Title: In-silico and in-vitro evaluation of the impact of mutations in non-severe haemophilia A patients on assays discrepancies
Journal name: Annals of hematology

Corresponding author:
Dr. rer. nat. Behnaz Pezeshkpoor,
Institute of Experimental Haematology and Transfusion Medicine,
Sigmund-Freud str. 25, 53127
University of Bonn, Bonn, Germany
Behnaz.pezeshkpoor@ukbonn.de

Thrombin generation potential of the FVIII variants

Due to the insensitivity of the TGT protocol to FVIII using 1 pM TF (Supplementary Figure 1A), a modified assay was established. Several parameters were tested: 1) Interference of the serum proteins in FBS in the media was tested using media collected from FVIIIWT variant expressed in serum free media composed of OptiMEM and 1% BSA. This analysis revealed similar results, excluding the effect of FBS (Supplementary Figure 1B). 2) TGT was triggered by 4 µM PL, and compared to 1pM TF in the presence and absence of NaAc. The analysis revealed a significant difference between the wild-type and mock reflected in the lag-time, endogenous thrombin potentiation (ETP) and the thrombin peak (Supplementary Figure 1C-D). The ideal concentration of PL was determined (Supplementary Figure 2). The 4 µM phospholipid was chosen and the pool plasma was tested at different FVIII concentrations (1.56%-100%) triggered with either 4 µM PL or 1 pM TF (Supplementary figure 3). The results are shown in supplementary figure 4 display clearly that substituting TF with 4 µM PL improved the sensitivity of the TGT assay to FVIII concentration in cell culture media.
Supplementary Figure 1: Determination of the test conditions for recombinant FVIII recombinant of thrombin generation. Pool plasma was diluted in FVIII deficient plasma (DP) from Siemens and thrombin generation was measured at A) 1 pM TF and B) 4 µM phospholipids (PL). Each curve is the average of two independent experiments performed in duplicates. Representative thrombin generation curves are shown.
Supplementary Figure 2: Thrombin generation of titration of pool plasma (PP) with different concentration of phospholipids (PL) the test conditions for recombinant FVIII recombinant of. Each curve is the average of two independent experiments performed in duplicates. Representative thrombin generation curves are shown.
Supplementary Figure 3: Pool plasma titration of thrombin generation. Pool plasma was diluted in FVIII deficient plasma (DP) from Siemens and thrombin generation was measured at A) 1 pM TF and B) 4 µM phospholipids (PL). Each curve is the average of two independent experiments performed in duplicates. Representative thrombin generation curves are shown.
**Supplementary Figure 4: Thrombin generation test curves.** Collected media from FVIII<sub>WT</sub> and mock were supplemented with 15 mM NaAc and FVIII concentration was adjusted to 0.05 U/ml. Samples were diluted 1:1 in FVIII-deficient plasma and thrombin generation was triggered by 1 pM tissue factor (TF) or 4 µM phospholipids (PL).