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**PROPOSED 1<sup>st</sup> INTERNATIONAL STANDARD FOR FACTOR VIII INHIBITOR  
PROGRESS REPORT ON COLLABORATIVE STUDY  
(Version 03/07/06)**

Sanj Raut, Louisa Faulkner and \*Alan Heath

*Haemostasis Section and Biostatistic Section, National Institute for Biological Standards  
and Control, Potters Bar, Hertfordshire, EN6 3QGUK*

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## SUMMARY

Following the recommendations from the ISTH/SSC SSC FVIII/FIX Subcommittee and the Factor VIII Inhibitor Standardisation Working Party (SWP), a collaborative study involving 21 laboratories was carried out to calibrate the FVIII inhibitor value for the proposed 1<sup>st</sup> International Standard for factor VIII (FVIII) Inhibitor.

5 candidate preparations (rabbit FVIII polyclonal antibody (PAb), 2 humanised monoclonal antibodies (MAbs I & II), human low titre (X) and high titre (Y) inhibitor plasmas) were assessed against 2 human inhibitor patient plasmas. Variability between Inhibitor methods and between the different FVIII assays were also assessed.

Overall, preparation Y (05/206) was the most suitable candidate material for the proposed 1st IS FVIII Inhibitor Plasma standard with an overall Bethesda Titre of 8.2 BU/vial and an overall GCV of 17.5%. Preliminary stability study showed a predicted loss of inhibitor titre potency of < 0.01% per year at -20°C.

Data from this study was discussed at the SSC FVIII/FIX Subcommittee meeting, and it was decided, that feedback from the participants be collated and then further discussions amongst the Factor VIII Inhibitor Standardisation Working Party (SWP) would take place as to the next steps to be taken.

## INTRODUCTION

The international need for a reference FVIII inhibitor standard was established at the FVIII/IX Subcommittee in 2001 and the project ratified by the Subcommittee in 2003.

Previous clinical and inhibitor studies had shown large inter-laboratory variabilities with CVs ranging from 33-202%, and that inter-laboratory CVs improved when measurements of FVIII inhibitors were carried out in the presence of an inhibitor reference standard. The aim of this study was therefore to develop a definitive WHO International Reference Preparation for measurement of FVIII Inhibitors in plasma.

## MATERIALS

There were 7 freeze-dried test materials provided:

- 99/658 Anti-Factor VIII Monoclonal Antibody Concentrate, potency < 40 BU/vial**
- 99/660 Anti-Factor VIII Monoclonal Antibody Concentrate, potency < 40 BU/vial**
- 01/460 Anti-Factor VIII Polyclonal Antibody Concentrate, potency < 40 BU/vial**
  
- 05/196 Anti-Factor VIII Antibody Concentrate (X), potency < 10 BU/vial**
- 05/206 INFECTIOUS Anti-Factor VIII Antibody Plasma (Y), potency < 40 BU/vial**
  
- 06/023PM Anti-Factor VIII Antibody Plasma (S), potency < 10 BU/ampoule**
- 06/024PM Anti-Factor VIII Antibody Plasma (T), potency < 40 BU/ampoule**

Laboratories were asked to use their routine assay methods for the measurement of FVIII inhibitor activity. Four independent FVIII inhibitor assays were requested from each laboratory (in replicates)

Samples were distributed to 25 expert laboratories. Data were received from 21 participants.

## RESULTS

### *Individual Laboratories' Estimation of Inhibitor Titres and Inter-Laboratory Variability*

Individual laboratory mean inhibitor levels for all 7 preparations are shown in the form of stacking histograms in Figures 1-7. Each box in the histograms represents the mean estimate (from an individual laboratory) as a percentage of the overall mean. Boxes are also labelled with the code number of the laboratory. Clear boxes represent one-stage FVIII assay data; shaded grey boxes represent chromogenic FVIII assay data.

The overall mean estimates of inhibitor levels for all 7 samples are given in Table 1. The variability between laboratories, expressed as coefficients of variation (CV's ) gave figures that were generally very good, particularly compared to the data found in previous collaborative inhibitor studies, ranging from 17-34% (Table 1). Preparation Y was with the candidate with the lowest CV of 17.6%.

On comparison of Inhibitor methods (Table 2), no significant differences in inhibitor titres were observed between the Classical Bethesda assays and the Nijmegen modification assays. However, no real differences in the CVs were observed between the Nijmegen and the Classical Bethesda assays. Preparation Y was with the candidate with the lowest CVs.

On comparison of FVIII potency assays (Table 3), only sample T and the rabbit polyclonal antibody (RPAb) showed a significantly higher inhibitor titres for the one stage assays compared to the chromogenic assays. The inter-laboratory variability, however was much improved with the chromogenic FVIII potency assays with CVs reduced for all the samples when assaying with the chromogenic method compared to the one-stage method. Preparation Y was with the candidate with the lowest CVs.

On comparing inter-laboratory variabilities for the 2 inhibitor patient samples (S & T) relative to each of the 5 candidates, Preparation Y again was found to be the candidate with the lowest reduced CVs when measuring inhibitors in both patient plasmas (Table 4).

## DISCUSSION

For all 7 samples, inhibitor titre results obtained without a reference standard gave CVs between 17-34%. This was remarkable as previously reported CVs were much greater.

For the overall mean inhibitor titres, comparison of the Nijmegen vs Bethesda assays, and also the comparison of one-stage vs chromogenic FVIII methods, the candidate with lowest

CVs was preparation Y. On assessing the patients' inhibitor plasma relative to the 5 candidate preparation, once again preparation Y gave the lowest CVs of 17 & 18%. It was interesting that no marked improvement in CVs were observed for the Nijmegen method compared to the Bethesda method, but a marked improvement in the CVs was observed for the chromogenic assay compared to the one-stage assay.

Overall preparation Y (05/206) was the most suitable candidate material for the proposed 1st IS FVIII Inhibitor Plasma standard with an overall Bethesda Titre of 8.2 BU/vial and an overall GCV of 17.5%. Preliminary stability studies show a predicted loss of inhibitor titre potency of < 0.01% per year at -20°C. This preparation, however, is HCV and HIV 1 & 2 positive. We are currently seeking feedback from participants.

**Table 1. Overall mean Bethesda inhibitor titres (BU/ml) for all samples and inter-laboratory CVs.**

BU Titre	99/658 MAb (I)	99/660 MAb (II)	01/460 RPAb	05/196 X	05/206 Y	06/023 S	06/024 T
Mean	11.4	24.5	12.9	2.6	8.2	4.2	9.0
Range	4 - 21	18 - 35	8 - 29	1.6 - 4.1	5 - 12	2.3 - 6.0	5.1 - 13.3
%CV	31.8	23.9	33.9	28.0	17.6	22.0	20.8

Table 2. Comparisons of Inhibitor titre estimates for all samples relative to different inhibitor methods. Parenthesis shows inter-laboratory CVs.

BU Titre	99/658 MAB (I)	99/660 MAB (II)	01/460 RPAb	05/196 X	05/206 Y	06/023 S	06/024 T
Nijmegen n=15 (%CV)	11.2 (37.9)	22.8 (20.8)	12.6 (38.0)	2.6 (26.1)	8.1 (16.4)	4.2 (22.7)	8.8 (19.4)
Bethesda n=6 (%CV)	12.0 (11.1)	28.7 (23.1)	13.5 (24.7)	2.5 (35.0)	8.5 (21.4)	4.3 (22.3)	9.6 (24.3)

*None of the differences were statistically significant  
(p<0.05 using Wilcoxon 2 sample test)*

**Table 3. Comparisons of Inhibitor titre estimates for all samples relative to different FVIII potency assays. Parenthesis shows inter-laboratory CVs.**

<b>BU Titre</b>	<b>99/658 MAb (I)</b>	<b>99/660 MAb (II)</b>	<b>01/460 RPAb</b>	<b>05/196 X</b>	<b>05/206 Y</b>	<b>06/023 S</b>	<b>06/024 T</b>
<b>1-Stage n=18 (%CVs)</b>	<b>11.2 (34.6)</b>	<b>23.6 (23.8)</b>	<b>13.5* (32.8)</b>	<b>2.7 (27.8)</b>	<b>8.1 (18.9)</b>	<b>4.2 (23.6)</b>	<b>9.4* (19.1)</b>
<b>Chromogenic n=3 (%CVs)</b>	<b>12.8 (7.2)</b>	<b>29.6 (17.5)</b>	<b>9.2 (2.5)</b>	<b>2.1 (20.1)</b>	<b>8.6 (10.2)</b>	<b>4.3 (10.9)</b>	<b>7.0 (8.5)</b>

*\* differences between methods were statistically significant (p<0.05 using Wilcoxon 2 sample test)*

Table 4. Inter-laboratory variabilities of inhibitor patient samples. Comparisons of CVs relative to different candidate standards.

Patient Sample Tested	Sample Used as Standard				
	99/658 MAB (I)	99/660 MAB (II)	01/460 RPAb	05/196 X	05/206 Y
Inhibitor S (06/023) %CV=22.0	49.3	19.3	26.4	25.4	18.3
Inhibitor T (06/024) %CV=20.8	44.4	26.0	16.9	25.2	17.6

Figure 1. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

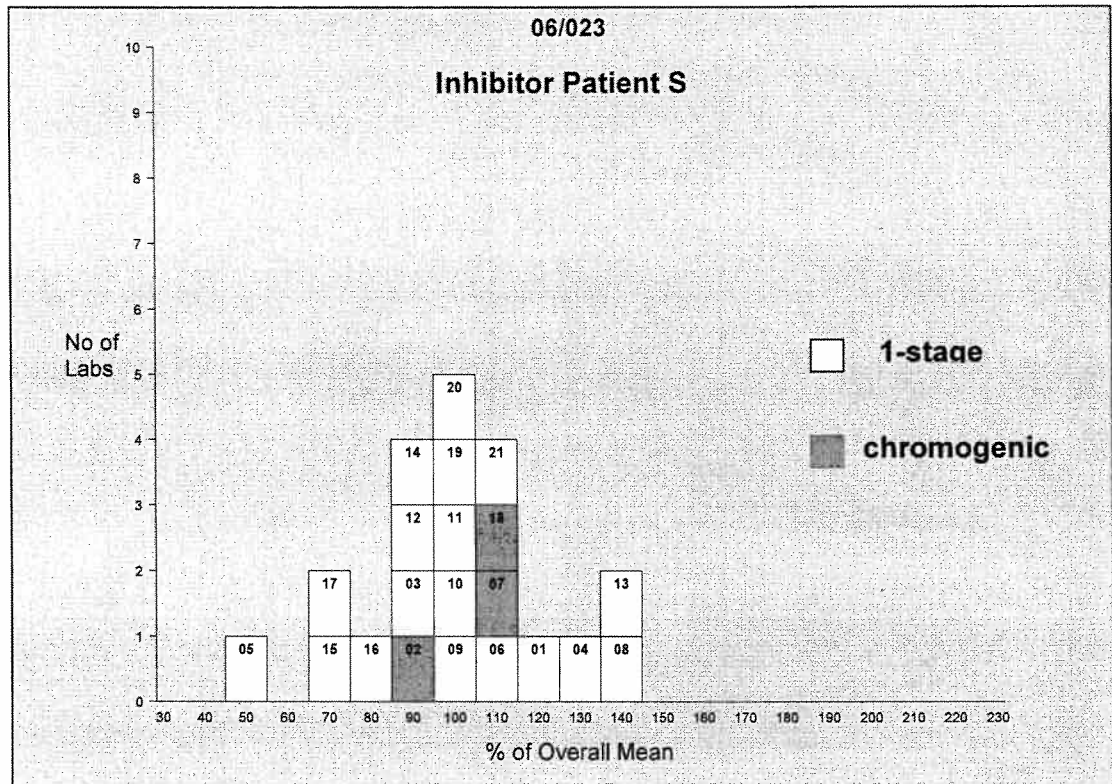


Figure 2. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

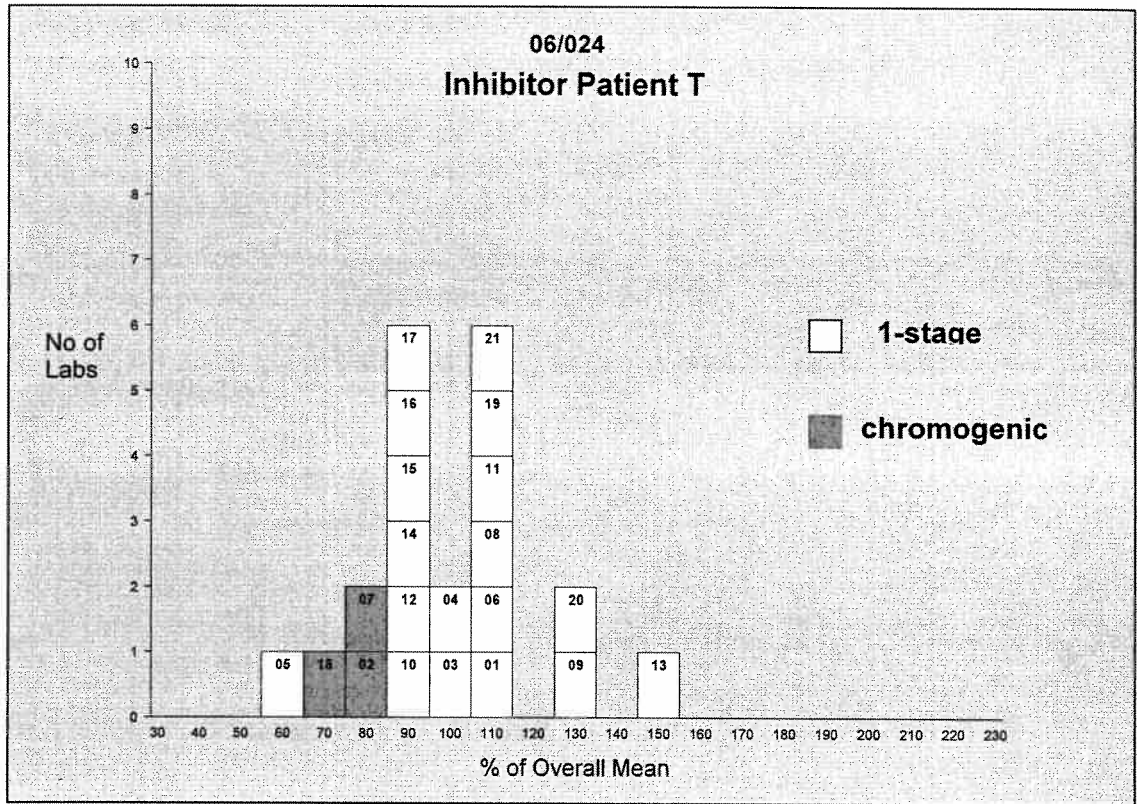


Figure 3. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

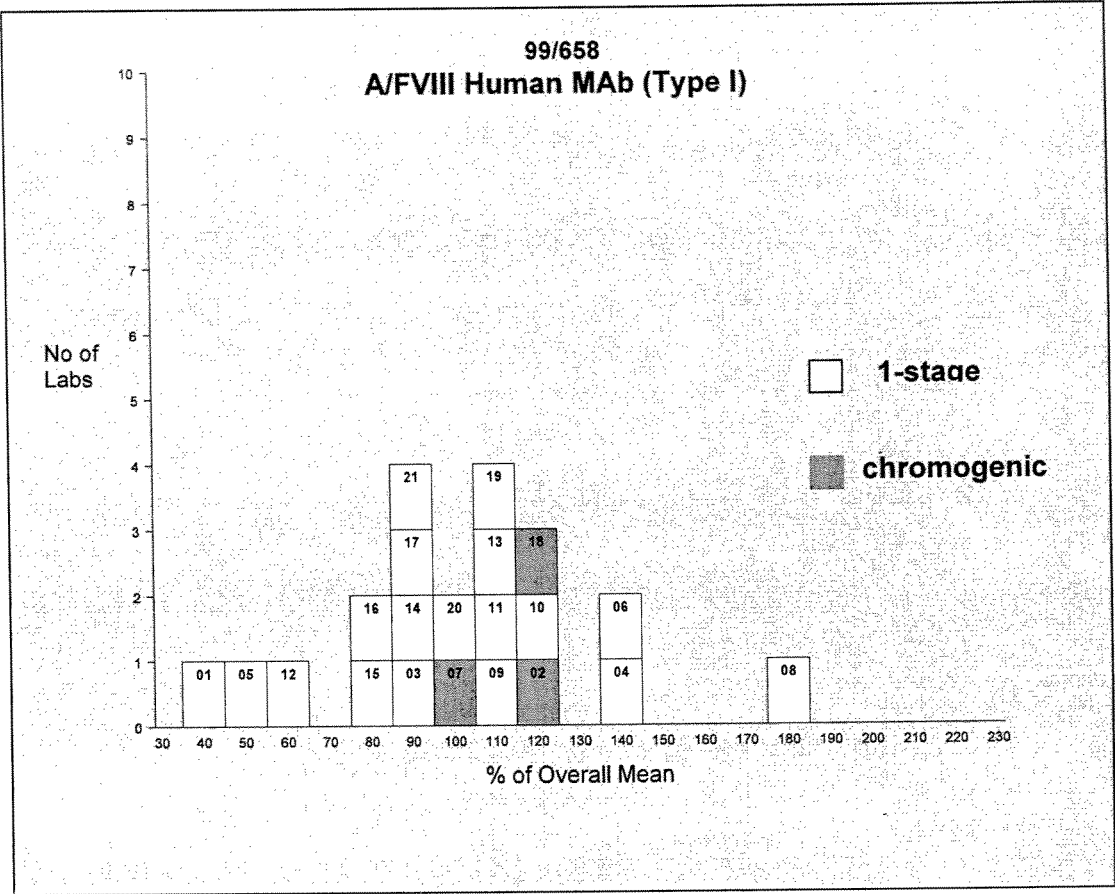


Figure 4. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

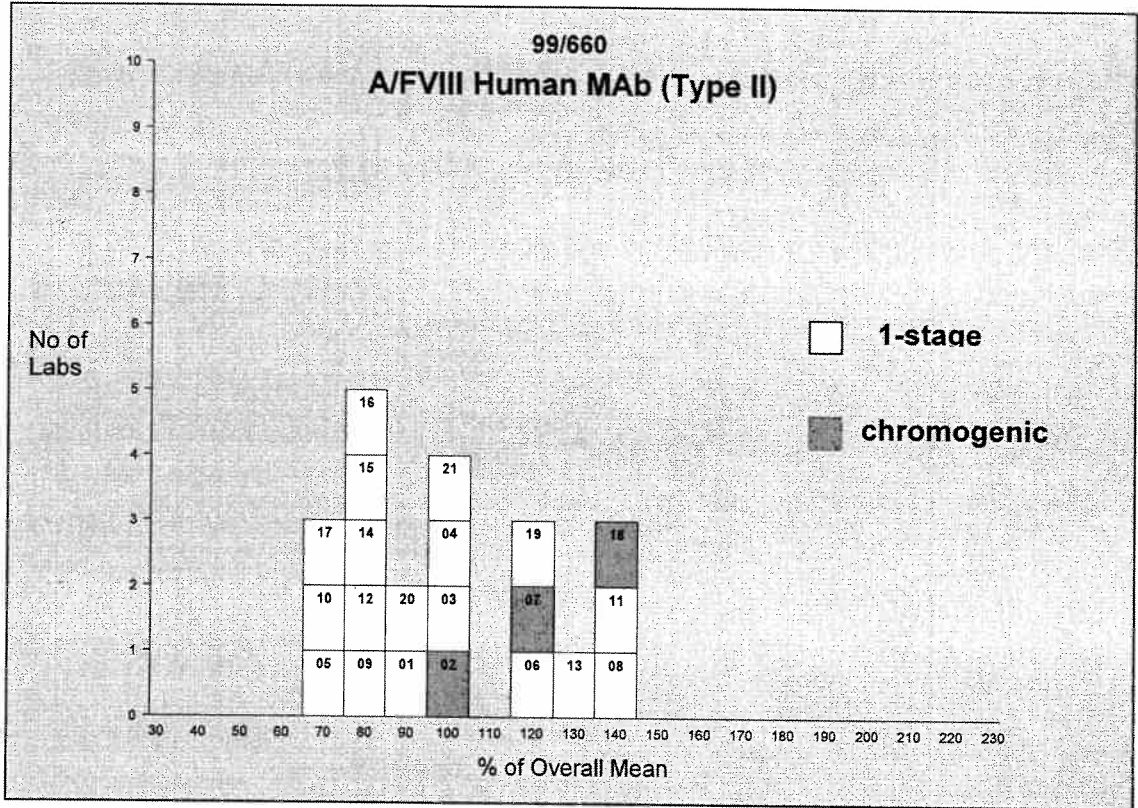


Figure 5. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

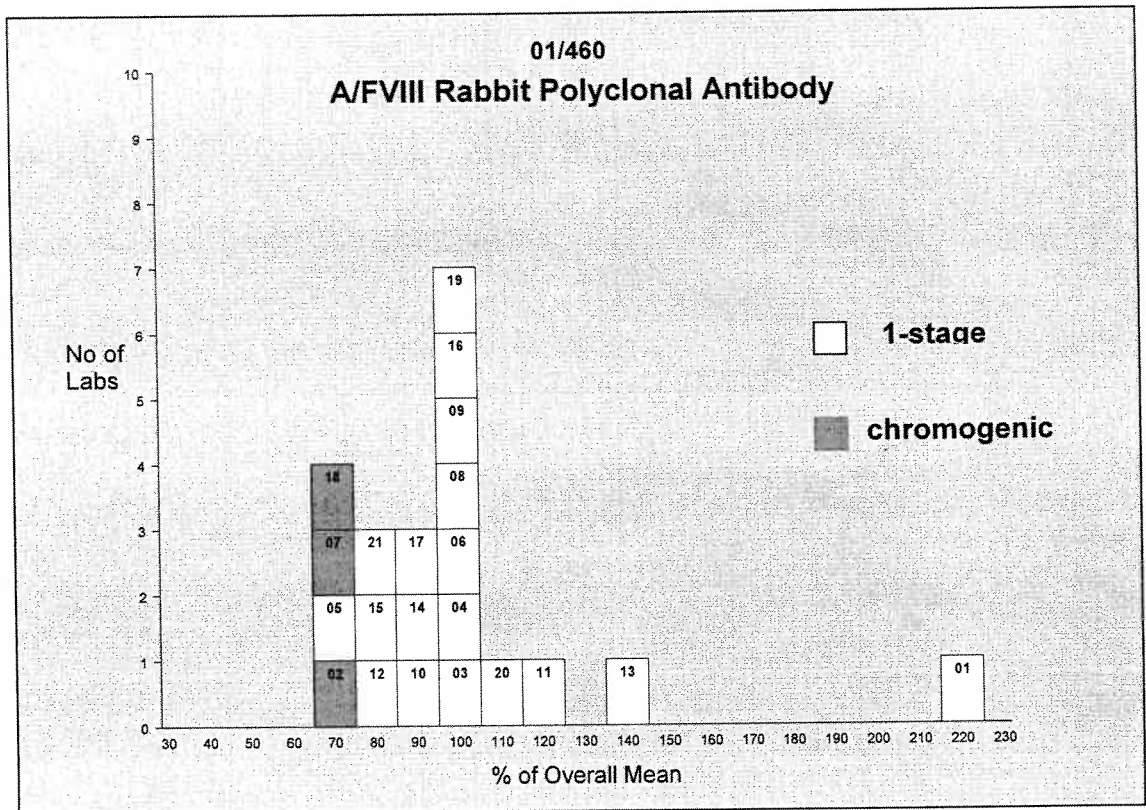


Figure 6. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)

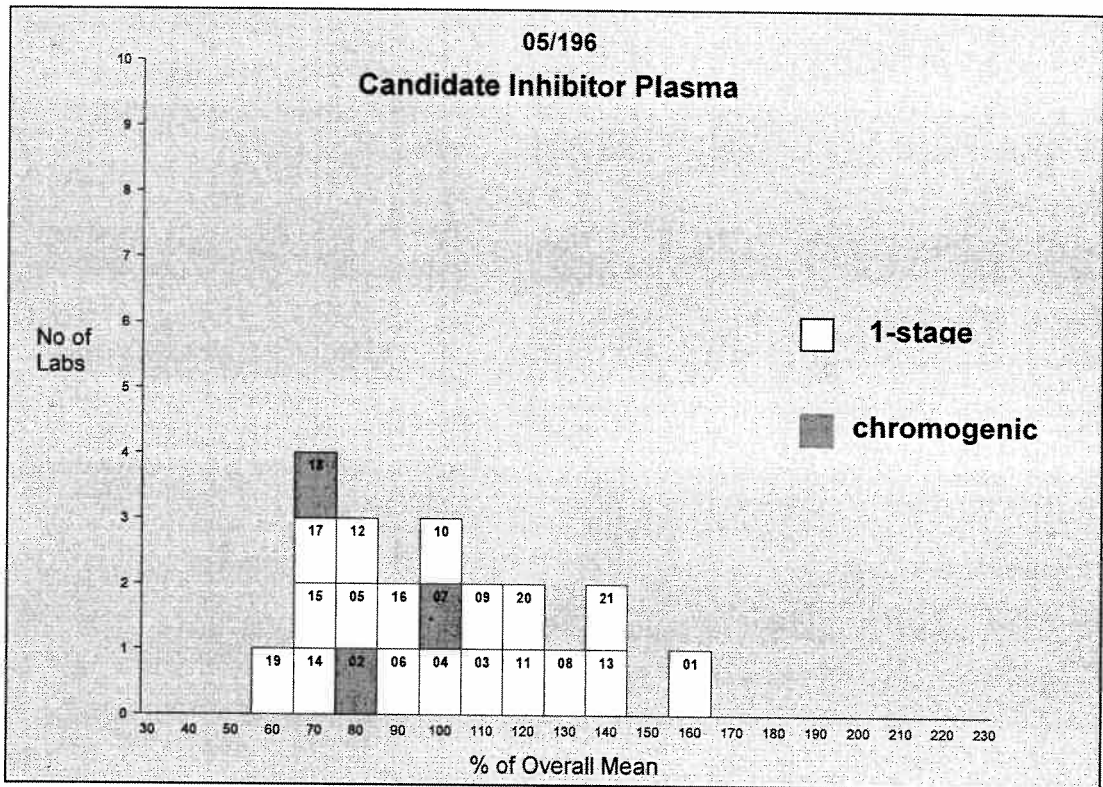
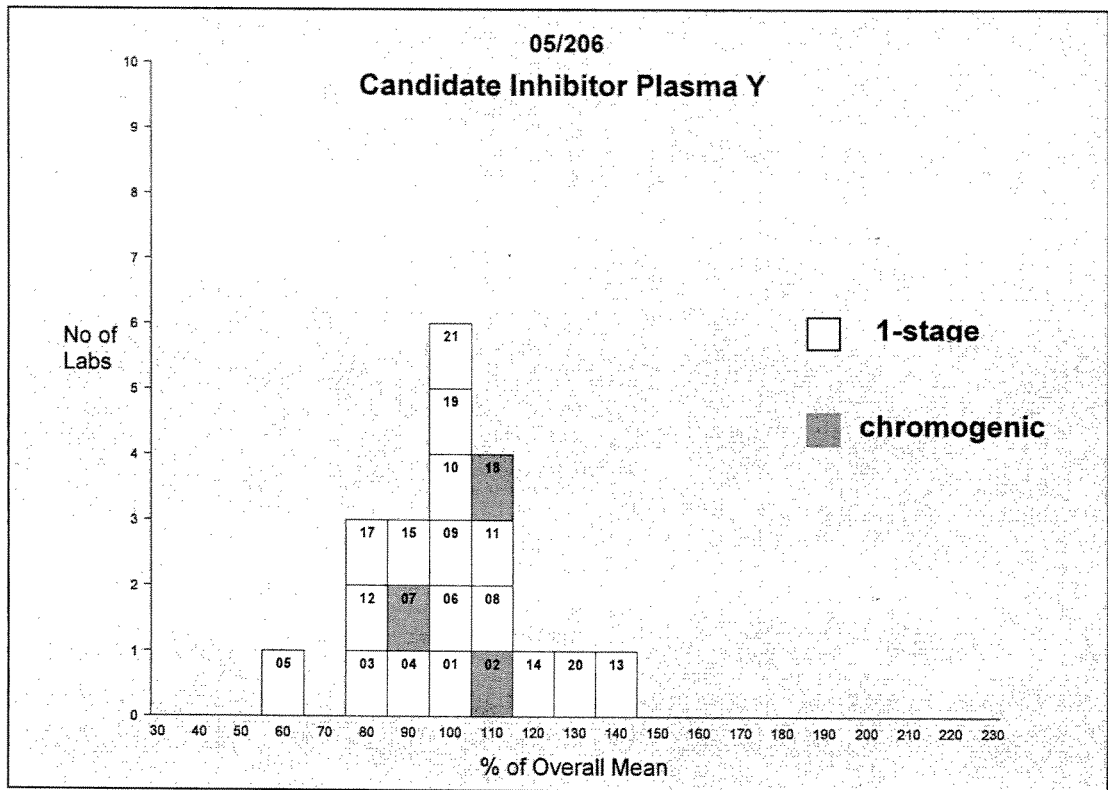


Figure 7. Mean laboratory estimates for FVIII inhibitor titres (BU/ml)



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