Factor VIII Inhibitor Testing: the way to better comparison of test results.

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Introduction

The inter-laboratory coefficient of variation (inter-laboratory CV) of the FVIII inhibitor assay in successive ECAT surveys appears to be more than 40% without a tendency of improvement. However, there is an urgent need for reliable inhibitor measurements, especially in the lower range, for early detection of inhibitors, monitoring of treatment, detection of eradication of inhibitors and for epidemiologic studies. Therefore a workshop on Factor VIII inhibitor testing was organized in order to investigate possible causes of the observed high inter-laboratory CV.

Prior to this workshop an external survey with a set of 7 different samples was organized (session 0) in 51 different laboratories. The set included a negative sample, 5 samples with an inhibitor titre between 0,6 and 2,0 BU/ml and a high titre sample.

Also in this survey in all samples a high inter-laboratory variation was observed (35 – 70%), and several participants measured a positive inhibitor titer in the negative control sample.

Based on these results we selected for the workshop 15 different laboratories which showed a wide variation in test results as well as variation in test methodology.

The samples of the external survey were also used in the workshop.

During the workshop four different sessions were performed. The figure shows a summary of the set-up of the different sessions.

SESSION	DESCRIPTION
0	Pre-workshop survey / home method
1	Repeat home method on central location with same reagents, dilutions and test conditions as used in the home situation in session 0. Universal analyzer (STA).
2	Use of buffered (own) NP / FVIII def plasma as control sample / dilutions as used in the pre-workshop survey / own reagents for FVIII measurement.
3	Use of buffered universal NP / universal FVIII def plasma as control sample/ universal dilutions/ own FVIII deficient plasma and reagents for FVIII measurement.
4	Use of Nijmegen assay with universal reagents / universal dilutions / universal reagents for FVIII measurement.

Results

The mean inhibitor activity of the inhibitor-free sample turned to zero when buffered normal plasma was used by all participants in sessions 2, 3 and 4. However in assays with non-buffered normal pool plasma (sessions 0 and 1) the mean inhibitor activity was 0.2 BU/mL with peak levels up to 1.25 BU/mL. These results clearly show that buffering of the normal pool plasma strongly increases the specificity of the assay.

The inter-laboratory CV of the different inhibitor-positive samples did not improve in session 2 compared to the pre-workshop survey. However, In all samples there was a clear correlation between the rate of the used dilution and the inhibitor activity indicating a major effect of the dilution step on the inhibitor assay results.
The C.V. of the inhibitor-containing samples significantly improved to 10-20% in session 3 where further standardization was reached by the use of universal dilutions in all samples. A further decrease of the C.V. to 5-13% was realized by also standardizing the reagents for the FVIII assay. The low C.V. in session 4 compared to session 3 may indicate an effect of the FVIII reagents on the measured inhibitor activity.
The conclusions of the workshop are: ***1.*** the use of buffered normal pooled plasma as a source for FVIII is needed for optimal specificity, ***2.*** The use the lowest possible dilution factor is necessary to obtain optimal reliable inhibitor results, and ***3,*** Factor VIII assay reagents may influence inhibitor results.
Recently, a post-workshop survey was organized with the same samples in the laboratories that participated in the workshop. The results will be discussed.