



**WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE**

**WHO/BS/03.1986
ENGLISH ONLY**

**Expert Committee on Biological Standardization
Geneva, 17 To 21 November 2003**

**COLLABORATIVE STUDY TO ESTABLISH THE
2ND WHO INTERNATIONAL STANDARD FOR
LOW MOLECULAR WEIGHT HEPARIN AND THE
LOW-MOLECULAR-MASS HEPARIN FOR ASSAY EUROPEAN
PHARMACOPOEIA BIOLOGICAL REFERENCE PREPARATION**

Elaine Gray¹, Peter Rigsby¹ and Marie-Emmanuelle Behr-Gross²

¹ National Institute for Biological Standards and Control, Blanche Lane, Potters Bar, Hertfordshire EN6 3QG, UK

² European Directorate for the Quality of Medicines, Council of Europe
224, avenue de Colmar, 67100 STRASBOURG, France

SUMMARY

Thirty laboratories participated in a collaborative study to calibrate replacements for the 1st International Standard for Low Molecular Weight Heparin and the European Pharmacopoeia Low-molecular-mass heparin for assay Biological Reference Preparation. Two freeze-dried materials and one liquid preparation were included in the study. All three samples gave excellent

./...

© World Health Organization 2003

All rights reserved. Publications of the World Health Organization can be obtained from Marketing and Dissemination, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 2476; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to Publications, at the above address (fax: +41 22 791 4806, email: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this publication is complete and correct and shall not be liable for any damages incurred as a result of its use.

intra- and inter-laboratory variations (majority of mean % geometric coefficient of variation < 10 %) when assayed against the 1st International Standard by both anti-Xa and anti-IIa assays. There were no major differences found between potency estimates using all methods and that obtained using European Pharmacopoeia method only. Overall, this study showed that the differences between the candidates are marginal. Based on a slightly lower inter-laboratory variation for the anti-Xa assays for sample B, we recommend that Sample B, 01/608 be considered as the 2nd International Standard for Low molecular Weight Heparin. Sample A, 01/592 and sample C, the liquid preparation, are to be considered as replacements for the European Pharmacopoeia 'Low-molecular-mass heparin for assay' Biological Reference Preparation.

INTRODUCTION

The 1st International Standard (IS¹) for Low Molecular Weight Heparin was established by the World Health Organisation (WHO) in 1987 (1). Since then, it has been used successfully for calibration of clinical preparations of low molecular weight heparin. It was calibrated against the 4th IS for Unfractionated Heparin and was assigned with potencies in anti-Xa and thrombin inhibitory (anti-IIa) units. Due to the depletion of the stocks of the 1st IS for Low Molecular Weight Heparin, 85/600, a replacement is required. As the number of clinical products has increased since the last calibration, a pilot study was carried out in 2001 to investigate the comparability of the current products and to select a couple of suitable candidates for the calibration study to replace the 1st IS.

The 4th IS for Unfractionated Heparin and low molecular weight heparin and eight clinical low molecular weight heparins were included in the pilot study. A detailed report of this pilot study was presented to the ECBS in November 2001 and is available from NIBSC (2). Briefly, the data indicated that due to non-parallelism and poor inter-laboratory agreement, unfractionated heparin should not be considered as a standard for anti-Xa and anti-IIa assays of low molecular weight heparins. All the low molecular weight heparins compared reasonably well against each other, and the two products (referred to as samples E and H in the study) that gave the lowest inter-laboratory variability were recommended and accepted by the participants of the study and the Expert Committee on Biological Standardization (ECBS) of the WHO to go forward as candidates for the replacement of the current IS.

As the European Directorate for the Quality of Medicine (EDQM) also required a replacement of their current European Pharmacopoeia (Ph. Eur.), Low-molecular-mass heparin for assay Biological Reference Preparation (BRP), it was agreed that it would be beneficial to carry out a joint WHO/EDQM study to assign potencies to both the replacement international and European standards.

¹ Abbreviations: IS : International Standard ; BRP : Biological Reference Preparation ; CL : Confidence limits ; ECBS: Expert Committee on Biological Standardization ; EDQM : European Directorate for the Quality of Medicines ; Ph. Eur. : European Pharmacopoeia ; gcv : Geometric coefficient of variation ; NIBSC : National Institute for Biological Standards and Control ; PEG : Polyethylene glycol ; WHO : World Health Organization ; SSC: Scientific and Standardization Committee ; ISTH: International Society on Thrombosis and Haemostasis

AIMS OF STUDY

The aim of the study was to calibrate 2 freeze-dried candidate low molecular weight heparin samples against the 1st IS for Low Molecular Weight Heparin, 85/600, with a view to establish one of the candidates as the 2nd IS for low molecular weight heparin and the other as Ph. Eur. *Low-molecular-mass heparin for assay BRP*. In addition, the potencies of a liquid preparation, intended to be a second Ph. Eur. BRP replacement batch were also to be determined.

SAMPLES

Coded samples in the study were:

- S - the 1st IS for Low Molecular Weight Heparin (85/600).
Potency : anti-Xa 1680 IU/ampoule; anti-IIa : 665 IU/ampoule.
- A - a freeze-dried porcine low molecular weight heparin (01/592).
Potency range : anti-Xa 800 – 1200 U/ampoule; anti-IIa 300 – 340 IU/ampoule.
- B - a freeze-dried porcine low molecular weight heparin (01/608).
Potency range : anti-Xa 800 – 1200 IU/ampoule, anti-IIa 300 – 340 IU/ampoule.
- C - a liquid preparation of porcine low molecular weight heparin (02/10-26)
Potency range : anti-Xa 100 - 140 IU/mL, anti-IIa 20 - 60 IU/mL.

Four sets of each S, A, B and C were provided. Each participant was asked to use the above figures as a guide and adjust the initial dilutions of each heparin so that the responses for the dilutions in the assays were in a similar range.

PARTICIPANTS

Thirty-five laboratories accepted the invitation to participate and 30 laboratories returned data for analysis. Each of the participants is referred to in this report by an arbitrarily assigned number, not necessarily representing the order of listing in appendix A.

ASSAY METHODS

Each laboratory was asked to perform their routine anti-Xa and anti-IIa chromogenic assays. Laboratories using commercial kits were advised to be aware that some kits do not provide antithrombin and rely on patient plasma as the source of antithrombin. For these laboratories, pooled normal plasma was to be used as the source of antithrombin. The details of

the assay methods used by the participants are listed in Table 1a (anti-Xa assays) and 1b (anti-IIa assays).

For the anti-Xa assays, 30 laboratories returned results. Lab 11 returned 2 sets of data, using different methods and these were treated as 2 separate sets of results and are referred to as Lab 11a and 11b. Sixteen laboratories used methods based on the Ph. Eur. monograph for low molecular weight heparins (3). The majority of modifications were the adjustment of reaction volume to allow the tests to be carried out on microtitre plates or automated instruments, while some laboratories have added Polyethylene glycol (PEG) and/or human albumin to their buffers. Five laboratories used in-house methods, while 10 laboratories used commercial kits (4 manufacturers, 5 different kits). Five laboratories used human plasma as source of antithrombin, while the rest of the participants used purified human antithrombin in their assays. Only one laboratory used human Factor Xa, the other laboratories used Factor Xa of bovine origin.

For the anti-IIa assays, 23 laboratories returned data. Six laboratories used in-house methods. Seventeen laboratories used modified Ph. Eur. methods, the modification being the addition of PEG, human serum albumin or aprotinin in the buffer and the adjustment of reaction volume for the tests to be carried out on microtitre plates or automated analysers. Two laboratories used human plasma as source of antithrombin, while the rest of the laboratories used purified human antithrombin. Six laboratories used bovine thrombin and 17 laboratories used human thrombin.

STUDY DESIGN

Participants were requested to perform four independent assays for each method, an independent assay being defined as one with a completely fresh set of ampoules and dilutions. To allow for day-to-day variation, the participants were asked to carry out the four assays, for each method, on four separate days.

Participants were asked to assay concurrently a series of at least three, preferably four dilutions of each of the 4 coded samples. The assay order of the materials (including replicates) was to be varied to give a balanced order overall. Some examples of different assay order schemes were given in the protocol for participants to choose from.

Raw assay data were to be returned together with a summary of their estimates for the potency of materials A, B and C using S, the 1st IS for Low Molecular Weight Heparin, as the standard.

RESULTS

Assay data returned

Thirty laboratories performed anti-Xa assays (180 assays in total). Twenty three laboratories additionally performed anti-IIa assays (140 assays in total).

The majority of participants were able to use scheme 1 ("8 place assay" as described in the protocol) and this accounted for 22 laboratories (anti-Xa assays) and 17 laboratories (anti-IIa assays). Almost all the remaining participants used scheme 2 ("4 place assay" as described in the protocol).

Some further points to note on the results are:

- No anti-IIa assay results were returned for ampoule set 1 in laboratory 2.
- Laboratory 11 performed two sets of anti-Xa assays – "ACL" and "Microtitre Plate". These have been assigned laboratory codes 11a and 11b respectively.
- In laboratory 24, samples 'A' and 'B' from ampoule set 1 were damaged during handling and so no results are given here.
- Laboratory 26 found that sample 'S' in ampoule set 4 was empty. This resulted in no sample 'S' being included in the ampoule set 4 assays, so no results are given here.

Statistical analysis

Based on the raw data reported, an independent, central statistical analysis was performed at NIBSC along the lines described hereafter.

Each run was treated as a separate assay. All assays were analysed using the principles of multiple parallel line bioassay (4) comparing transformed assay response to log concentration. Here, an analysis of variance gives an assessment of the linearity and parallelism of the response lines. All results were also plotted and the validity of the assays was assessed both visually and by analysis of variance, with tests for deviations from linearity and parallelism being performed at the 1 % level of significance. Responses at the extremes of the response range that showed no change with further increase or decrease of dose were omitted.

For the majority of assays, the untransformed assay responses were found to give the best linearity with log concentration. In four laboratories, a log transformation of the responses was found to be more appropriate. In one laboratory, responses were transformed to percentages relative to the estimated upper and lower limits of the dose-response curve and weighted regression of logit response on log concentration was used.

All mean potencies given in this report are unweighted geometric mean potencies. Corresponding 95 % confidence limits (CL) were also calculated. Variability between assays and laboratories has been expressed using geometric coefficients of variation (% gcv) (5).

Assay validity

It was possible to assess linearity and parallelism in almost all laboratories. An exception was laboratory 32, where a single dilution only was used for the test samples.

The majority of assays passed all validity criteria. Deviations from linearity were detected in 10 % of anti-Xa assays and 4 % of anti-IIa assays. Deviations from parallelism were detected in 2 % of anti-Xa assays and 4 % of anti-IIa assays. In most cases, these deviations were probably due to inadequate replication in the assay design. One assay by laboratory 23 (sample 'C', ampoule set 1) was excluded due to non-linearity of the standard. Following visual assessment, all other assays were included.

Potency estimates

Potency of candidate standards using the 1st IS for Low Molecular Weight Heparin, as standard

The potency estimates by the participants (data not shown) were mostly in agreement with the estimates calculated by NIBSC, with the exception of the following laboratories: Lab 5: anti-Xa potency estimates for B and C by assay 3; Lab 11: no agreement for all potency estimates and Lab 16: anti-Xa potencies for A and B. The causes for these discrepancies are not clear.

The calculated potency estimates relative to sample 'S', the 1st IS, for the anti-Xa assays are shown in tables 2 - 4 and figures 1 - 3 show the potencies expressed as % of the mean. Anti-IIa assay results are given in tables 5 - 7 and figures 4 - 6 show the potencies expressed as % of the mean. The overall mean potency estimates by all methods were 1273 anti-Xa IU/ampoule and 306 anti-IIa IU/ampoule, 1097 anti-Xa IU/ampoule and 326 anti-IIa IU/ampoule and 113 anti-Xa IU/mL and 33 anti-IIa IU/mL for samples A, B and C respectively (Table 11a). The overall mean potency estimates by Ph. Eur. Methods only were 1271 anti-Xa IU/ampoule and 306 anti-IIa IU/ampoule, 1096 anti-Xa IU/ampoule and 330 anti-IIa IU/ampoule and 112 anti-Xa IU/mL and 33 anti-IIa IU/mL for samples A, B and C respectively (Table 11b).

In general, between-assay variability was low, with gcv's being ≤ 10 % in most cases. As shown in Tables 10a and 10b, the inter-laboratory or between-laboratory variability, including all methods or Ph. Eur. method only, was of similar magnitude, with gcv's ranging from 5 % to 8 %.

The Shapiro-Wilk test (6) for normality was applied to the log potencies in each case. All showed no deviations from the normal distribution, apart from the anti-IIa results for sample 'A'. In this case, satisfactory normality was achieved by excluding the results from laboratory 13, so the mean potency has also been calculated excluding this laboratory.

In figures 1-3, laboratories have been shaded according to the kit used in their anti-Xa assays. The 'Stachrom' kit gives significantly lower results for samples 'A' and 'B' when compared to all other kits ($p < 0.001$ and $p = 0.004$ respectively in unpaired t-test on log potencies).

The potency estimates and the anti-Xa to anti-IIa ratios for all 3 samples obtained taking results from all methods were similar to the potencies taking from assays using Ph. Eur. Methods only, with no statistically significant differences found between these estimates (Tables 11a and 11b).

Potency of Sample B using Sample A as the putative standard

The potency estimates of sample B relative to sample A, assuming the assigned potencies for 'A' are 1273 IU/ampoule and 306 IU/ampoule for anti-Xa and anti-IIa activities respectively (Table 11a), are shown in Tables 8a and 9a. The overall mean potency estimates by all methods were 1090 anti-Xa IU/ampoule and 325 anti-IIa IU/ampoule. Similar overall potency estimates were obtained using Ph. Eur. methods alone: 1090 anti-Xa IU/ampoule and 331 anti-IIa/ampoule. Note that laboratories where 'B' was not included in the same assay as 'A' have been excluded. These potency estimates are non-significantly 0.6 % and 0.3 % lower, for anti-Xa and anti-IIa activities respectively, than those estimated by calibration against the IS. Inter- and intra-laboratory variability were also similar to those obtained using the IS as the standard.

Potency of Sample A using Sample B as the putative standard

The potency estimates of sample A relative to sample B, assuming the assigned potencies for 'B' is 1097 IU/ampoule and 326 IU/ampoule for anti-Xa and anti-IIa activities respectively (Table 11a), are shown in Tables 8b and 9b. The overall mean potency estimates by all methods were 1281 anti-Xa IU/ampoule and 307 anti-IIa IU/ampoule. Similar overall potency estimates were obtained using Ph. Eur. methods alone: 1281 anti-Xa IU/ampoule and 301 anti-IIa/ampoule. Note that laboratories where 'A' was not included in the same assay as 'B' have been excluded. These potency estimates are non-significantly 0.6 % and 0.3 % higher, for anti-Xa and anti-IIa activities respectively, than those estimated by calibration against the IS. Inter- and intra-laboratory variability were also similar to those obtained using the IS as the standard.

Potency of Sample C using Sample A or B as putative standards

The potency estimates for sample 'C' have also been calculated relative to samples 'A' and 'B'. These are shown in tables 8c and 9c. Note that laboratories where 'C' was not included in the same assay as 'A' or 'B' have been excluded. Results have been converted to IU/mL using the mean potencies already calculated 1273 and 1097 anti-Xa IU/ampoule, 306 and 326 anti-IIa IU/ampoule for 'A' and 'B' respectively. The overall mean potency estimates by all methods relative to A were 113 anti-Xa IU/ampoule and 33 anti-IIa IU/ampoule; and relative to B were 112 anti-Xa IU/ampoule and 33 anti-IIa IU/ampoule. The overall potency estimates obtained using Ph. Eur. Methods alone relative to A were 114 anti-Xa IU/ampoule and 33 anti-IIa/ampoule and relative B were 113 anti-Xa IU/ampoule and 33 anti-IIa IU/ampoule. The results

agree with those calculated using the current IS. The magnitude of between-assay and between-laboratory variability is also the same.

DEGRADATION STUDY

Preliminary accelerated degradation study, monitored using anti-Xa assay, by NIBSC of both candidates A and B showed no sign of change after 12 months storage as assessed by fitting to the Arrhenius equation. Ampoules for sample A stored at +20, +37 and +45°C were found to have 92.0, 95.7 and 94.8 % activity respectively when compared to the -70°C ampoules. Similar activities were found with sample B: 96.5, 92.4 and 92.8 % activity was obtained for the +20, +37 and +45°C ampoules when assayed against the -70°C material. Continual real time degradation study of the -20°C against ampoules stored at -70°C and further accelerated degradation study at elevated temperature will be carried out to monitor the stability of the replacement standard.

DISCUSSION

The aim of this collaborative study was to calibrate 2 freeze-dried candidate low molecular weight heparin samples against the 1st IS for Low Molecular Weight Heparin, with a view to establish one of the candidates as the 2nd IS for Low molecular weight heparin and the other as Ph. Eur. *Low-molecular-mass heparin for assay BRP*. In addition, the potencies of a liquid preparation, intended to be a second Ph. Eur. BRP replacement batch were also to be determined.

Similar to results found in the pilot study, there was good parallelism between the candidates and the 1st IS, thus giving good comparison and valid potency estimates for all 3 samples.

Intra-laboratory (between assay) variations for all the samples assayed by both anti-Xa and anti-IIa assays were good, with the majority of the % gcvs well under 10 % (Tables 2 - 7). This indicates that the majority of the participants were able to perform their choice of assays with precision. For the anti-Xa assay, there was a tendency for some of the laboratories using commercial kits to have higher % gcvs (Tables 2 - 4). However, there was no correlation of high between assay % gcv with the use of any particular kit, suggesting that the higher variability was not due to the poor performance of the kits. There was also no other obvious correlation between performance and parameters such as methods, reagents or instrumentation.

The inter-laboratory variations were excellent (Tables 10a and 10b) and were similar when taking into account of all methods or the Ph. Eur. methods alone. Percent gcv were found to range from 5 - 8 %. For the anti-Xa assays, including all methods, the % gcv for sample B when assayed against the IS is slightly lower than that obtained with sample A, 5.2 % as opposed to 8.2 %. There were no differences in the % gcv obtained with the anti-IIa assays.

In terms of potency estimates, there was close agreement between those obtained by all methods and the Ph. Eur. methods alone for all 3 samples (Tables 11a-c, Fig 1-6). Although one of the commercial kits did give significantly lower anti-Xa potencies for samples A and B than

other methods (Fig 1 and 2), overall these results indicate that the in-house methods and the commercial kits gave comparable results and performed similarly to the pharmacopoeial methods.

In terms of potency estimates, there was also close agreement between those obtained by using either the 1st IS or preparation B for samples A and C, the candidate Ph. Eur. BRPs (Tables 11a-c, Fig 7-10).

CONCLUSIONS AND RECOMMENDATIONS

Both freeze dried candidate materials, samples A and B, gave low intra- and inter-laboratory variability. Based on the slightly lower inter-laboratory variability for the anti-Xa assays, sample B is to be recommended as the replacement IS with assigned potencies using results by all methods.

In line with the pharmacopoeial policy, samples A and C, the proposed Ph. Eur. BRPs are recommended to be assigned with potencies by Ph. Eur. methods. Taking into account that the 1st IS should be replaced in the near future by preparation B and that the calculated potency estimates relative to sample B and to sample S (1st IS) do not differ significantly for A and C, it is proposed to assign potencies for the replacement BRP batches relative to sample B.

Therefore it will be recommended:

- i) to the ECBS to establish Preparation B (NIBSC code 01/608) as the 2nd IS for Low Molecular Weight Heparin with following unitages:

- anti-Xa potency: 1097 IU/ampoule
- anti-IIa potency: 326 IU/ampoule

and, provided that Preparation B becomes the 2nd IS for Low Molecular Weight Heparin,

- ii) to the Ph. Eur. Commission to adopt:

Preparation C (liquid, 02/10-26) and Preparation A (Freeze-dried preparation, 01/592) as *Low-molecular-mass heparin for assay BRP* replacement batches with following unitages:

Preparation C

- anti-Xa potency: 113 IU/mL
- anti-IIa potency: 33 IU/mL

Preparation A

- anti-Xa potency: 1281 IU/ ampoule
- anti-IIa potency: 301 IU/ampoule

COMMENTS FROM THE PARTICIPANTS AND THE MEMBERS OF THE SCIENTIFIC AND STANDARDIZATION COMMITTEE (SSC) OF THE INTERNATIONAL SOCIETY ON THROMBOSIS AND HAEMOSTASIS (ISTH)

Twenty-three out of the 30 participants agreed with the proposal that preparation B should be recommended to be the replacement international standard. The other 7 participants have not sent any comments or responses. The members of the ISTH/SSC Subcommittee on Control of Anticoagulation have also approved the recommendation. There was only a minor comment from one member of the Subcommittee and it was related to the title of the report. The recommendation has also been subsequently approved at the business meeting of the SSC on 16 July 2003.

PRODUCT SUMMARY FOR THE PROPOSED 2ND INTERNATIONAL STANDARD FOR LOW MOLECULAR WEIGHT HEPARIN, 01/608

Proposed 2nd International Standard for Low Molecular Weight Heparin	
Code : 01/608	
Presentation	Sealed glass din ampoules
Number of ampoules available	5000
Excipient	water
Coefficient of variation of the fill	0.06%
Residual moisture after lyophilisation and secondary desiccation	0.28%
Mean dry weight	9.0 mg/ampoule

REFERENCES

- (1) Barrowcliffe TW, Curtis AD, Johnson EA and Thomas DP. An international standard for low molecular weight heparin. *Thrombosis and Haemostasis*. 1988; 60 (1): 1-7.
- (2) Gray E, Sands, AD and Barrowcliffe TW. Report on the pilot study on proposed candidate materials for the 2nd international standard for low molecular weight heparin. WHO ECBS report 2001.
- (3) Monograph 0828 *Heparins, Low-Molecular Mass*. Ph.Eur. 4th Ed. 2003 ;Sup. 4.5 : 3713-3715.
- (4) Finney DJ. *Statistical methods in biological assay*. 3rd edn London: Charles Griffin 1978.
- (5) Kirkwood TBL. Geometric means and measures of dispersion. *Biometrics* 1979; 35: 908-909.
- (6) Armitage P and Berry G. *Statistical Methods in Medical Research*. Blackwell Scientific Publications. 1994.

ACKNOWLEDGEMENTS

We would like to thank the participants of the study and the following manufacturers for their kind donation of candidate samples:

Sanofi Synthelabo (Notre Dame de Bondeville, France)

Nordmark Arzneimittel GmbH & Co.KG (Uetersen, Germany)

APPENDIX A : List of Participants

Dr B Parma
R & D Department
Opocem SpA
via Pacinotti, 3
41040 Corlo di Formigine
Modena
Italy

Dr PN Shaklee
Biocascade Incorporated
107 Skyline Drive PO Box 98
Arlington
Wisconsin 53911-0098
USA

Dr G Rautmann and Ms G Cozie
European Directorate for the Quality of Medicines
Council of Europe
224 - 226 Avenue de Colmar
67100 Strasbourg
France

Dr H Nagel
Nordmark Arzneimittel GmbH & Co.KG
Analysis of Natural materials
Pinnauallee 4
D25436 Uetersen
Germany

Dr M Schroder
Biological research laboratory
Leo Pharma A S
55 Industriparken
DK-2750 Ballerup
Denmark

Drs K MacKinnon and M Kovacs
Department of Hematology
London Health Sciences Centre
Room 2111 800 Commissioners Road E
London
Ontario N6A 4G5
Canada

Ms V Dupont
Aventis Pharma
9 quai Jules Guesde, CPV SAS - Bat 38 - Laboratoire 118
94400 Vitry sur Seine
France

Dr C Alonso
Division de Productos Biologicos y Biotecnologia
Agencia Espanola del Medicamento
Carretera de Majadahonda-Pozuelo Km2
E-Majadahonda 28220 Madrid
Spain

Mr B van Genugten
Diosynth bv
Building RR 2031
5340 BH Oss
The Netherland

Professor J Fareed
Loyola University Medical Center, Hemostasis Research Labs
Loyola University Medical Center
2160 South First Avenue, Bldg 102 Room 2652
Maywood
IL 60153
USA

Professor J Harenberg
Internal Department of Medicine
University Hospital Mannheim
Theodor-Kutzer-Ufer 1-3
D68167 Mannheim
Germany

Dr E Sandberg
Biological Department
Danish Medicines Agency
Frederikssundsvej 378
DK-2700 Bronshoj
Denmark

Drs ME Albertengo, L Oliva and M Esnaola
Departamento Productos Biologicos
Instituto Nacional de Medicamentos
Av Caseros 2161
1264 Buenos Aires
Argentina

Dr C Loh
Therapeutics Goods Administration
Po Box 100
Woden ACT 2606
Australia

Dr JJ Descombe
Sanofi-synthelabo
1 rue de l'Abbaye
76960 Notre Dame de Bondeville
France

Dr R Domanig
Development Department
Sandoz GmbH
Schaftenau Plant
Biochemiestrasse 10
A-6336 Langkampfen
Austria

Professor JI Weitz
Henderson Research Center
711 Concession Street
Hamilton
Ontario L8V 1C3
Canada

Dr AC Fylling
Biopharmaceutical Development
Biovitrum
Lindhagensg 133, Bldg S34/5
11276 Stockholm
Sweden

Dr Y Gourmelin
Diagnostica Stago
2 rue Pierre Fossati
95130 Franconville
France

Dr F Lackner
BIFA
Possingergasse 38
1160 Vienna
Austria

Dr J Tapon-Bretonniere
Laboratoire d'Hematologie, Service Du Pr Anne-Marie Fischer
Hopital Europeen Georges Pompidou
20 rue Leblanc
75908 Paris
Cedex 15
France

Dr J Dufaux
Service de Controle des Medicaments de l'Association Pharmaceutique Belge
Rue Stevin, 137
1000 Brussels
Belgium

Dr J Dayan-Kenigsberg
Unite Biotechnologie, Biochimie de Proteins, Macromolecules
Direction des Laboratoires et des Contrôles
AFSSAPS
143, Bd A France
93285 St Denis
France

Dr S Kitchen
University Department of Haematology/Coagulation Unit
Royal Hallamshire Hospital
Glossop Road
Sheffield S10 2JF
UK

Mr FC Arntzen
Biological Laboratory, Norwegian Medicines Agency
Norwegian Medicines Agency
Sven Oftedals vei 6
N-0950 Oslo
Norway

Mrs K Erlandsson-Persson
Medical Products Agency
Box 26
SE-75103 Uppsala
Sweden

Dr ML Wiesel
Etablissement Francais du Sang
10 rue Spielmann
67065 Strasbourg
France

Dr S Thomas
Division of Haematology
NIBSC
Blanche Lane
Potters Bar
Hertfordshire EN6 3QG
UK

Dr F Nicham
Serbio
PAE Parispace 3
125 Av. Louis Roche
92635 - Gennevilliers Cedex
France

Dr E Holmer
Carmeda AB
Kanalvägen 3B
SE-194 61 Upplands Väsby
Sweden

FIGURES LEGEND

Figures 1 - 3. Potency estimates of the 3 candidates, samples A to C, respectively, relative to sample S, the 1st IS for Low Molecular Weight Heparin, 85/600 by anti-Xa assays.

The number in the square denotes the laboratory code. Each square represents the geometric mean estimate from the laboratory expressed as a percentage of the overall mean. The shading represents the different methods.

Figures 4 - 6. Potency estimates of the 3 candidates, samples A to C, respectively, relative to sample S, the 1st IS for Low Molecular Weight Heparin, 85/600 by anti-IIa assays.

The number in the square denotes the laboratory code. Each square represents the geometric mean estimate from the laboratory expressed as a percentage of the overall mean. The shading represents the different methods.

Figures 7 - 8. Potency estimates of the 2 candidate BRPs, samples A and C, respectively, relative to sample B, the candidate 2nd IS for Low Molecular Weight Heparin, 01/608, by anti-Xa assays.

The number in the square denotes the laboratory code. Each square represents the geometric mean estimate from the laboratory expressed as a percentage of the overall mean. The shading represents the different methods.

Figures 9 - 10. Potency estimates of the 2 candidate BRPs, samples A and C, respectively, relative to sample B, the candidate 2nd IS for Low Molecular Weight Heparin, 01/608, by anti-IIa assays.

The number in the square denotes the laboratory code. Each square represents the geometric mean estimate from the laboratory expressed as a percentage of the overall mean. The shading represents the different methods.

Table 1a. Details of Anti-Xa Assay Methods

Lab	Method	FXa	Antithrombin	Measurement	Instrument
1	Ph. Eur.	B	H PUR	E	ACL7000
2	in-house	B	H PUR	K	STACompact
3	Ph. Eur. *	B	H PUR	E	PI Reader
4	Ph. Eur. *	B	H PUR	K	ACL300R
5	Ph. Eur. *	B	H PUR	K	Cobas Mira
6	IL-Test Heparin	B	H PUR	K	ACL300+
7	Ph. Eur. *	B	H PUR	E	PI Reader
8	Ph. Eur. *	B	H PUR	K	PI Reader
10	in-house	B	H PUR	K	PI Reader
11a	in-house	B	H PLA	K	ACL300+
11b	Spectrozyme fXa	B	H PUR	E	PI Reader
12	in-house	B	H PUR	E	PI Reader
13	Ph. Eur. *	B	H PUR	E	PI Reader
15	Coatest Heparin	B	H PUR	E	PI Reader
16	Ph. Eur. *	B	H PUR	K	Cobas Farall
17	Ph. Eur.	B	H PUR	E	Spectrometer
18	Ph. Eur. *	B	H PUR	E	Spectrometer
20	Stachrom	B	H PUR	E	Amax 190+
21	in-house	B	H PUR	K	STACompact
23	Stachrom	B	H PUR	E	Spectrometer
24	Ph. Eur. *	H	H PUR	K	Amax 190+
26	Rotachrom	B	H PLA	K	STAR
27	Ph. Eur. *	B	H PUR	E	PI Reader
28	Coatest Heparin	B	H PUR	K	PI Reader
29	Coamatic	B	H PLA	K	Sysmex CA6000 and CA7000
30	Ph. Eur. *	B	H PUR	E	PI Reader
31	Ph. Eur. *	B	H PUR	E	PI Reader
32	Rotachrom	B	H PLA	K	STACompact
33	Ph. Eur. *	B	H PUR	K	IL-Futura
34	Stachrom	B	H PUR	E	Spectrometer
35	Ph. Eur. *	B	H PUR	K	PI Reader

* = modified Ph. Eur. methods (see text for details)

B = bovine, H = human, PLA = plasma, PUR = purified, E = endpoint, K = kinetic, PI Reader = plate reader

Table 1b. Details of Anti-IIa Assay Methods

Lab	Method	Thrombin	Antithrombin	Measurement	Instrument
1	Ph. Eur. *	H	H PUR	E	ACL7000
2	in-house	H	H PUR	K	STACompact
3	Ph. Eur. *	H	H PUR	E	PI Reader
4	Ph. Eur. *	H	H PUR	E	PI Reader
5	Ph. Eur. *	B	H PUR	K	Cobas Mira
7	Ph. Eur. *	H	H PUR	E	PI Reader
8	Ph. Eur. *	H	H PUR	K	PI Reader
10	in-house	H	H PUR	K	PI Reader
11	in-house	H	H PLA	K	ACL300+
12	in-house	H	H PLA	E	PI Reader
13	Ph. Eur. *	B	H PUR	E	Spectrometer
15	Ph. Eur. *	B	H PUR	E	PI Reader
16	Ph. Eur. *	B	H PUR	K	Cobas Fara II
17	Ph. Eur.	H	H PUR	E	Spectrometer
18	Ph. Eur. *	B	H PUR	E	Spectrometer
20	Spectrolyse IIa	B	H PUR	K	ACL7000
21	in-house	H	H PUR	K	STACompact
24	Ph. Eur. *	H	H PUR	K	Amax 190+
27	Ph. Eur. *	H	H PUR	E	PI Reader
30	Ph. Eur. *	H	H PUR	E	PI Reader
31	Ph. Eur. *	H	H PUR	E	PI Reader
33	Ph. Eur. *	H	H PUR	K	IL-Futura
35	Ph. Eur. *	H	H PUR	K	PI Reader

* = modified Ph. Eur. methods (see text for details)

B = bovine, H = human, PLA = plasma, PUR = purified

E = endpoint, K = kinetic, PI Reader = plate reader

Table 2. Sample A: anti-Xa potencies expressed in IU/ampoule relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	<i>gcv</i> (%)
1	1331	1328	1316	1353	1332	1.0
2	1173	1194	1223	1223	1203	2.1
3	1452	1385	1297	1398	1382	4.8
4	1274	1258	1278	1284	1274	0.9
5	1218	1190	1201	1188	1199	1.1
6	1354	1270	1332	1308	1316	2.8
7	1240	1223	1184	1230	1219	2.0
8	1138	1176	1197	1236	1186	3.5
10	1221	1221	1235	1180	1214	2.0
11a	1232	1463	1244	1436	1340	1.0
11b	1314	1746	1218	1491	1429	17.0
12	1521	1403	1404	1539	1465	9.6
13	1221	1322	1241	1251	1258	5.1
15	1440	1189	1256	1191	1265	3.5
16	1306	1254	1325	1317	1300	9.4
17	1331	1277	1344	1298	1312	2.5
18	1286	1329	1492	1191	1320	2.4
20	1123	1101	1115	1102	1110	9.8
21	1168	1188	1181	1154	1173	1.0
23	1105	1389	1176	929	1138	1.3
24		1589	1383	1421	1462	18.1
26	1307	1314	1480		1365	7.6
27	1283	1212	1099	1309	1223	7.3
28	1288	1306	1327	1355	1319	8.2
29	1240	1054	1323	1129	1182	2.2
30	1152	1176	1164	1245	1184	10.5
31	1183	1189	1149	1222	1186	3.5
32	1281	1915	1355	1642	1528	2.6
33	1261	1300	1290	1202	1263	20.2
34	1116	1139	1188	1202	1161	3.6
35	1296	1266	1286	1250	1274	3.6

Overall geometric mean: 1273 (95 % CL: 1237 – 1310)
Between-laboratory *gcv*: 8.2 %
Ph. Eur. method only geometric mean: 1271 (95 % CL: 1232 – 1312)
n = 16; Between-laboratory *gcv*: 6.1 %

Table 3. Sample B: anti-Xa potencies expressed in IU/ampoule relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	gcv (%)
1	1137	1107	1072	1127	1111	2.6
2	1029	1060	1038	1135	1065	4.5
3	1158	1038	1190	1269	1161	8.7
4	1078	1057	1089	1112	1084	2.1
5	1036	1024	864	1027	985	9.1
6	1177	1119	999	1160	1112	7.7
7	1066	1063	1068	1081	1069	0.8
8	1078	1032	1073	1138	1079	4.1
10	1075	1081	1115	1083	1088	1.7
11a	1065	1049	1221	1090	1104	7.1
11b	1110	1270	1059	1134	1141	8.0
12	1211	1156	932	1277	1136	14.8
13	1002	1150	1066	1069	1070	5.8
15	1176	1020	1064	1093	1087	6.1
16	1114	1120	1104	1103	1110	0.7
17	1056	1081	1187	1118	1109	5.2
18	1055	1128	1199	1019	1098	7.4
20	1042	1024	1024	992	1020	2.1
21	1031	1033	1032	1040	1034	0.5
23	1124	1263	964	895	1052	16.7
24		1171	1252	1175	1199	3.8
26	1159	1127	1188		1158	2.7
27	993	1124	1201	1133	1110	8.3
28	1106	959	1155	1174	1095	9.6
29	998	1414	1213	956	1131	19.9
30	1071	1044	994	1150	1063	6.3
31	1153	1185	1110	1108	1138	3.3
32	1099	2010	996	1190	1272	36.8
33	1124	1129	1147	1094	1123	2.0
34	855	1065	1082	1074	1014	12.1
35	1094	1001	1039	1038	1042	3.7

Overall geometric mean: 1097 (95 % CL: 1077 - 1118)
 Between-laboratory gcv: 5.2 %
 Ph. Eur. method only geometric mean: 1096 (95 % CL: 1070 - 1123)
 n = 16; Between-laboratory gcv: 4.9 %

Table 4. Sample C: anti-Xa potencies expressed in IU/mL relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	<i>gcv</i> (%)
1	114	111	107	113	111	2.7
2	110	112	109	113	111	1.9
3	120	123	107	121	117	6.6
4	108	110	111	114	111	2.0
5	106	107	126	103	110	9.6
6	116	109	112	117	113	3.3
7	120	118	115	118	118	1.7
8	111	107	102	106	108	3.4
10	101	108	120	115	111	7.5
11a	96	98	110	106	102	6.6
11b	134	143	100	135	127	17.4
12	134	117	116	136	125	8.9
13	99	108	105	113	106	5.7
15	110	107	101	101	105	4.4
16	115	116	118	115	118	1.1
17	112	114	117	114	114	1.9
18	104	124	128	112	117	10.2
20	119	113	116	115	116	2.2
21	106	109	106	108	107	1.4
23		128	108	98	111	14.4
24	124	130	133	119	128	5.1
26	129	126	144		133	7.5
27	113	117	112	117	115	2.4
28	108	108	107	108	108	0.5
29	100	127	108	98	108	12.3
30	112	108	94	118	108	10.4
31	113	100	100	110	108	6.5
32	103	198	85	95	113	46.3
33	113	112	114	104	111	4.6
34	98	106	106	109	105	4.7
35	115	124	106	102	111	9.1

Overall geometric mean: 113 (95 % CL: 110 – 115)
Between-laboratory *gcv*: 6.4%

Ph. Eur. method only geometric mean: 112 (95 % CL: 109 – 115)
n=16; Between-laboratory *gcv*: 8.0 %

Table 5. Sample A: anti-Ila potencies expressed in IU/ampoule relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	gcv (%)
1	310	333	304	316	316	4.0
2		306	282	314	300	5.8
3	312	328	304	310	313	3.4
4	322	304	286	292	300	5.4
5	294	306	303	309	303	2.1
7	308	301	303	313	307	1.8
8	314	279	311	282	298	6.5
10	365	298	295	311	316	10.4
11	314	262	205	348	277	26.2
12	296	286	277	291	288	2.9
13	468	435	397	447	436	7.2
15	284	314	307	317	305	5.1
16	337	321	311	313	321	3.7
17	299	305	306	296	301	1.7
18	290	270	290	291	285	3.8
20	360	355	320	426	363	12.6
21	307	312	306	323	312	2.5
24		340	366	336	347	4.7
27	266	282	299	279	281	4.9
30	324	303	285	307	305	5.3
31	302	296	289	283	292	2.9
33	328	318	317	295	314	4.6
35	303	318	295	320	309	4.0
Overall geometric mean: 311 (95 % limits: 298 – 324)						
Between-laboratory gcv: 10.0 %						
Excluding laboratory 13: 306 (95 % limits: 298 – 315)						
Between-laboratory gcv: 6.3 %						
Ph. Eur. method only geometric mean: 306 (95 % CL: 298 - 314)						
n = 16; Between-laboratory gcv: 5.1 %						

Table 6. Sample B: anti-Ila potencies expressed in IU/ampoule relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	gcv (%)
1	339	358	323	334	338	4.4
2		304	326	305	312	4.1
3	344	316	361	352	343	5.9
4	325	281	307	315	306	6.4
5	323	337	342	322	331	3.2
7	332	333	334	331	332	0.5
8	319	358	332	330	334	5.0
10	361	301	316	328	326	8.0
11	292	309	224	405	301	27.5
12	276	286	314	311	296	6.5
13	367	405	350	370	372	6.3
15	308	339	291	327	316	6.8
16	340	346	330	356	343	3.2
17	300	303	326	321	312	4.2
18	313	332	338	331	328	3.4
20	389	315	323	348	343	9.9
21	311	323	323	326	321	2.2
24		337	356	341	345	2.9
27	289	302	309	299	300	2.9
30	369	331	347	361	352	5.0
31	307	296	305	315	306	2.5
33	353	345	352	340	347	1.8
35	324	291	320	339	318	6.6

Overall geometric mean: 326 (95 % CL: 318 – 335)
Between-laboratory gcv: 6.1 %

Ph. Eur. method only geometric mean: 330 (95 % CL: 321 - 340)
n = 17; Between-laboratory gcv: 6.0 %

Table 7. Sample C: anti-IIa potencies expressed in IU/mL relative to the 1st IS (S)

Lab	Ampoule 1	Ampoule 2	Ampoule 3	Ampoule 4	Geometric Mean	gcv (%)
1	37	37	34	33	35	5.6
2	.	30	30	33	31	6.0
3	33	34	33	35	34	3.1
4	36	34	31	32	33	6.9
5	34	33	32	33	33	1.7
7	38	37	37	36	37	2.5
8	34	32	31	35	33	5.0
10	36	32	33	33	33	5.4
11	27	33	27	41	31	21.7
12	32	32	29	31	31	5.3
13	32	39	35	39	36	10.0
15	28	32	30	32	30	5.3
16	37	32	32	35	34	8.5
17	32	33	32	32	32	1.2
18	31	34	34	34	33	4.9
20	42	38	39	34	38	9.2
21	33	32	35	34	33	3.5
24	32	34	37	34	34	6.1
27	28	28	33	30	30	7.2
30	39	33	31	32	34	10.9
31	35	33	37	33	35	6.1
33	37	34	37	31	35	8.7
35	34	29	34	30	32	8.7

Overall geometric mean: 33 (95 % CL: 32 – 34)
Between-laboratory gcv: 6.4 %

Ph. Eur. method only geometric mean: 33 (95 % CL: 32 – 34)
n=17; Between-laboratory gcv: 5.8 %

Table 8a. Sample B: anti-Xa potencies expressed in IU/ampoule relative to preparation A

Lab	Geometric Mean	gcv (%)
1	1061	1.9
2	1126	3.8
3	1069	10.4
5	1046	9.2
6	1076	8.3
7	1117	2.1
10	1141	1.9
11a	1049	15.1
11b	1016	8.8
12	987	11.0
13	1083	2.6
15	1094	5.0
16	1087	3.2
18	1059	3.0
20	1170	1.5
21	1122	1.6
24	1044	10.9
26	1080	5.2
27	1155	15.6
28	1057	8.5
29	1218	26.0
30	1143	4.0
32	1059	18.8
33	1133	1.9
Overall geometric mean: 1090 (95 % CL: 1068 – 1113)		
Between-laboratory gcv: 4.9 %		
Ph. Eur. method only geometric mean (n=11): 1090		
(95 % CL: 1064 – 1117)		
Between-laboratory gcv: 4.4 %		

Table 8b. Sample A: anti-Xa potencies expressed in IU/ampoule relative to preparation B

Lab	Geometric Mean	<i>gcv</i> (%)
1	1316	1.9
2	1240	3.8
3	1306	10.4
5	1336	9.2
6	1298	8.3
7	1251	2.1
10	1224	1.9
11a	1331	15.1
11b	1374	8.8
12	1415	11.0
13	1289	2.6
15	1277	5.0
16	1285	3.2
18	1319	3.0
20	1194	1.5
21	1244	1.6
24	1338	10.9
26	1293	5.2
27	1209	15.6
28	1321	8.5
29	1146	26.0
30	1221	4.0
32	1318	18.8
33	1233	1.9
<p>Overall geometric mean: 1281 (95 % CL: 1255 – 1307) Between-laboratory <i>gcv</i>: 4.9 %</p> <p>Ph. Eur. method only geometric mean (n=11): 1281 (95 % CL: 1250 – 1313) Between-laboratory <i>gcv</i>: 4.4 %</p>		

Table 8c. Sample C: anti-Xa potencies expressed in IU/mL relative to preparations A and B

Lab	Relative to A:		Relative to B:	
	Geometric Mean	gcv (%)	Geometric Mean	gcv (%)
1	106	2.0	110	0.5
2	117	2.4	114	2.9
3	108	3.6	111	12.6
5	110		111	1.5
6	110	2.6	112	6.6
7	123	0.5	121	1.9
10	116	8.0	108	5.6
11a	97	12.2	102	3.9
11b	109	3.6	121	9.1
12	107	5.1	109	5.0
13	105	7.2	106	5.7
15	114	2.5	115	1.4
16	111	2.7	113	3.4
18	133	1.3	124	2.1
20	116	1.8	113	1.2
21	123	8.4	118	5.3
24	124	1.6	126	5.0
26	119	7.2	113	8.3
27	104	2.2	108	10.0
28	116	20.6	104	7.7
29	116	8.8	111	5.0
30	114	5.5	102	7.9
32	112	2.0	108	2.8
33	115	2.5	113	7.5
	Overall geometric mean: 113 (95 % CL: 110 - 117)	Between-laboratory gcv: 6.9 %	Overall geometric mean: 112 (95 % CL: 109 - 115)	Between-laboratory gcv: 5.8 %
	Ph. Eur. method only geometric mean (n=11): 114 (95 % CL: 108 - 120)	Between-laboratory gcv: 7.7 %	Ph. Eur. method only geometric mean (n=11): 113 (95 % CL: 108 - 118)	Between-laboratory gcv: 7.2 %

Note: no % gcv available for lab 5 when C was assayed against A, as only one assay was performed where C was assayed against A.

Table 9a. Sample B: anti-Ila potencies expressed in IU/ampoule relative to preparation A

Laboratory	Geometric Mean	gcv (%)
1	328	1.6
2	317	10.0
3	335	9.4
5	334	3.4
7	332	2.1
10	316	3.8
11	333	11.5
12	315	8.8
15	317	6.3
16	327	5.0
18	352	5.6
20	389	13.4
21	314	2.1
24	304	2.2
27	327	2.1
30	354	4.8
33	338	3.0
<p>Overall geometric mean: 325 (95 % CL: 317 – 333) Between-laboratory gcv: 5.2 %</p> <p>Ph. Eur. method only geometric mean (n=11): 331 (95 % CL: 322 – 341) Between-laboratory gcv: 4.2 %</p>		

Table 9b. Sample A: anti-Ila potencies expressed in IU/ampoule relative to preparation B

Laboratory	Geometric Mean	<i>gcv</i> (%)
1	304	1.6
2	314	10.0
3	298	9.4
5	299	3.4
7	301	2.1
10	316	3.8
11	300	11.5
12	316	8.8
15	315	6.3
16	305	5.0
18	283	5.6
20	346	13.4
21	317	2.1
24	328	2.2
27	305	2.1
30	282	4.8
33	295	3.0
<p>Overall geometric mean: 307 (95 % CL: 299 – 315) Between-laboratory <i>gcv</i>: 5.2 %</p> <p>Ph. Eur. method only geometric mean (n=11): 301 (95 % CL: 293 – 310) Between-laboratory <i>gcv</i>: 4.2 %</p>		

Table 9c. Sample C: anti-I₂a potencies expressed in IU/mL relative to preparations A and B

Lab	Relative to A:		Relative to B:	
	Geometric Mean	<i>gcv</i> (%)	Geometric Mean	<i>gcv</i> (%)
1	34	5.0	34	3.8
2	31	5.2	32	8.7
3	33	4.5	32	6.6
5	33	4.1	33	5.6
7	37	3.6	36	2.5
10	32	5.2	33	2.9
11	35	22.8	34	13.3
12	33	3.7	34	11.3
15	31	1.0	31	5.3
16	32	6.3	32	8.4
18	36	7.6	33	2.3
20	32	21.0	36	10.7
21	33	4.2	34	3.4
24	31	0.7	33	2.2
27	32	3.6	32	5.1
30	34	6.2	31	8.7
33	34	5.3	33	6.8
	Overall geometric mean: 33 (95 % CL: 32 – 34) Between-laboratory <i>gcv</i> : 5.0%		Overall geometric mean: 33 (95 % CL: 32 – 34) Between-laboratory <i>gcv</i> : 4.4%	
	Ph. Eur. method only (n=11): 33 (95% CL: 32 – 34) Between- laboratory <i>gcv</i> : 5.6%		Ph. Eur. method only (n=11): 33 (95% CL: 32 – 34) Between- laboratory <i>gcv</i> : 4.1%	

Table 10a. Summary of Inter-laboratory variation of potency assays relative to 1st IS (S) expressed as gcv %, by all methods

Assay Type	A	B	C
Anti-Xa	8.2	5.2	6.4
Anti-IIa	6.3	6.1	6.4

Table 10b. Summary of Inter-laboratory variation of potency assays relative to 1st IS (S) expressed as gcv %, by Ph. Eur. methods

Assay Type	A	B	C
Anti-Xa	6.1	4.9	6.0
Anti-IIa	5.1	6.0	5.8

Table 11a. Summary of potency estimates relative to 1st IS (S) by all methods

Assay Type	IU/ampoule		IU/mL
	A	B	C
Anti-Xa	1273	1097	113
Anti-IIa	306	326	33
Anti-Xa: Anti-IIa Ratio	4.16	3.37	3.42

Table 11b. Summary of potency estimates relative to 1st IS (S) by Ph. Eur. methods

Assay Type	IU/ampoule		IU/mL
	A	B	C
Anti-Xa	1271	1096	112
Anti-IIa	306	330	33
Anti-Xa: Anti-IIa Ratio	4.15	3.32	3.39

Table 11c. Summary of potency estimates of samples A and C relative to preparation B

Assay Type	Methods	Potency Geometric mean	
		A (IU/ampoule)	C (IU/mL)
Anti-Xa	All	1281	112
	Ph. Eur.	1281	113
Anti-IIa	All	307	33
	Ph. Eur.	301	33
Anti-Xa: Anti-IIa Ratio	All	4.17	3.39
	Ph. Eur.	4.25	3.42

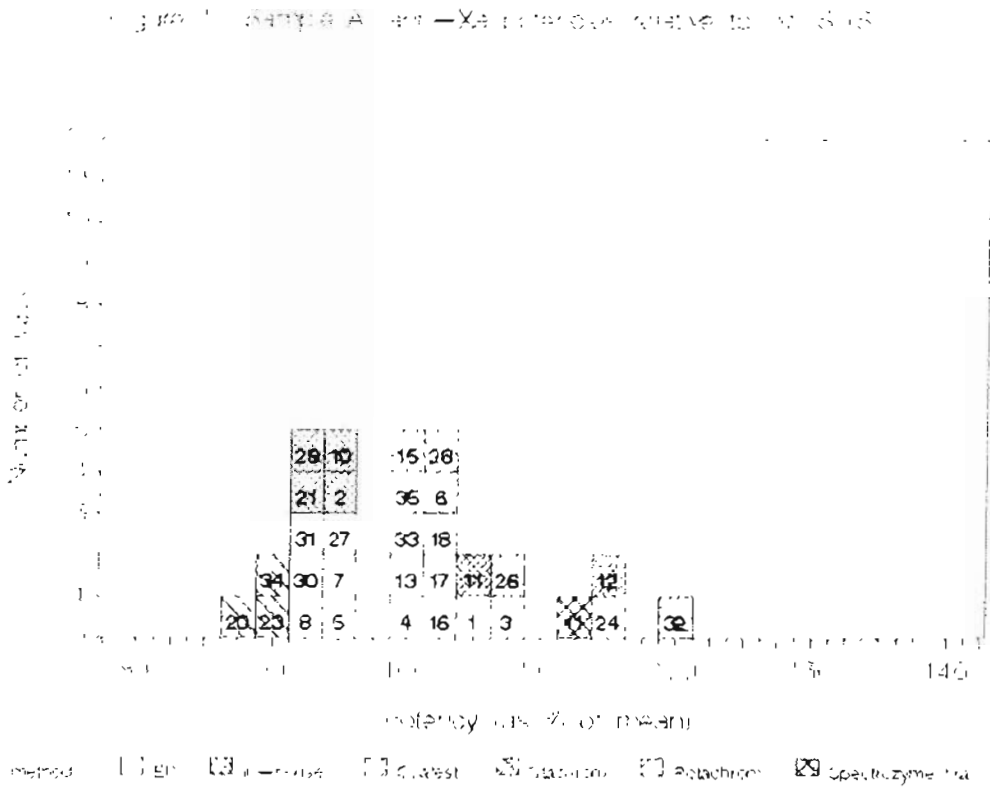


Figure 2. Sample B: anti-Xa potencies relative to 1st IS (S)

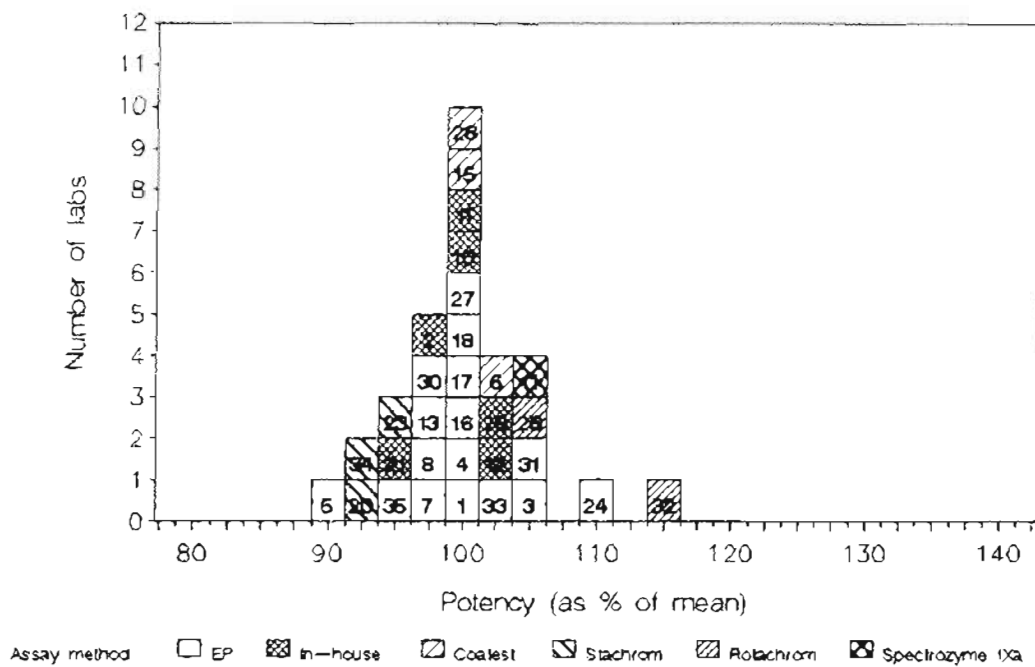


Figure 3. Sample C: anti-Xa potencies relative to 1st IS (S)

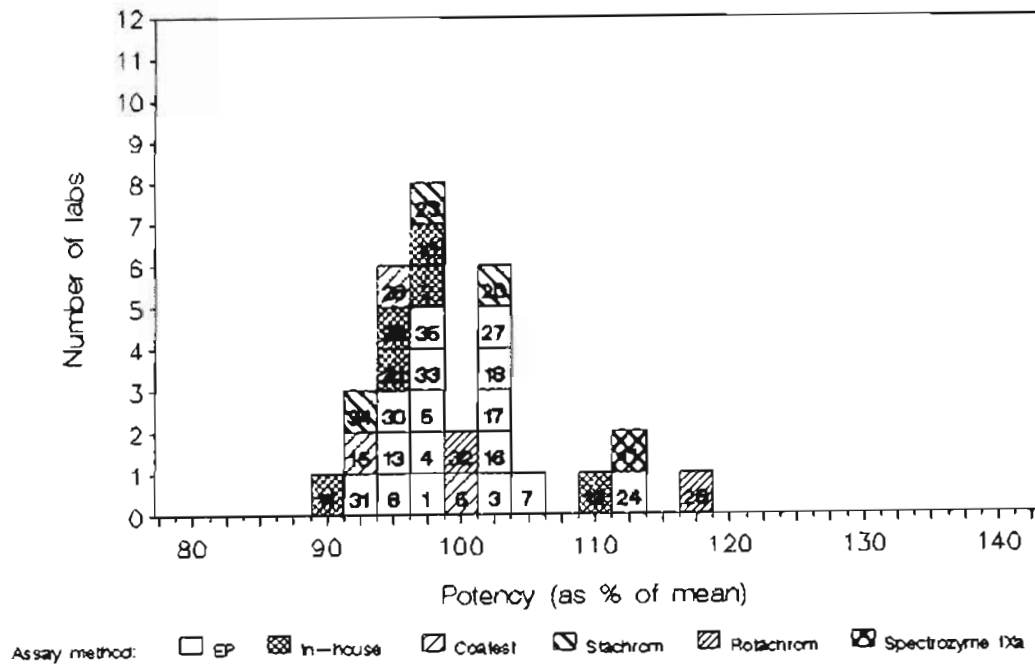


Figure 4. Sample A: anti-Ila potencies relative to 1st IS (S)

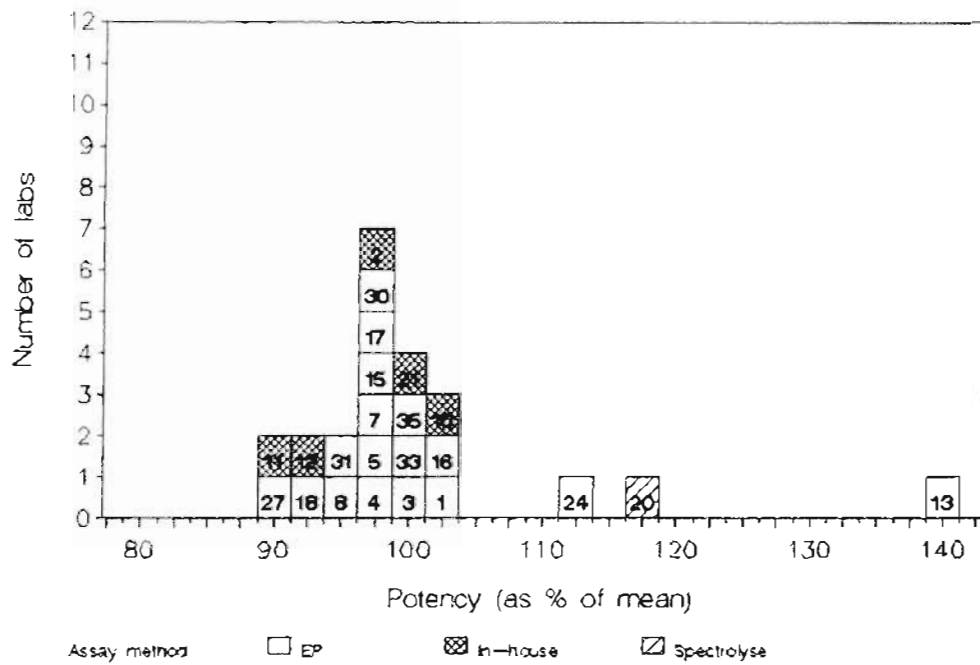


Figure 5 Sample 5 part - 1a potencies relative to 1st S (1st)

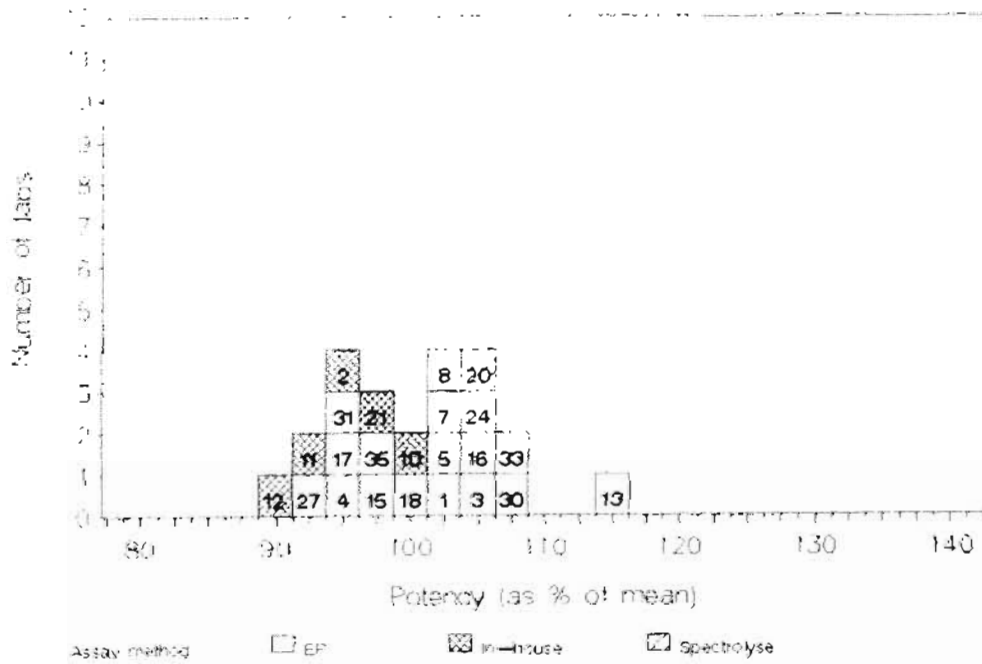


Figure 6. Sample C anti- β potencies relative to 1st IS (S)

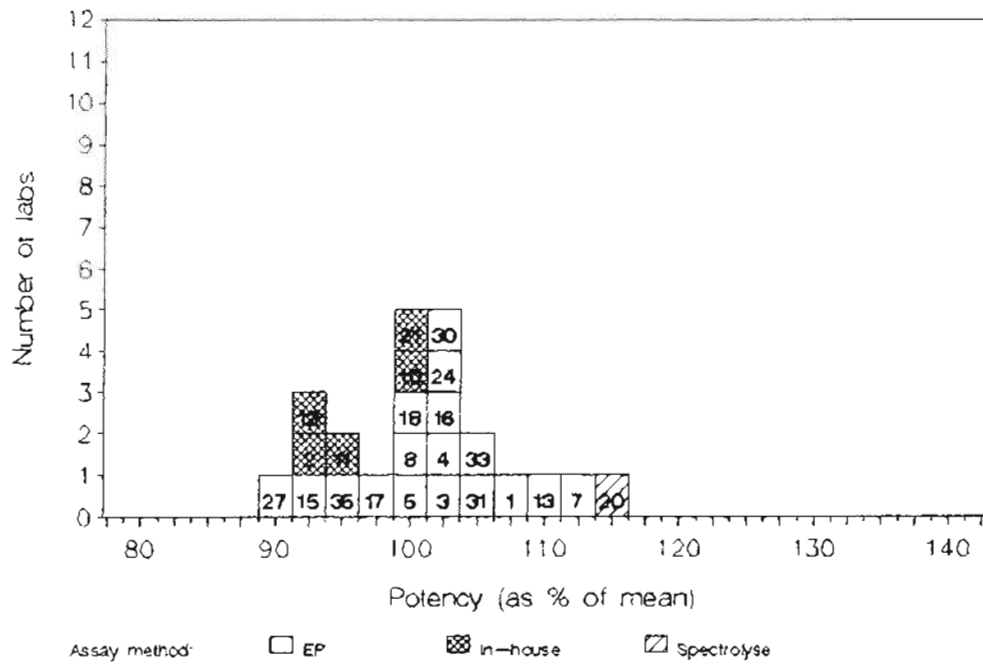


Figure 7. Comparison of the X-ray spectra of the two samples.

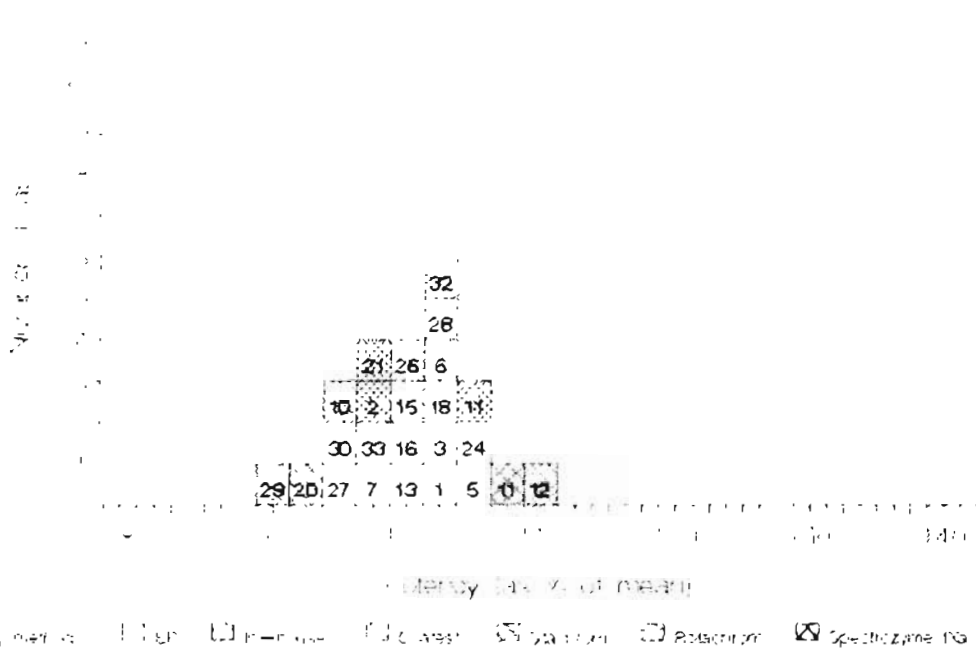


Figure 8 Sample C anti-Xa potencies relative to sample B

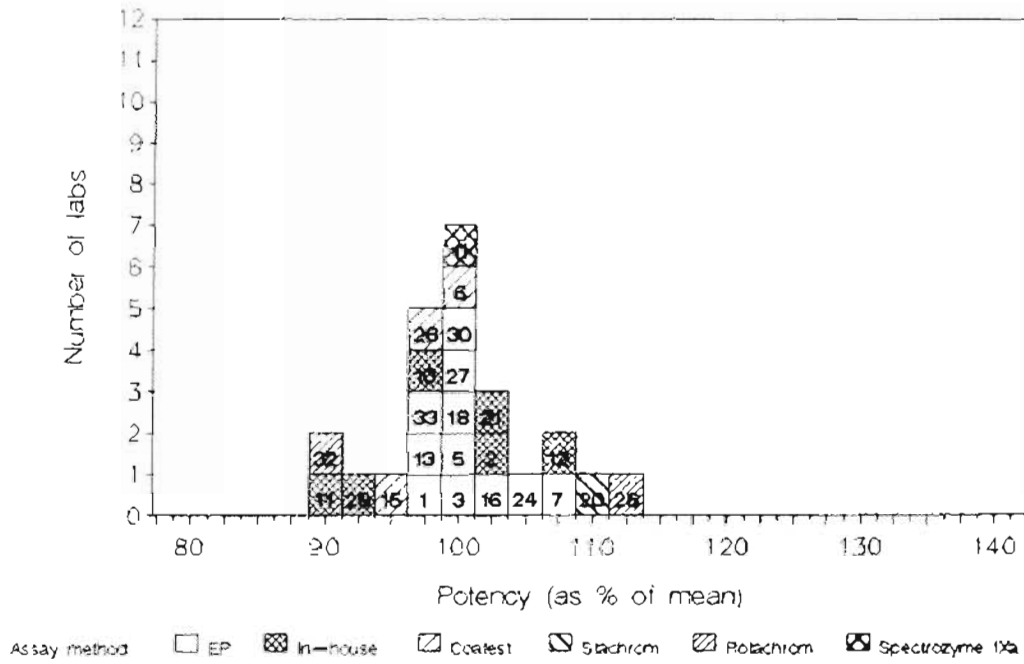


Figure 9. Sample A. anti-IIIa potencies relative to sample B

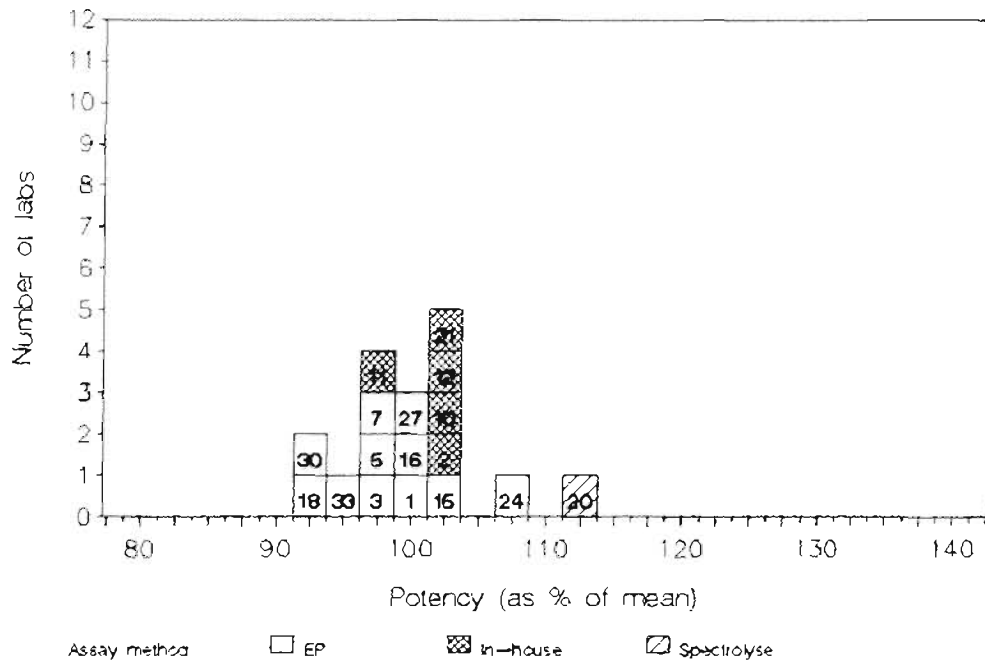
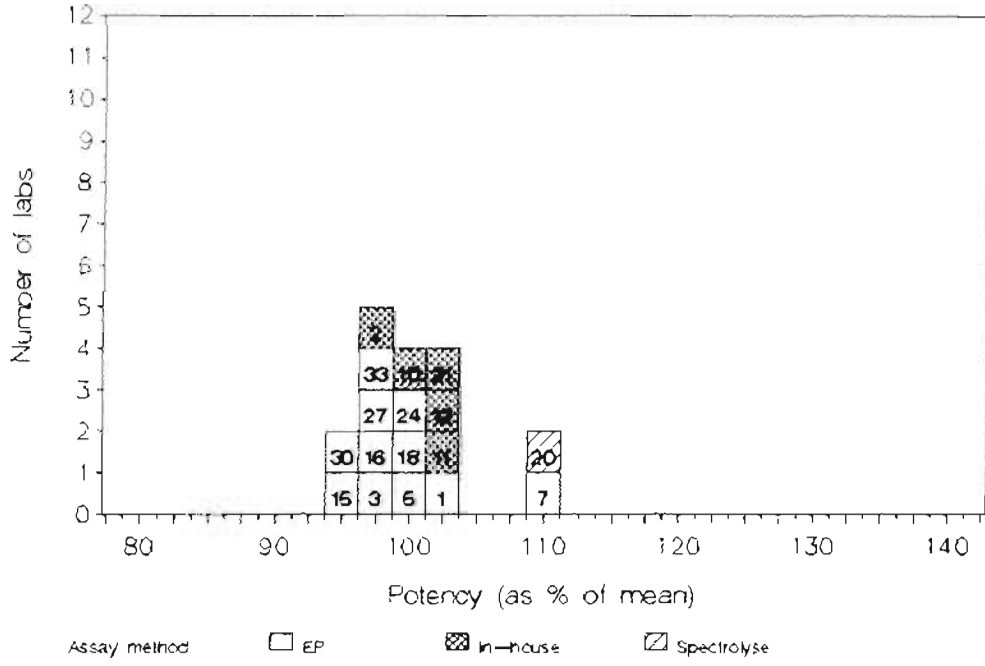


Figure 10 Sample C anti-Ila potencies relative to sample B



===