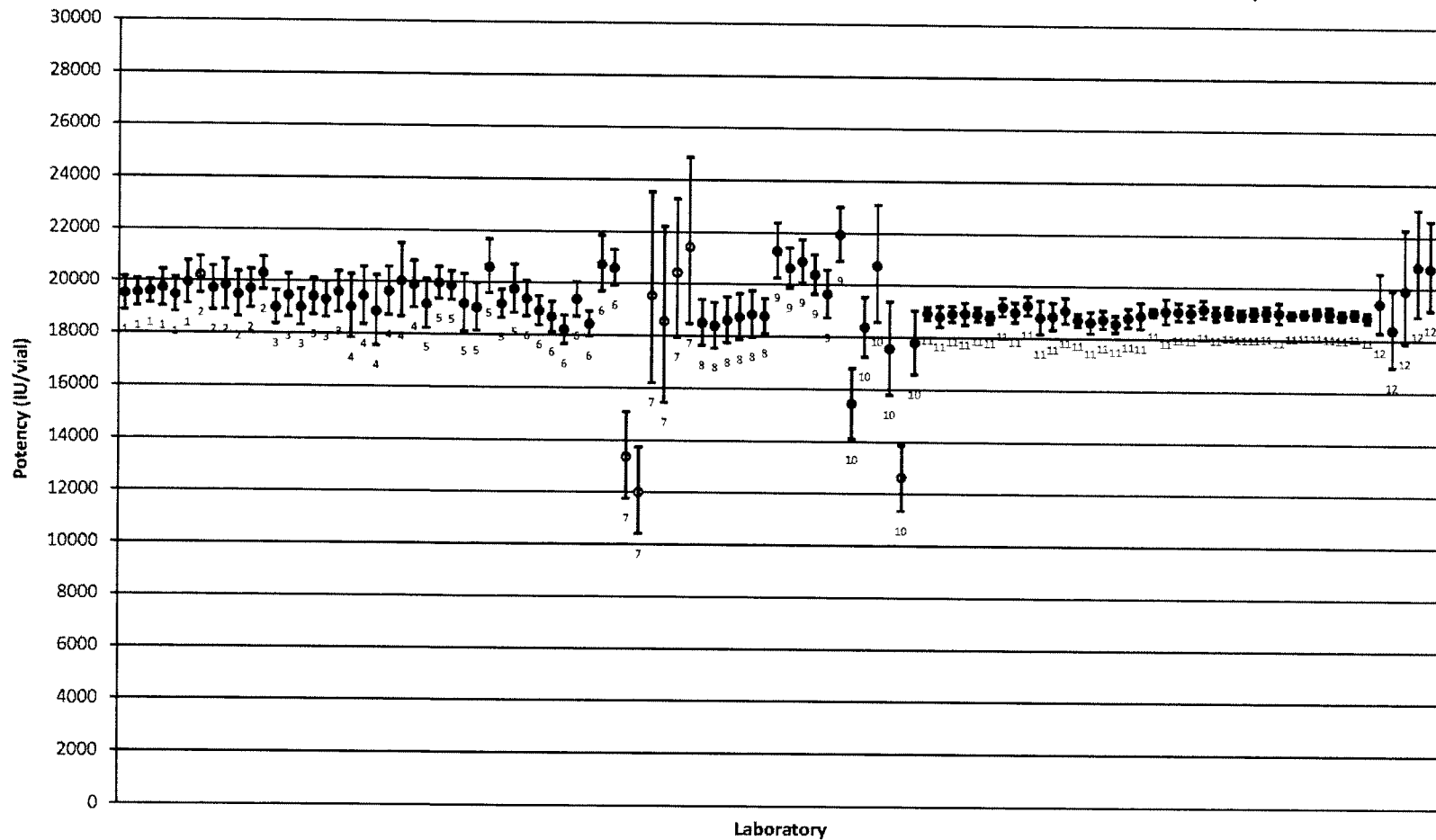
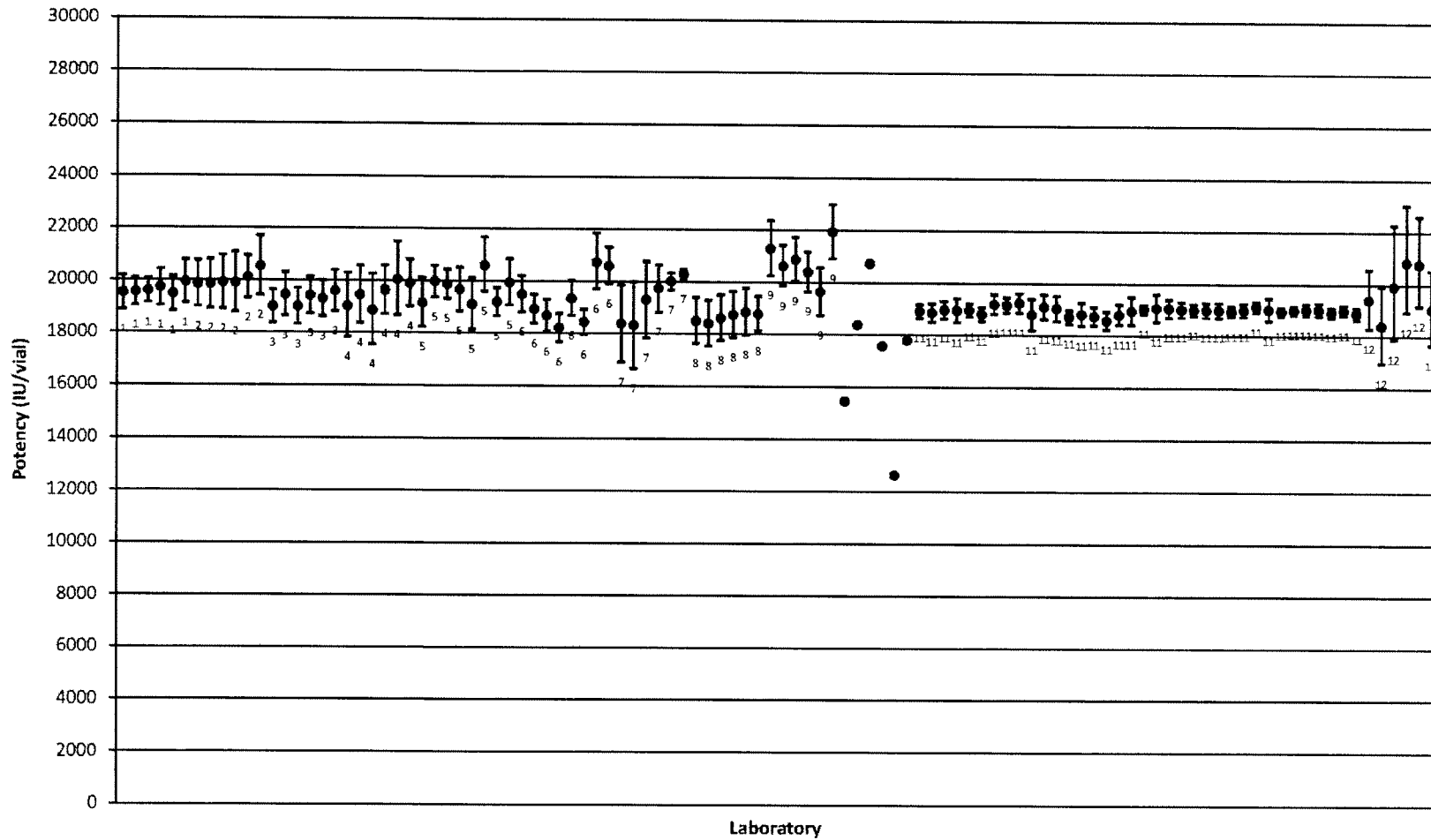


Figure 2
Individual potency estimates per assay and 95% confidence intervals (calculated at EDQM)



The numbers below the 95% confidence intervals are the laboratory codes. Invalid assays are shown with an empty dot.

Figure 3
Individual potency estimates per assay and 95% confidence intervals (calculated by participants)



The numbers below the 95% confidence intervals are the laboratory codes. Assays considered invalid by the participants themselves are shown with an empty dot (none in this study).

Figure 4 - Histogram of final potency estimates per assay

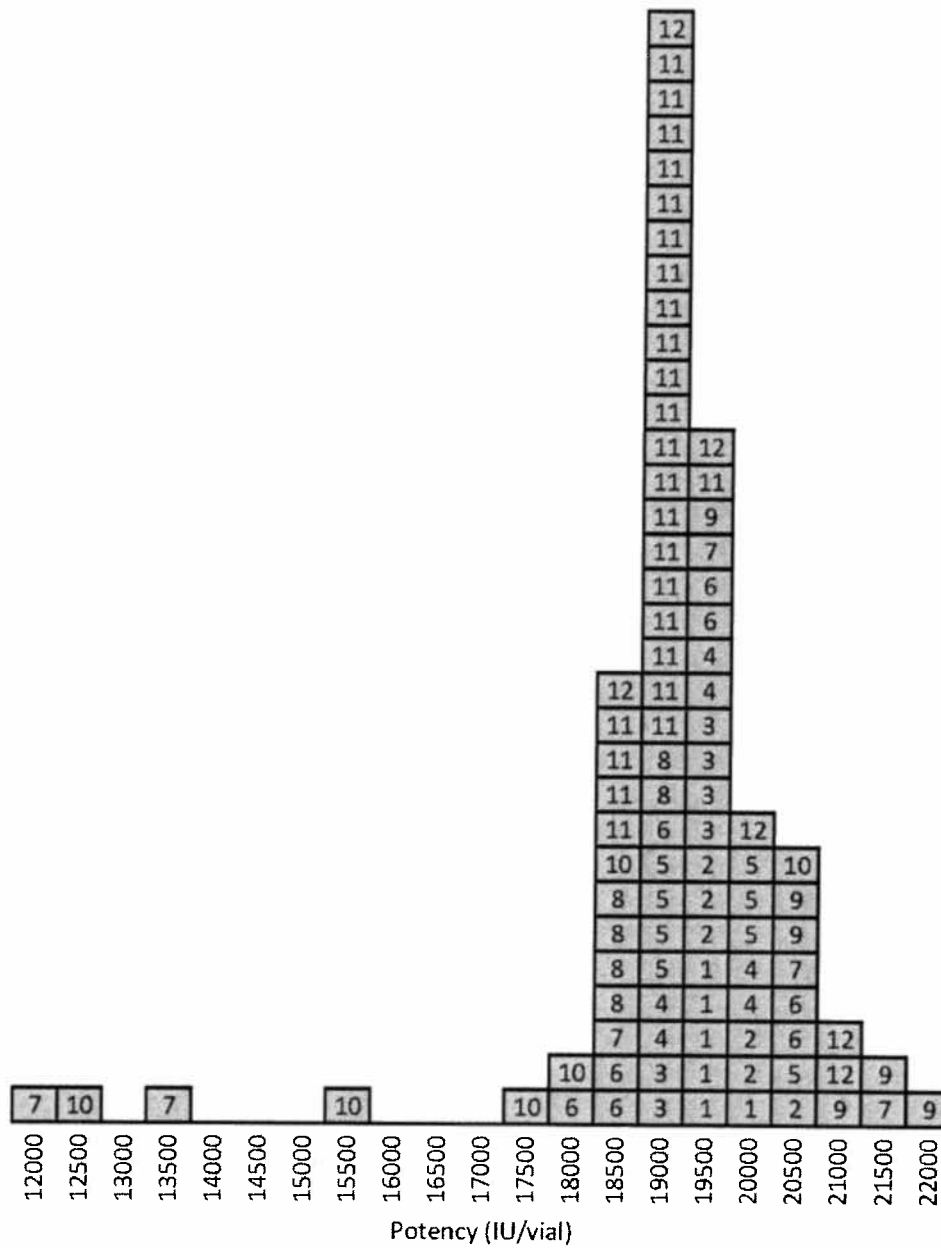
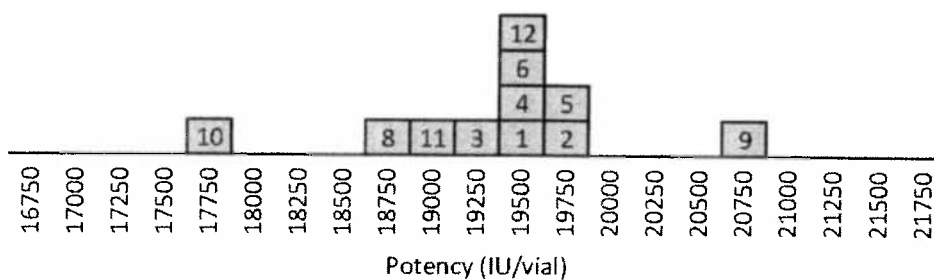
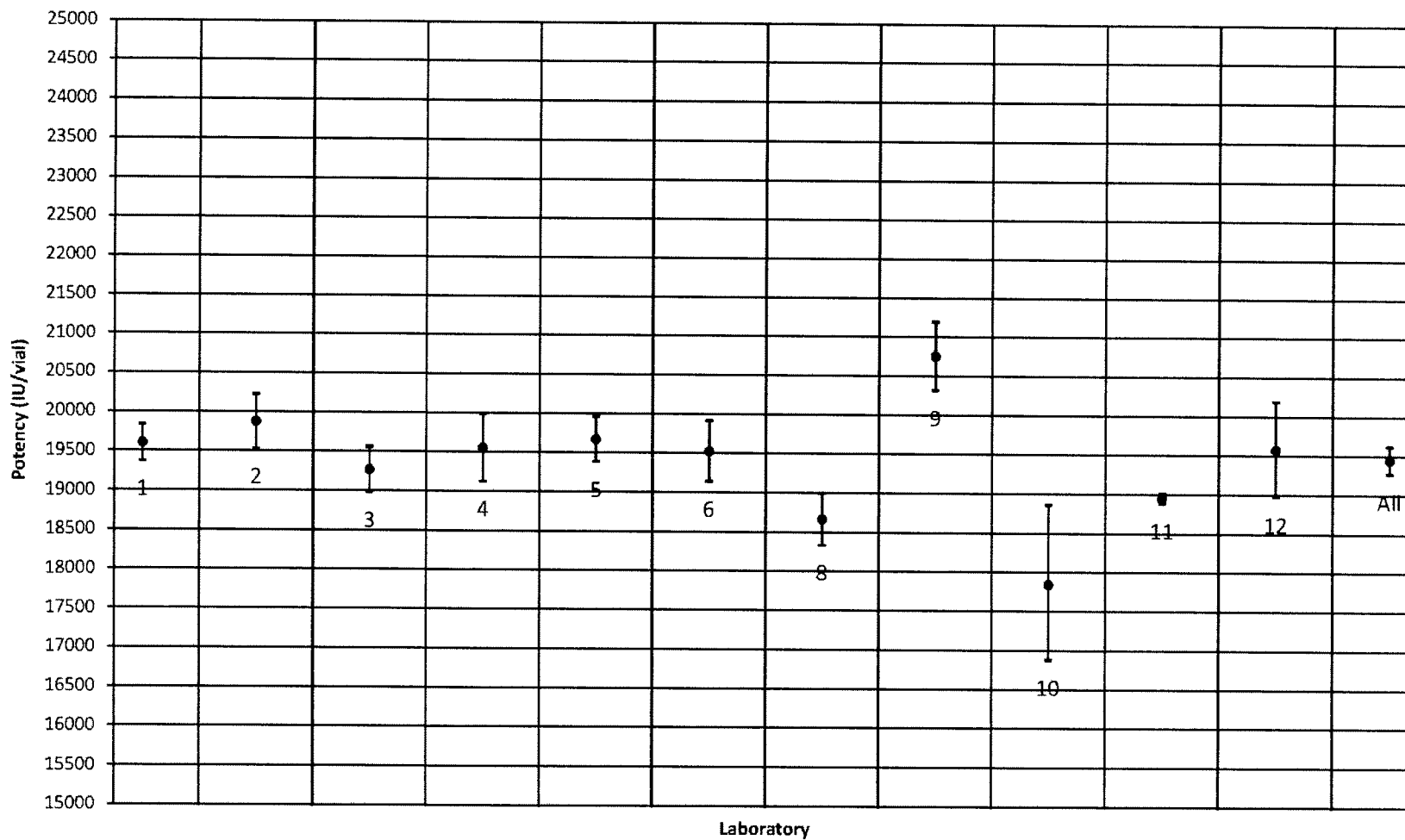


Figure 5 - Histogram of final potency estimates per laboratory



Numbers in the boxes are the laboratory codes.

Figure 6
Potency estimates per assay and 95% confidence intervals per laboratory

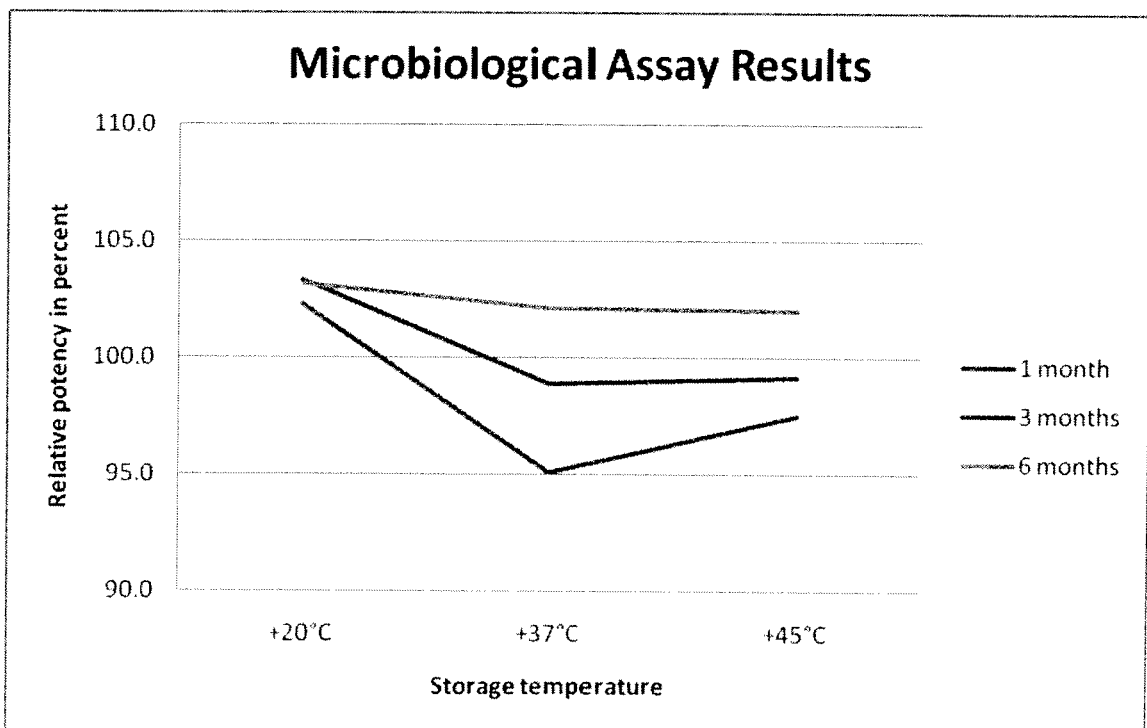


The numbers below the 95% confidence intervals are the laboratory codes.

ANNEX 1: Accelerated Degradation, Microbiological Assay Results

Relative Potency in per cent versus samples stored at -80°C

Storage temperature		+20°C	+37°C	+45°C
1 month	vial 1	101.5	90.0	97.0
	CI $P=0.95$	95.4% - 104.9%	94.9% - 105.3%	95.1% - 105.1%
	vial 2	102.8	99.9	97.9
	CI $P=0.95$	96.5% - 103.7%	96.6% - 103.6%	96.0% - 104.1%
	mean	102.3	95.1	97.5
	CI $P=0.95$	97.2% - 102.8%	92.3% - 108.3%	97.0% - 103.1%
3 months	vial 1	104.2	97.8	95.5
	CI $P=0.95$	94.8% - 105.6%	95.3% - 105.0%	92.2% - 108.4%
	vial 2	102.3	99.4	99.6
	CI $P=0.95$	94.0% - 106.4%	96.7% - 103.4%	97.2% - 102.9%
	mean	103.4	98.9	99.2
	CI $P=0.95$	96.1% - 104.0%	97.3% - 102.7%	97.4% - 102.7%
6 months	vial 1	100.1	101.4	104.5
	CI $P=0.95$	94.2% - 106.3%	94.4% - 106.1%	94.6% - 105.8%
	vial 2	105.9	102.4	98.1
	CI $P=0.95$	94.6% - 105.8%	96.4% - 103.8%	93.0% - 107.5%
	mean	103.2	102.1	102.0
	CI $P=0.95$	96.1% - 104.1%	97.0% - 103.1%	95.8% - 104.4%



ANNEX 2: Accelerated Degradation, Liquid Chromatography Results

Mean Impurity Peak Areas in Per Cent after 1 Month

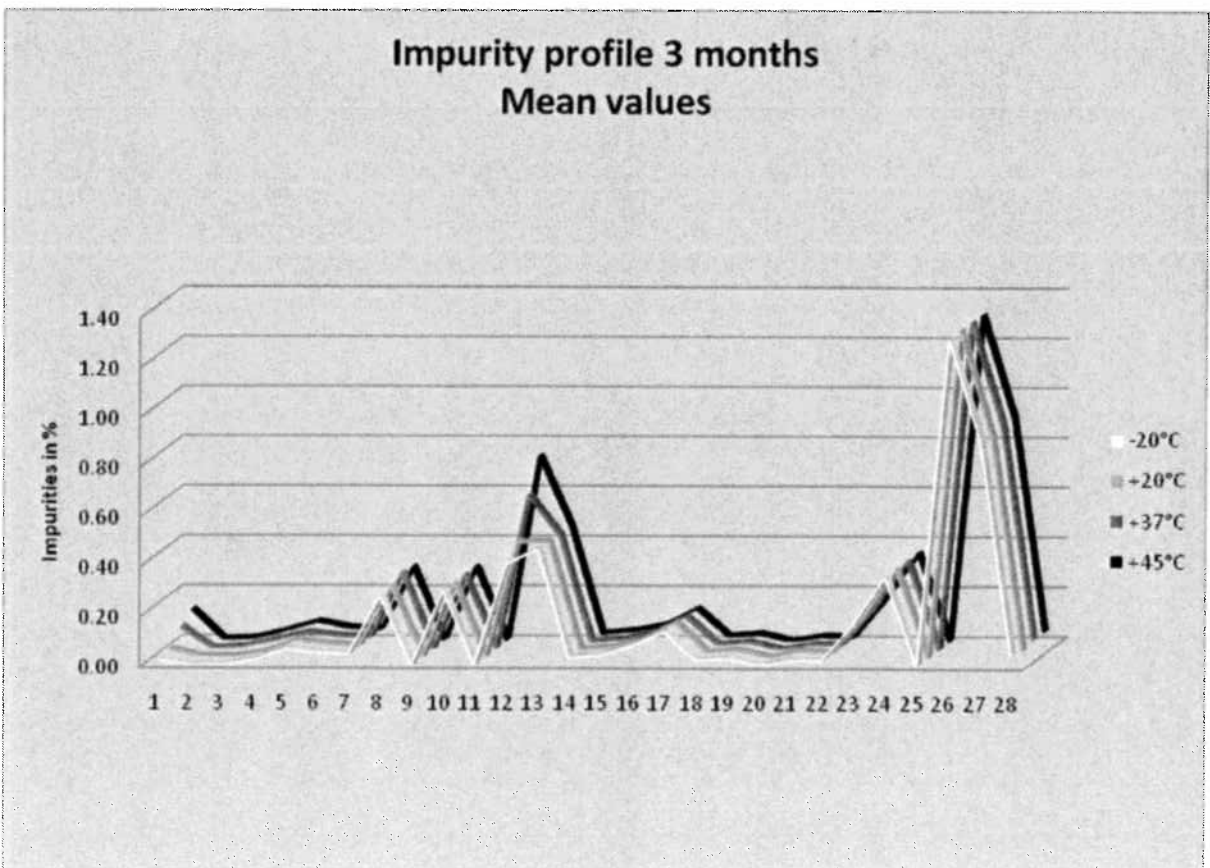
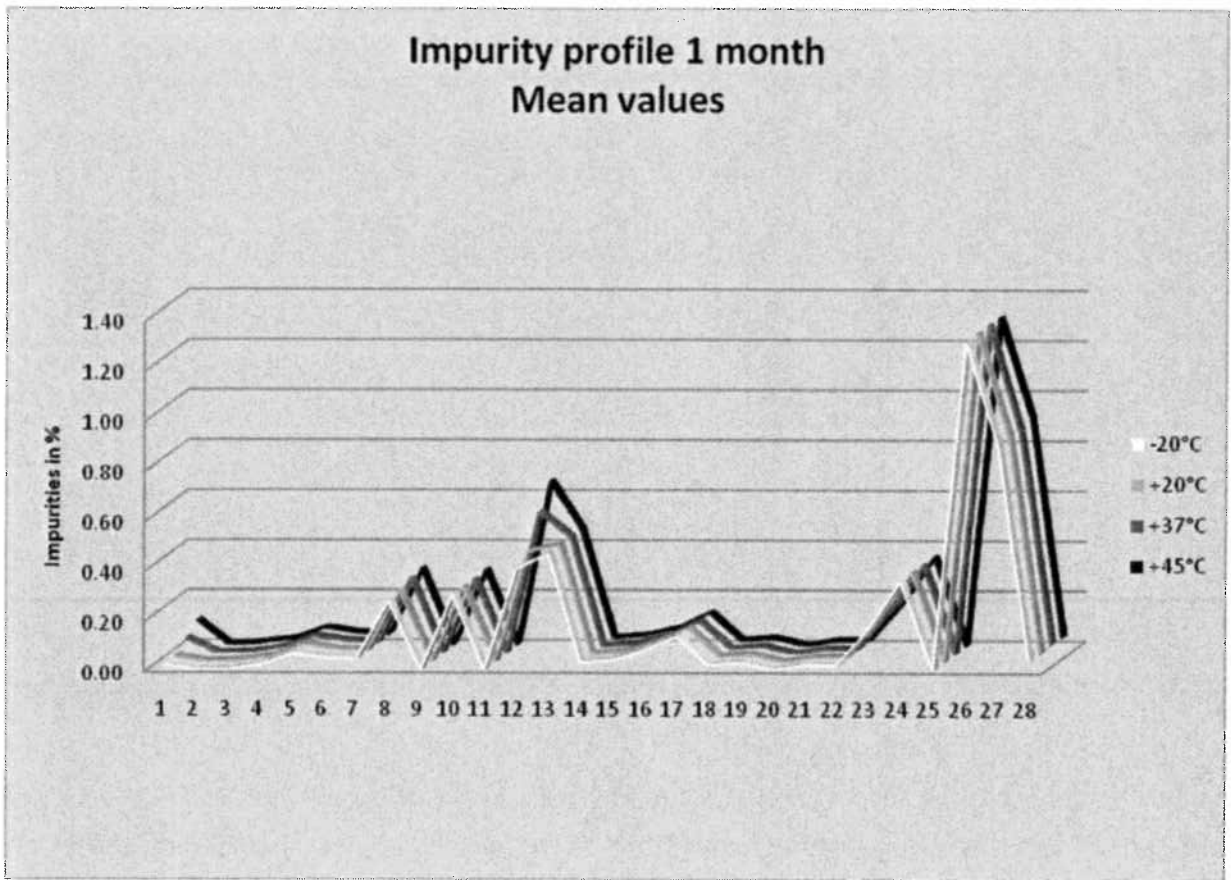
1 month	-20°C			+20°C			+37°C			+45°C		
	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean
Peak1	0,02	0,02	0,02	0,02	0,02	0,02	0,06	0,06	0,06	0,10	0,10	0,10
Peak2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Peak3	0,00	0,00	0,00	0,01	0,00	0,01	0,01	0,00	0,01	0,01	0,01	0,01
Peak4	0,02	0,02	0,02	0,02	0,02	0,02	0,03	0,02	0,03	0,03	0,02	0,03
Peak5	0,07	0,06	0,07	0,07	0,07	0,07	0,07	0,07	0,07	0,08	0,06	0,07
Peak6	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Peak7	0,04	0,05	0,05	0,04	0,05	0,05	0,04	0,05	0,05	0,04	0,05	0,05
Peak8	0,27	0,28	0,28	0,29	0,28	0,29	0,30	0,30	0,30	0,30	0,31	0,31
Peak9	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Peak10	0,30	0,30	0,30	0,30	0,30	0,30	0,29	0,30	0,30	0,30	0,30	0,30
Peak11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Impurity A	0,41	0,40	0,41	0,44	0,44	0,44	0,57	0,56	0,57	0,66	0,65	0,66
Peak13	0,47	0,46	0,47	0,47	0,46	0,47	0,47	0,46	0,47	0,46	0,46	0,46
Peak14	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Peak15	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Peak16	0,08	0,08	0,08	0,08	0,08	0,08	0,07	0,07	0,07	0,07	0,07	0,07
Peak17	0,13	0,14	0,14	0,14	0,13	0,14	0,13	0,13	0,13	0,13	0,13	0,13
Peak18	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Peak19	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Peak20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Peak21	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Peak22	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Peak23	0,16	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,17	0,16	0,17
Impurity B	0,36	0,35	0,36	0,36	0,36	0,36	0,36	0,35	0,36	0,36	0,35	0,36
Streptomycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Impurity C	1,31	1,31	1,31	1,31	1,32	1,32	1,31	1,32	1,32	1,31	1,31	1,31
Peak28	0,91	0,89	0,90	0,90	0,89	0,90	0,90	0,88	0,89	0,90	0,92	0,91
Peak29	0,04	0,04	0,04	0,03	0,04	0,04	0,04	0,04	0,04	0,03	0,04	0,04

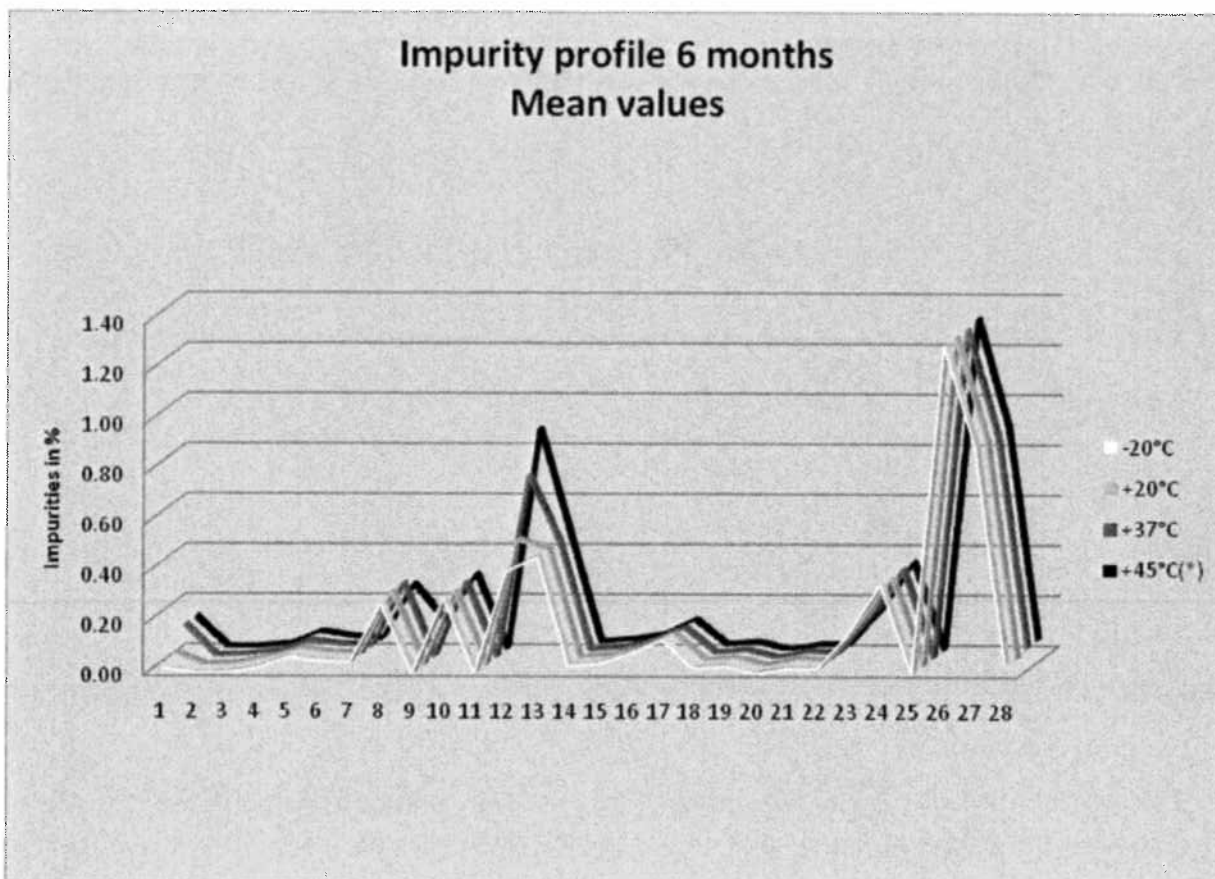
Mean Impurity Peak Areas in Per Cent after 3 Months

3 months	-20°C			+20°C			+37°C			+45°C		
	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean
Peak1	0,02	0,02	0,02	0,03	0,03	0,03	0,09	0,08	0,09	0,12	0,13	0,13
Peak2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,00	0,01
Peak3	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01	0,01	0,01	0,01
Peak4	0,02	0,02	0,02	0,03	0,01	0,02	0,03	0,04	0,04	0,04	0,04	0,04
Peak5	0,07	0,06	0,07	0,07	0,06	0,07	0,07	0,06	0,07	0,08	0,07	0,08
Peak6	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05
Peak7	0,04	0,05	0,05	0,04	0,05	0,05	0,04	0,05	0,05	0,04	0,05	0,05
Peak8	0,27	0,28	0,28	0,28	0,29	0,29	0,30	0,31	0,31	0,26	0,33	0,30
Peak9	ND	ND	ND	ND	ND	ND	ND	ND	ND	0,06	ND	ND
Peak10	0,30	0,30	0,30	0,29	0,30	0,30	0,29	0,30	0,30	0,29	0,30	0,30
Peak11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Impurity A	0,41	0,40	0,41	0,46	0,46	0,46	0,61	0,62	0,62	0,74	0,74	0,74
Peak13	0,47	0,46	0,47	0,46	0,46	0,46	0,45	0,46	0,46	0,46	0,46	0,46
Peak14	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Peak15	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04
Peak16	0,08	0,08	0,08	0,07	0,08	0,08	0,07	0,07	0,07	0,06	0,07	0,07
Peak17	0,13	0,14	0,14	0,14	0,14	0,14	0,13	0,13	0,13	0,13	0,13	0,13
Peak18	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02
Peak19	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03
Peak20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Peak21	0,02	0,02	0,02	0,03	0,02	0,03	0,02	0,02	0,02	0,02	0,02	0,02
Peak22	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,01	0,02
Peak23	0,16	0,17	0,17	0,17	0,16	0,17	0,17	0,16	0,17	0,17	0,17	0,17
Impurity B	0,36	0,35	0,36	0,36	0,35	0,36	0,36	0,35	0,36	0,36	0,35	0,36
Streptomycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Impurity C	1,31	1,31	1,31	1,31	1,32	1,32	1,32	1,31	1,32	1,31	1,31	1,31
Peak28	0,91	0,89	0,90	0,89	0,89	0,89	0,90	0,90	0,90	0,90	0,90	0,90
Peak29	0,04	0,04	0,04	0,03	0,03	0,03	0,04	0,04	0,04	0,04	0,04	0,04

Mean Impurity Peak Areas in Per Cent after 6 Months

6 months	-20°C			+20°C			+37°C			+45°C		
	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean	Vial 1	Vial 2	Mean
Peak1	0,02	0,02	0,02	0,05	0,05	0,05	0,13	0,13	0,13	0,13		
Peak2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,01	0,01	0,01		
Peak3	0,00	0,00	0,00	0,01	0,00	0,01	0,01	0,01	0,01	0,01		
Peak4	0,02	0,02	0,02	0,02	0,04	0,03	0,02	0,03	0,03	0,02		
Peak5	0,07	0,06	0,07	0,07	0,06	0,07	0,07	0,06	0,07	0,07		
Peak6	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05	0,05		
Peak7	0,04	0,05	0,05	0,04	0,05	0,05	0,04	0,05	0,05	0,04		
Peak8	0,27	0,28	0,28	0,29	0,30	0,30	0,26	0,33	0,30	0,26		
Peak9	ND	ND	ND	ND	ND	ND	0,07	ND	ND	0,11		
Peak10	0,30	0,30	0,30	0,29	0,30	0,30	0,30	0,30	0,30	0,30		
Peak11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
Impurity A	0,41	0,40	0,41	0,50	0,50	0,50	0,72	0,73	0,73	0,88		
Peak13	0,47	0,46	0,47	0,46	0,46	0,46	0,46	0,46	0,46	0,46		
Peak14	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03		
Peak15	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04	0,04		
Peak16	0,08	0,08	0,08	0,07	0,08	0,08	0,07	0,07	0,07	0,06		
Peak17	0,13	0,14	0,14	0,13	0,13	0,13	0,12	0,12	0,12	0,12		
Peak18	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02		
Peak19	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03	0,03		
Peak20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
Peak21	0,02	0,02	0,02	0,03	0,02	0,03	0,03	0,02	0,03	0,02		
Peak22	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02	0,02		
Peak23	0,16	0,17	0,17	0,17	0,17	0,17	0,17	0,16	0,17	0,16		
Impurity B	0,36	0,35	0,36	0,35	0,35	0,35	0,36	0,35	0,36	0,35		
Streptomycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		
Impurity C	1,31	1,31	1,31	1,31	1,31	1,31	1,31	1,31	1,31	1,32		
Peak28	0,91	0,89	0,90	0,90	0,90	0,90	0,89	0,89	0,89	0,89		
Peak29	0,04	0,04	0,04	0,03	0,03	0,03	0,04	0,04	0,04	0,04		





Annex 3: SAS-Script used for the calculations

```

/* This is the essential script to perform the analysis of variance.
It expects a SAS-dataset "Dihydrostrepto" with the following fields:
prep: 1 for standard, 2 for test.
dose: on log-scale with the primary dose set to 0.
cdose: copy of dose.
row: indicates the row in Latin square designs.
block: the column in Latin square designs or the petri-dish in randomised block designs.
block and/or row are set to 1 if not applicable for their respective designs.
obs: the value of the observation (possibly transformed).
*/

ods select none;
proc glm data=Dihydrostrepto;
  /* Perform the Anova by progressively relaxing model assumptions */
  class prep cdose block row;
  model obs=block row prep dose dose(prepare) dose*dose cdose*prep / ss1;
  ods output OverallAnova=OverallAnova ModelAnova=ModelAnova;

data Anova(keep=source df ss ms fvalue probf);
  /* Non-linearity has to be calculated in a separate datastep */
  retain dfLin ssLin;
  set ModelAnova OverallAnova;
  if df>0 then output;
  if Source='dose*dose' then do; dfLin=df; ssLin=SS; end;
  if Source='prep*cdose' then do; dfLin=dfLin+df; ssLin=ssLin+ss; end;
  if Source='Error' then do;
    Source='Non-linearity'; FValue=(ssLin/dfLin)/ms; ProbF=1-ProbF(FValue,dfLin,df);
    ss=ssLin; df=dfLin; ms=ss/df;
    if df>0 then output;
  end;

ods select all;
proc print data=Anova noobs;
run;

/* This is the essential script to perform the potency calculations.
It expects a SAS-dataset "info" with the following fields:
Assigned: The assigned potency of the standard
mgS: weight taken of the Standard
mlS: Dilution used to prepare the primary dose of the Standard.
mgT: weight taken of the Test
mlT: Dilution used to prepare the primary dose of the Test.
*/

ods select none;
proc glm data=Dihydrostrepto;
  /* Fit the parallel line model and output the parameter estimates and covariance matrix */
  class block row;
  model obs=prep dose block row / inverse solution;
  ods output InvXPX=CovB ParameterEstimates=ParmEst;

data Estimate(keep=Low Est High);
  /* calculate the relative potency (m) */
  set ParmEst; where Parameter='prep'; a=Estimate;
  set ParmEst; where Parameter='dose'; b=Estimate;
  m=a/b;
  /* Use Fieller's theorem to compute the confidence limits */
  set CovB; where Parameter='prep'; v11=prep;
  set CovB; where Parameter='dose'; v12=prep; v22=dose;
  set Anova; where source='Error'; t=tinv(0.975,df); s=sqrt(ms);
  g=(t*t*s*s*v22)/(b*b);
  root=v11-2*m*v12+m*m*v22-g*(v11-v12*v12/v22);
  mL=(m-g*v12/v22-t*s/b*sqrt(root))/(1-g);
  mU=(m-g*v12/v22+t*s/b*sqrt(root))/(1-g);
  /* Transform the relative potency to IU by correcting for the pre-dilutions */
  set info; Correction=Assigned*mgS/mlS*mlT/mgT;
  Low=Correction*exp(mL); Est=Correction*exp(m); High=Correction*exp(mU);
  output;
  stop;

ods select all;
proc print data=Estimate noobs;
run;

```

Annex 4: Safety Data Sheet and Leaflet



SAFETY DATA SHEET

DIHYDROSTREPTOMYCIN

ISA_42688

Revised edition no : 1

Date : 4 / 7 / 2011

Supersedes : 0 / 0 / 0



Xn - Harmful

Warning

**SECTION 1 Identification of the substance/mixture and of the company/undertaking****1.1. Product identifier**

Trade name : DIHYDROSTREPTOMYCIN
 Identification of the product : Dihydrostreptomycin
 CAS No : 000128-46-1
 EC No : 204-888-2

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use : For laboratory tests and assays only.

1.3. Details of the supplier of the safety data sheet

Company identification : European Directorate for the Quality of Medicines & Healthcare
 EDQM, Council of Europe,
 7 Allée Kastner CS 30026
 F-67081 Strasbourg FRANCE
 Tel. +33 (0)3 88 41 20 35
 Fax. + 33 (0)3 88 41 27 71
 Emergency phone: +44 (0) 1235 239670

Directions for use : For any questions: www.edqm.eu/hd (HelpDesk)

1.4. Emergency telephone number

: +44(0)1235 239670

SECTION 2 Hazards identification**2.1. Classification of the substance or mixture**

Classification 67/548/EEC or 1999/45/EC

: Repr. Cat. 3; R63
 Xi; R36/37

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SECTION 2 Hazards identification (continued)

Hazard Class and Category Code Regulation (EC) N° 1272/2008 (CLP)

Health hazards : Eye irritation - Category 2A - Warning - (CLP : Eye Irrit. 2) - H319
 Reproductive toxicity - Unborn Child - Category 2 - Warning - (CLP : Repr. 2) - H361d
 Specific Target Organ Toxicity - Single exposure - Respiratory tract irritation - Category 3 - Warning - (CLP : STOT SE 3) - H335

Adverse human health effects : Deafness. Blurred vision. Exposure may produce an allergic reaction.

2.2. Label elements

Labelling 67/548/EEC or 1999/45/EC

Symbol(s)



R Phrase(s)

: Xn : Harmful
 : R36/37 : Irritating to eyes and respiratory system.
 R63 : Possible risk of harm to the unborn child.

S Phrase(s)

: S22 : Do not breathe dust.
 S36/37 : Wear suitable protective clothing and gloves.
 S45 : In case of accident or if you feel unwell, seek medical advice immediately (show the label when possible).

Labelling Regulation (EC) N°1272/2008 (CLP)

Hazard pictograms



Hazard pictograms code

: SGH09 - SGH07

Signal words

: Warning

Hazard statements

: H319 - Causes serious eye irritation.
 H335 - May cause respiratory irritation.
 H361U - Suspected of damaging the unborn child.

Precautionary statements

- General : P202 - Do not handle until all safety precautions have been read and understood.

- Prevention : P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
 P271 - Use only outdoors or in a well-ventilated area.
 P280 - Wear protective gloves/protective clothing/eye protection/face protection.

- Response : P304+P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
 P305+P351+P338+P337+P313 - IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists : Get medical advice.
 P308+P313 - IF exposed or concerned: Get medical advice/attention.



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SECTION 2 Hazards identification (continued)

- Storage : P233 - Keep container tightly closed.
P235 - Keep cool.
P403 - Store in a well-ventilated place.
P405 - Store locked up.
- Disposal considerations : P501A - Dispose of this material and its container to hazardous or special waste collection point.
P501C - Dispose of this material and its container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

2.3. Other hazards

: None under normal conditions.

SECTION 3 Composition/information on ingredients

Substance name	Contents	CAS No	EC No	Annex No	REACH	Classification
Dihydrostreptomycin		128-48-1	204-668-2	---	----	Repr. Cat. 3 (R03) Xi (R06) Repr. 2 (H361D) Eye Irr. 2 (H319) STOT SE 3 (H355)

SECTION 4 First aid measures

4.1. Description of first aid measures

First aid measures

- Inhalation : Assure fresh air breathing. Rest. If you feel unwell, seek medical advice.
- Skin contact : Remove affected clothing and wash all exposed skin area with mild soap and water, followed by warm water rinse.
- Eye contact : Rinse immediately with plenty of water. Obtain medical attention if pain, blinking, tears or redness persist.
- Ingestion : Rinse mouth. If swallowed, seek medical advice immediately and show this container or label.

4.2. Most important symptoms and effects, both acute and delayed

: See Heading 2.

4.3. Indication of any immediate medical attention and special treatment needed

In case of reactions described in hazards identification or other severe, immediate or persisting symptoms seek medical advice and call the nearest poison centre. Show the label and this safety data sheet.

SECTION 5 Fire-fighting measures

5.1. Extinguishing media

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SECTION 5 Fire-fighting measures (continued)

Extinguishing media

- Suitable extinguishing media : Water spray. Carbon dioxide. Dry powder.
- Unsuitable extinguishing media : Do not use a heavy water stream.

5.2. Special hazards arising from the substance or mixture

- Hazardous combustion products : Incomplete combustion will generate carbon monoxide and other toxic gases.

5.3. Advice for fire-fighters

- Protection against fire : Do not enter fire area without proper protective equipment, including respiratory protection.
- Surrounding fires : Use water spray or fog for cooling exposed containers.

SECTION 6 Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

- General precautions : Remove ignition sources. Evacuate area.
- Personal precautions : Spill should be handled by trained cleaning personnel properly equipped with respiratory and eye protection.

6.2. Environmental precautions

- : Do not empty into drains ; dispose of this material and its container in a safe way.

6.3. Methods and material for containment and cleaning up

- Clean up methods : Clean spills promptly. To clean the floor and all objects contaminated by this material, use : sodium hypochlorite solution. Ensure adequate ventilation.

6.4. Reference to other sections

- : See Heading 8. & 13.

SECTION 7 Handling and storage

7.1. Precautions for safe handling

- Handling : Handle in accordance with good industrial hygiene and safety procedures.
- Personal protection : Avoid all unnecessary exposure. Ensure prompt removal from eyes, skin and clothing.
- Technical protective measures : Material should be handled in a laboratory hood whenever possible.

7.2. Conditions for safe storage, including any incompatibilities

- Storage : Not intended for long-term storage. Keep container tightly closed in a cool, well ventilated place.



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SECTION 7 Handling and storage (continued)

Storage - away from : All heat sources, including direct sunlight. Open flame. Sources of ignition. Sparks. Incompatible materials, see §10

7.3. Specific end use(s)

: See Heading 1.

SECTION 8 Exposure controls/personal protection

8.1. Control parameters

: No data available.

8.2 Exposure controls

Pictograms (Precautionary statements)



Respiratory protection

: Wear approved mask. (P2)
In case of insufficient ventilation, wear suitable respiratory equipment.

Skin protection

: Wear suitable protective clothing.

Eye protection

: Chemical goggles or safety glasses.

Hand protection

: Wear suitable gloves resistant to chemical penetration.

Industrial hygiene

: Provide local exhaust or general room ventilation.
Material should be handled in a laboratory hood whenever possible.

SECTION 9 Physical and chemical properties

9.1. Information on basic physical and chemical properties

- Physical state at 20 °C : Powder.
- pH value : No data available.
- Melting point [°C] : No data available.
- Boiling point [°C] : No data available.
- Flash point [°C] : No data available.
- Solubility : No data available.
- Solubility in water : No data available.
- Log P octanol / water at 20°C : -7.510
- Auto-ignition temperature [°C] : No data available.
- Thermal decomposition [°C] : No data available.
- Explosive Properties : See Heading 2.

9.2. Other information

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SECTION 9 Physical and chemical properties (continued)

- Chemical formula : C₂₁H₄₁N₇O₁₂
- Molecular weight : 583.69

SECTION 10 Stability and reactivity

10.1. Reactivity

: See below.

10.2. Chemical stability

: Stable under normal conditions.

10.3. Possibility of hazardous reactions

Hazardous reactions : None under normal conditions.
Hazardous polymerization : Will not occur.

10.4. Conditions to avoid

Conditions to avoid : Moisture.

10.5. Incompatible materials

Materials to avoid : Acids. Bases.

10.6. Hazardous decomposition products

Hazardous decomposition products : Carbon monoxide. Nitrogen oxides. Carbon dioxide.
When heated to decomposition, emits dangerous fumes.

SECTION 11 Toxicological information

11.1. Information on toxicological effects

RTECS nr : WK2450000 (See actual entry in RTECS for complete information.)
Rat oral LD₅₀ [mg/kg] : No data available.
Rabbit dermal LD₅₀ [mg/kg] : No data available.
Rat inhalation LC₅₀ [mg/L/4h] : No data available.
Health hazards : Eye irritation - Category 2A - Warning - (CLP : Eye Irrit. 2) - H319
Reproductive toxicity - Unborn Child - Category 2 - Warning - (CLP : Repr. 2) -
H361d
Specific Target Organ Toxicity - Single exposure - Respiratory tract irritation -
Category 3 - Warning - (CLP : STOT SE 3) - H335
Risk Phrases : Irritating to eyes and respiratory system. - Possible risk of harm to the unborn child.
Acute toxicity : Deafness. Eye disorders.
Exposure may produce an allergic reaction.



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SECTION 11 Toxicological information (continued)

Chronic toxicity : No data available.
Sensitization : No data available.
Toxic for reproduction : unborn child : Category 3 : Substances which cause concern for humans owing to possible developmental toxic effects.

SECTION 12 Ecological information

12.1. Toxicity

EC50-48 Hour-Daphnia magna [mg/L] : No data available.
IC50-72h-Algae [mg/L] : No data available.
LC50-96 Hour - fish [mg/L] : No data available.

12.2. Persistence - degradability

Persistence - degradability : No data available.
Biodegradation [%] : No data available.

12.3. Bioaccumulative potential

Bioaccumulative potential : No data available.

12.4. Mobility in soil

Log P octanol / water at 20°C : -7.510

12.5. Results of PBT and vPvB assessment

: Not required.

12.6. Other adverse effects

SECTION 13 Disposal considerations

13.1. Waste treatment methods

General : Dispose of this material and its container at hazardous or special waste collection point.
Dispose in a safe manner in accordance with local/national regulations.

SECTION 14 Transport information

General information : Not regulated.

14.1. UN Number

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SECTION 14. Transport information (continued)

- UN No. : Not regulated.

14.2. Proper shipping name

- Proper shipping name : Not regulated.

14.3. Transport Hazard Classification

- ADR Class : Not regulated.

14.4. Packing group

- ADR Packing group : Not regulated.

14.5. Environmental hazards

: See Heading 2.

14.6. Special precautions for user

: Good housekeeping is needed during storage, transfer, handling, and use of this material to avoid excessive dust accumulation.

14.7. Bulk transport - annex II Marpol 73/78 - IBC

: Not applicable.

SECTION 15 Regulatory information

15.1. Safety, health and environmental regulations/legislation specific for the substance or mixture

: Ensure all national/local regulations are observed.

15.2. Chemical Safety Assessment

: See Heading 2.

SECTION 16 Other information

List of relevant R phrases	: R36/37 : Irritating to eyes and respiratory system. R63 : Possible risk of harm to the unborn child.
S Phrase(s)	: S22 : Do not breathe dust. S36/37 : Wear suitable protective clothing and gloves. S45 : In case of accident or if you feel unwell, seek medical advice immediately (show the label when possible).
Hazard statements	: H319 - Causes serious eye irritation. H335 - May cause respiratory irritation. H361U - Suspected of damaging the unborn child.
Precautionary statements	



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SECTION 16 Other information (continued)

- **General** : P202 - Do not handle until all safety precautions have been read and understood.
 - **Prevention** : P261 - Avoid breathing dust/fume/gas/mist/vapours/spray.
P271 - Use only outdoors or in a well-ventilated area.
P280 - Wear protective gloves/protective clothing/eye protection/face protection.
 - **Response** : P304+P340 - IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+P338+P337+P313 - IF IN EYES : Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists : Get medical advice.
P308+P313 - IF exposed or concerned: Get medical advice/attention.
 - **Storage** : P233 - Keep container tightly closed.
P235 - Keep cool.
P403 - Store in a well-ventilated place.
P405 - Store locked up.
 - **Disposal considerations** : P501A - Dispose of this material and its container to hazardous or special waste collection point.
P501C - Dispose of this material and its container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.
- Further information** : Revision - See : *

This document has been prepared in accordance with the MSDS requirements of the national codes of practice declared by NOHCS under s.38(1) of the National Occupational Health and Safety Commission Act 1985 (Cwlth).

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The 3RD International Standard for Dihydrostreptomycin

1. The Standard

The 3rd International Standard (IS) for Dihydrostreptomycin (ISA_39036) consists of vials of freeze-dried Dihydrostreptomycin. This preparation was established as the 3rd IS for Dihydrostreptomycin by the Expert Committee on Biological Standardization of the World Health Organization in 2011.

2. Biological Activity

The standard was calibrated in an international collaborative study involving 12 laboratories from different countries, against the 23rd IS for Dihydrostreptomycin.

The assigned potency is 19425 IU per vial for the 3rd IS for Dihydrostreptomycin.

3. Use of the Standard

Dissolve the entire content of the vial with an exact amount of solvent using gentle shaking. Transfer the solution to a plastic tube and keep at room temperature during the assay. The solution should be used as soon as possible and should be kept at 25°C maximum during assays. Unused material must be discarded and not frozen for later use. Unopened vials should be stored at -20°C.

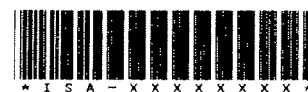
The product in the vial is freeze-dried. Do not weigh out portions of the product; dissolve it preferably by injecting solvent through the rubber stopper while avoiding the generation of pressure within the vial which might lead to a loss of material when retracting the needle. The cake should dissolve rapidly. Care should be taken to avoid any loss and rinsing steps are recommended to ensure quantitative transfer into the volumetric flask.

4. Stability

Accelerated degradation studies have shown that the standard is stable when stored in unopened vials at -20°C, with no predictable loss of potency over a period of at least 60 months. It is therefore recommended that the unopened vials are stored at -20°C or below until immediately before use.

5. References

Collaborative Study for the Establishment of the Third International Standard for Dihydrostreptomycin, WHO/BS/11.xxxx



6. Caution

This material is not for administration to humans. Safety Data Sheet is available on the EDQM website (www.edqm.eu) or on request.

7. Citation

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

8. Product liability

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