Introduction & Aim

FXIII deficiency is a rare bleeding disorder. Severe deficiency (FXIII activity <5%) is associated with spontaneous intracranial bleeding and death.1,2 Testing guidelines recognise that ammonia release assays require a blank step to prevent miss-diagnosis at low levels of FXIII activity.

This verification study aimed to assess the suitability of the Technofluor FXIII activity assay (fluorescence resonance energy transfer, FRET method) - blanking not indicated - and compare it to the currently utilised Siemens Berichrom FXIII activity assay (chromogenic ammonia release assay, un-blanked).

Method

- Normal and abnormal controls
- 6x replicates
- Single run

Results

The Technofluor FXIII activity assay demonstrated excellent stability, precision, repeatability and accuracy. A LLOQ of 1.4 IU/dL was demonstrated during the validation process. This is a significant improvement on the LLOQ of the currently utilised Siemens Berichrom FXIII activity assay (un-blanked) which is locally defined as 10 IU/dL. This difference in sensitivity at the lower levels is demonstrated in Fig. 3-5.

Observations and Conclusions

The clinical significance of the difference in LLOQ between the assays was demonstrated in two separate trough FXIII levels from a known FXIII deficient patient; see Fig. 5. FRET results for the FXIII deficient patient are in keeping with genetic diagnosis (compound heterozygosity) and initial clinical presentation (umbilical stump bleeding and intracranial haemorrhage). As this finding was mirrored in the NEQAS exercise, the robustness of this specific EQA survey appears weak given our history of successful returns with the Berichrom assay. We are unaware of any UK NEQAS participants performing a blanking step, therefore, as EQA target values are determined from median responses, inaccurate reporting is likely. Given that FXIII levels of 11% have been associated with significant bleeding3 the assay discrepancy is clinically significant - improved accuracy at this clinically important level will aid patient safety.

The results of this comparison study are supported by published literature which states that the Berichrom FXIII assay is affected by endogenous ammonia and elevated fibrinogen levels and it does not require blanking. Additionally, it is specified by the manufacturer that calibration is only required once per lot. From this study it is concluded that the Technofluor FXIII activity assay offers a user-friendly and accurate method for the assessment of FXIII activity.

References

2. Bossuyt J. et al. 2010
4. Mager M. et al. 2010
5. Wijnen J. et al. 2010

Figure 1: FRET Method
1. Thrombin + Ca2+ = FXIIIa
2. FXIIIa cleaves side-chain of the assay's substrate
3. Releases the dark quencher
4. Light emission from fluorophore
5. Fluorescence is proportional to the FXIII activity

Figure 2: Chromogenic Ammonia Release
1. Thrombin + Ca2+ = FXIIIa
2. Glycine-ethyl ester and peptide substrate reaction is catalysed by FXIIIa-release of ammonia
3. Ammonia is incorporated into an α-keto glutarate with NADPH by glutamate dehydrogenase
4. The decrease of NADPH is measured photometrically

Figure 3: Passing-Bablok regression analysis (n=27) demonstrates greater difference between the two assays at the lower concentrations with an intercept estimate of -27.33

Figure 4: Bland-Altman difference analysis suggests that at lower concentrations, the FRET assay is producing smaller values than the ammonia release assay. However, at higher concentrations, the FRET assay is producing higher values than the ammonia release assay.