# Factor VIII inhibitor testing - a way to comparable test results

H.W. Verbruggen, Laboratory of Clinical Chemistry and Haematology, Jeroen Bosch Hospital, ´s Hertogenbosch, The Netherlands

## Introduction

In 2005 the ECAT started external quality control surveys of the factor VIII-inhibitor assay. A rather high between-laboratory variability of the results (>40%) was observed from the start, that unfortunately did not improve during the years (1).

## Methods

In 2009 the ECAT started a cycle of activities, as described below, with the aim to decrease the inter-laboratory variation of factor VIII inhibitor assay results. In each stage of the cycle, an identical set of 7 samples was used.

***Stage 1.***

A survey in 51 laboratories in 2009 (zero-measurement). After evaluation of the results, 15 out of 51 participants were invited for a workshop in November 2009. The laboratories were selected on basis of a wide variation of methods, techniques and assay results.

***Stage 2***

At start of the workshop, the participants performed the FVIII inhibitor assay on the same samples, using their own reagents and methods.

***Stage 3***

At the final day of the workshop, the participants performed the FVIII inhibitor assay on the same samples, using uniform reagents and a standard procedure.

***Stage 4***

In 2010, shortly after the workshop, an external survey was organized among the workshop participants in order to test the direct effect of the workshop on the inter-laboratory variation. Participants were asked to use their current in-house method.

***Stage 5***

All 51 laboratories, that also participated in the survey in 2009, were asked to participate in a final survey thereby using a standard assay protocol including buffered normal pool plasma, FVIII deficient plasma as reference sample and a standardized sample dilution rate. 22/51 laboratories agreed and participated.

## Results

The means and coefficients of variation (CV) of the results of the assays of inhibitor positive samples at the various stages of the study are shown in table 1.

The high inter-laboratory variation of the assay was confirmed in the initial survey and the first session of the workshop. The CVs decreased dramatically in the final session of the workshop when all participants used universal reagent and a standardized protocol (2).

Table 1. Inhibitor activity in BU/ml and between brackets the inter-laboratory Coefficient of Variation.

| | Pre-Workshop Survey ( 2009) | | Workshop results (2009) | | Post-workshop survey (2010) | Standardized final survey 2012 |
|---|---|---|---|---|---|---|
| Sample no. and nominal inhibitor activity | 51 Laboratories | 15 laboratories selected for the workshop | First Session | Last Session | 13 Laboratories | 22/51 Laboratories |
| 1<br>1.6 BU/ml | 2.32<br>(36%) | **2.69**<br>**(43%)** | 2.97<br>(39%) | 1.93<br>(8 %) | 2.9<br>(41%) | 2.7<br>(33%) |
| 2<br>0.8 BU/ml | 0.79<br>(49%) | **1.02**<br>**(31%)** | 1.33<br>(69%) | 0.94<br>(5%) | 1.1<br>(88%) | 0.7a<br>(17%) |
| 3<br>1.4 BU/ml | 0.97<br>(41%) | **1.16**<br>**(39%)** | 1.17<br>(30%) | 1.16<br>(6%) | 1.1<br>(31%) | 1.0<br>(23%) |
| 4<br>0.7 BU/ml | 0.44<br>(70%) | **0.59**<br>**(69%)** | 0.61<br>(45%) | 0.50<br>(13%) | 0.6<br>(61%) | 0.5a<br>(30%) |
| 5<br>2.0 BU/ml | 1.74<br>(36%) | **1.74**<br>**(37%)** | 2.34<br>(41%) | 2.22<br>(12%) | 1.9<br>(31%) | 1.8<br>(25%) |
| 6<br>15.0 BU/ml | 11.0<br>(36%) | **11.5**<br>**(44%)** | 14.9<br>(41%) | 14.6<br>(6%) | 12.0<br>(36%) | 12.4<br>(23%) |
| *Mean CV* | *45%* | ***44%*** | *44%* | *8%* | *48%* | *25%* |

a One outlier excluded.

However, the results of the post-workshop survey in 2010 among workshop participants showed a high variation of results indicating that the outcome of the workshop had not lead to improvement of the inter-laboratory variation.
The final survey in 2012 with 22/51 participants of the initial survey, using reagents and procedures that were standardized according to the recommendations of the workshop, yielded VCs ranging from 23-33%. Two results (one of sample 2 and one of sample 4) were excluded as outliers. The mean CV of the final survey was about half of the mean CV of the other surveys with exception of the final survey during the workshop. The high CV of sample 1 in the final survey was mainly caused by the dilution-rate dependent inhibitor activity in a number of laboratories.
The inhibitor activity of the negative sample in the final survey was below the cut-off value in all participants but one.
No significant differences in inhibitor activity were found between the results of the final survey and the results of any of the other surveys.

## Conclusion

Further standardization of the FVIII inhibitor assay has proven to be possible by performing a cycle of activities that is described in this abstract.

(1) Meijer P, Verbruggen B. The between-laboratory variation of factor VIII inhibitor testing: the experience of the external quality assessment program of the ECAT foundation.
Semin Thromb Hemost. 2009; 35(8): 786-93.

(2) Verbruggen B, Dardikh M, Polenewen R, van Duren C, Meijer P. The factor VIII inhibitor assays can be standardized: results of a workshop.
J Thromb Haemost. 2011; 9(10):2003-8.