Collaborative study for the establishment of Korean standard for blood products(factor IX)

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OBJECTIVES

Hemophilia B is severe bleeding problem due to blood coagulation factor IX deficiency and it is an essential protein for coagulation pathway system. The determination of Factor IX activity is employed for monitoring substitution therapy with factor IX concentrates in hemophilia B. The accurate potency estimation measured against the reference standard in coagulant products plays also an important role for the effective treatment and the reliable reference standard is an urgent need. However National Standard for Factor IX had not been established yet in Korea and the working standard plasma material was used as replacement for the reference standard to measure factor IX potency. It has different factor IX content on every batch and there are other coagulant factors besides IX. Factor IX activity's wide variation occurred in Korea and it might be caused by not only the reagent but the working standard material. Such analysis prompted us to prepare a National Standard for factor IX through a collaborative study. The aim of this study was to assess the stability, to assay factor IX standard candidate against 4th International Standard for Blood Coagulation Factor IX (07/182), Concentrate and to establish the 1st Korean national standard for factor IX.

METHODS

One-stage Clotting assay

1) The total contents *The 4th International Standard for FIX concentrate*(coded 07/182, with assigned potency of 7.9 IU/ampoule)
and the candidate standard preparation should be reconstituted for 30 min at room temperature with 1mL WFI and indicated on the label (the candidates).

2) Pre-dilution in FIX-deficient plasma to 1 IU/mL and serial dilution of standard and the candidate using working buffer as 1/10, 1/20, 1/40, 1/80.

3) React with FIX-deficient plasma, the candidate, actin and $CaCl_2$ and determine the coagulation time using coagulator(CL8).

Stability study

The stability of the candidate material was estimated by the stability after dissolution, the long-term stability and accelerated thermal degradation study to predict during a long-term storage at low temperature [6]. For the accelerated thermal degradation study, it was stored at different temperature at -20, 4, 20, 37, 45℃ for 6 months.

Statistical analysis

Each assay results was analyzed by parallel-line bioassays.

RESULTS

Products Summary

Code	The candidate preparation (11/037)
Presentation	Sealed glass 5 ml vial
Number of vials produced	2800
Date of filling	20 Jun 10
Residual moisture	0.8 (n=3,034)
Storage condition	-20 ℃

The raw material for this candidate standard was plasma-derived factor IX concentrate prepared by ion-exchange chromatography and containing factor II, VII and X. It was manufactured by Korean manufacturer, according to the WHO guideline for the preparation of international and reference materials for biological substances

Stability study

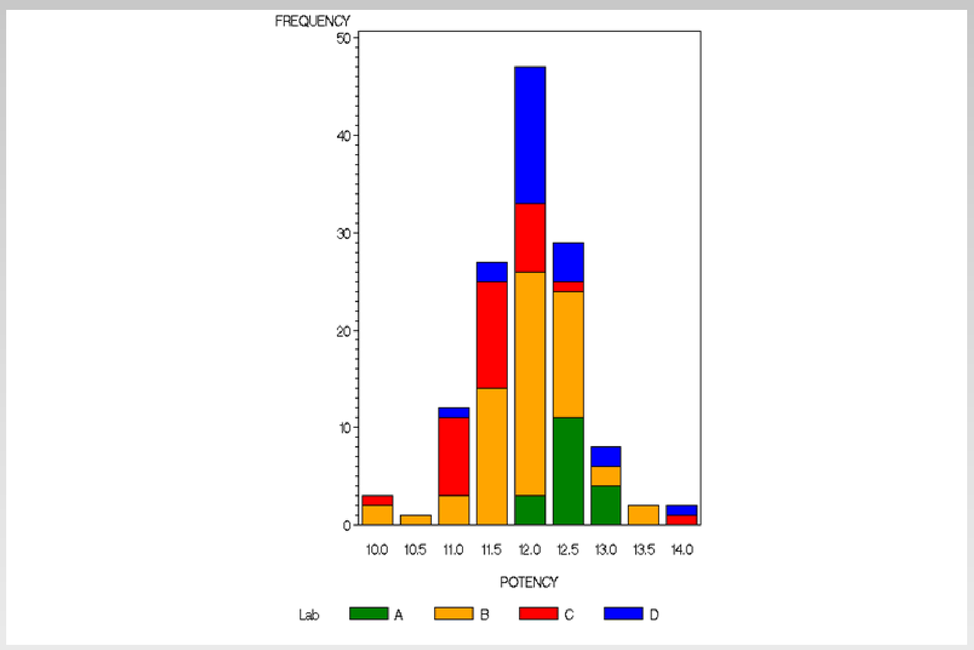
The candidate standard's stability data was obtained from elevated temperatures over a period of six months by the clotting method. It is evident that the proposed standard is stable at the recommended storage temperature (-20℃) while a loss of activity was lost when stored at 37 and 45℃. For evaluating the real time stability, the potency value would be monitored through its lifetime.

Descriptive statistics of potency estimates by laboratory

Lab	No. of Assays	Mean	STD	CV(%)	Min	Median	Max	95% Confidence Limits		Geometric Mean	Geometric CV(%)
A	18	12.55	0.28	2.22	12.11	12.56	13.04	12.41	12.69	12.55	2.21
B	60	11.95	0.63	5.29	9.96	11.97	13.41	11.77	12.10	11.93	5.41
C	29	11.56	0.63	5.46	10.24	11.51	13.85	11.32	11.78	11.55	5.29
D	24	12.20	0.61	4.96	11.01	12.13	14.08	11.94	12.44	12.19	4.86
Grand mean	131	11.99	0.66	5.51	9.96	12.02	14.08	11.86	12.09	11.97	5.55

The potency from each laboratory, expressed as IU/vial relative to the 4th International Standard for FIX concentrate and to Standard human plasma, was estimated. Assays that did not meet the statistical validity were not excluded from the overall mean potency estimation. Results from each participant, together with the overall means and %GCV are given in Table .
One hundred thirty-one sets of clotting assay results were analyzed. Based on the data collected from all participants, intra-laboratory variability was found to range from 2 -5.55% and good inter-laboratory agreement with the majority of GCV around 5% was obtained.

Histogram for final potency estimation by laboratory



Based on the histogram, there is a little standard deviation among laboratories, but the overall potency estimation showed normal distribution.
The estimated mean value obtained from the one-stage clotting assay was 12 international units (IU)/vial.

CONCLUSIONS

1. The candidate standard is adopted as the 1st Korean National Standard for Blood coagulation factor IX, concentrate.

2. The potency discrepancy obtained by assay method was significant based on statistical data, and the potency was determined on one-stage clotting method

3. Stability studies indicated that the candidate was very stable. The way to measure the clotting time depends on the equipment showed little differences on the potency value.

4. The estimated mean value obtained from the one-stage clotting assay was 12 international units against the 4th International Standard (NIBSC, 07/182)

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DOI: 10.3252/pso.eu.WFH2012.2012
Poster presented at: WFH 2012 WORLD CONGRESS PARIS, FRANCE
PosterSessionOnline.com