How to measure low levels of Factor VIII and IX

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Accurate determination of low levels of FVIII and FIX activity is of importance not only for diagnostic classification into severe, moderate and mild hemophilia A and B but also for monitoring activity after administration of factor concentrates. Furthermore, in case of pharmacokinetic studies it is crucial to determine a true trough level of factor activity.

For specific factor activity determination, the one-stage (OS) clotting method, utilizing intrinsic activation of coagulation and an excess of factor deficient plasma, is still used in the majority of coagulation laboratories. Two-stage chromogenic substrate (CS) methods, relying upon FXa generation and hydrolysis of a chromogenic FXa substrate, are slowly increasing. Two-stage clotting methods are only rarely used. A highly sensitive clot waveform (CWF) method utilizing either intrinsic or both intrinsic and extrinsic activation offers an alternative method as well as a modified thrombin generation (TG) method utilizing FIXa as activator. Both the CWF and the FIXa-TG methods seem to discriminate below 0.01 IU/mL.

Global methods such as the common TG method and the overall hemostatic potential (OHP) method may provide valuable information for phenotypic characterization and for tailoring treatment and serve as supplemental methods to specific factor determinations.

OS methods are deceptively simple in their design but complex from a biochemical point of view since coagulation activation, thrombin generation and fibrin formation all occurs within 40 -120 s and hence considerably faster than in vivo. Chromogenic methods do not utilize factor deficient plasmas for analysis of plasma samples. The validity of this approach has been shown but also puts high demands on manufacturers regarding consistent lot-to-lot performance of chromogenic kits.

OS methods may not always be accurate below 0.02 IU/mL. There may also be a systematic overestimation at low activity levels, a deviation which may be avoided by keeping the level of factor deficient plasma constant for all calibrator doses.

CS methods claim to manage 0.005 – 0.01 IU/mL and both the CWF and the FIXa-TG methods seem to discriminate below 0.01 IU/mL.

For diagnosis of new hemophilia A patients, both OS and CS methods should be used due to that certain mutations will not be reflected in either of the two methods. Furthermore, samples should be analysed at preferably three dilutions to check for conformity with the calibrator plasma.

The advent of new, so called long-life, rFVIII and rFIX concentrates, has increased the analytic complexity. Thus, large variations have been shown to occur in potency assignments when using OS methods with different APTT reagents and this may well be translated to analysis of plasma from patients receiving such factor concentrates. One cause of discrepancy on analysis of FIX is that under certain conditions FIXa is formed already during contact activation, thus causing overestimation of FIX activity. CS FIX methods, using a defined amount of FXIa as activator of FIX, appear to be more promising in this respect.