Comparison of factor deficient plasmas for measuring Factor VIII and IX

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Introduction
Factor dosages are part of the diagnostic work up in patients with bleeding tendency (factor (F) VIII and Fix) or in thrombophilia screening (FVIII). The correct diagnosis of FVIII and FIX deficiency and the assessment of severity of the disease are essential for a patient-tailored treatment strategy. Persistently high levels of FVIII are regarded as a thrombophilia risk marker. FVIII can be measured using the one-stage clotting assay, two-stage clotting assay and the chromogenic (amidolytic) assay. The one-stage method suffers from diversity in the reagents and assay conditions used: difference in activated partial thromboplastin time (aPTT) reagents, deficient plasmas, reference plasmas, and instruments.

Materials and Methods
We compared two one-stage factor assays with difference in factor deficient plasma and activator for FVIII and FIX on citrated plasma samples of patients with low, normal and high factor levels on a STA-R Evolution® (Diagnostica Stago, Asnières, France). STA®-Deficient VIII and IX (Diagnostica Stago) and the recently available STA®-ImmunoDef VIII and IX (Diagnostica Stago) were compared. In daily practice, we use STA®-Deficient VIII and IX activated by STA®- PTT-A with two calibration curves covering the whole measurement range (curve for values 0-20%, a routine curve for the whole range) and with calibration for each run. The one stage clotting assay performed with STA®-ImmunoDef was activated by STA®- CK-Prest® according to the manufacturer’s recommendations and calibrated once per lot of reagent. First, to obtain the limit of detection (LOD) we performed the STA®-ImmunoDef assays according to the manufacturer’s recommendation (6 points calibration curve) and compared it with a 7 points calibration curve. Then, we determined within-run and between-run imprecision and linearity. Finally, a method comparison was made for factor dosage as well as for quantifying antiFVIII and antiFIX (Bethesda method).

Results
The LOD for FVIII was 0.20% and 0.25% with STA®-Deficient and STA®-ImmunoDef, respectively. FIX had a LOD of 0.28% and 0.72%, respectively for both plasmas. Between-run and within-run CV’s for FVIII ranged between 7.18% - 9.89% and 3.30% - 3.93% , respectively. For FIX between-run and within-run CV’s ranged between 6.93% - 9.43% and 4.04% - 4.06% , respectively. Method comparison (n= 106 FVIII; n=54 FIX) resulted in a correlation coefficient of 0.96 for both FVIII and IX. Results obtained with STA®-ImmunoDef were 12.0% and 12.9% higher than with STA®-deficient plasma for FVIII and FIX, respectively. Method comparison for the Bethesda dosage for antiFVIII (13 samples) and antiFIX (8 samples) showed a correlation of 0.99 for both inhibitors. Linearity (up to 200%) showed excellent correlation between expected and measured values for FVIII with both plasmas ($r^2= 0.99$). For FIX we obtained a correlation coefficient of 0.99 and a correlation coefficient between 0.97 and 0.99 with STA®-Deficient plasma and STA®-ImmunoDef plasma, respectively.

Conclusions
The LOD for the STA®-ImmunoDef VIII and IX is a little higher than the LOD for the STA®-Deficient
VIII and IX, although both methods are able to measure accurately below 1%. Between-run imprecision of STA®-ImmunoDef for FVIII and FIX is comparable to the imprecision obtained with STA-Deficient plasma with a historical CV of our laboratory of 8.6% and 7.2% for FVIII, 8.0% and 7.1% for FIX for two levels of control material. Method comparison resulted in good correlation coefficients for both FVIII and IX. Results obtained with STA®-ImmunoDef tended to be higher than with STA-Deficient plasma for FVIII and FIX. Linearity for FVIII and FIX was excellent for both plasmas. Method comparison for the Bethesda method quantifying antiFVIII and antiFIX showed for both an excellent correlation without a significant difference between both factor deficient plasmas. The recently available STA®-ImmunoDef VIII and IX plasmas have advantages compared to the STA®-deficient plasmas: a 8 hour on-board stability (in stead of 4 hours), one calibration needed per lot (in stead of 1 per run), smaller lot-to-lot differences and a large measurement range allowing the use of only one calibration curve for low and high values.

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