Review of Factor XIII Screen Versus Assay: Results from the RCPAQAP

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Introduction
Factor XIII (FXIII) is a protransglutaminase which, when activated, is involved in cross-linking fibrin monomers to form a stable clot. In the absence of FXIII the clot is unstable and more susceptible to breakdown. Although deficiency in this plasma protein is rare, clinical manifestations of this condition can be life-threatening. Currently, clot lysis methods are used as a screening tool when FXIII deficiency is suspected. Assays are used less frequently to determine levels of FXIII. The Royal College of Pathologists Australasia Quality Assurance Programs (RCPAQAP) provides external proficiency testing for FXIII. This review aims to summarise results of a three-year period for the relevance of screening and quantitation of FXIII.

Methods
The FXIII proficiency program contains four samples per year. Survey data from 2015 to 2017 (12 samples), were analysed with FXIII levels ranging from 1% to 110%. Survey materials were lyophilised plasma and comprised both patient samples and combinations of commercial FXIII deficient plasma mixed in different proportions with a normal plasma pool. Clot lysis screens were performed either using Calcium chloride or Thrombin for clotted the samples and Acetic acid or Urea as the lysing agent. Assays were performed based on Chromogenic or Latex principles. Chromogenic methods involve the use of a blank procedure to account for background interference by the sample in the assay.

Results
FXIII clot lysis results:
Three of the 12 survey samples had target FXIII levels of 1 to 3%. Only 62% (21/34) of participants using a screening test correctly interpreted a severe deficiency in these samples. Nine of the 12 survey samples had target FXIII levels of 4-28.9%.

Summary of FXIII Screen results (FXIII target 1-2%)

<table>
<thead>
<tr>
<th>Clot lysis method</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid</td>
<td>11</td>
</tr>
<tr>
<td>Urea</td>
<td>11</td>
</tr>
</tbody>
</table>

In the 12 samples, 70% (85/121) of all Chromogenic tests were performed without use of a blanking procedure.

Discussion
The FXIII lysis results for samples with 1-3% FXIII showed 38% (33/90) of participants incorrectly excluded FXIII deficiency (Figure 1). The clot lysis screening test results when FXIII levels were 4-8%, showed 50% (99/190) of participants correctly excluded severe FXIII deficiency (Figure 2). However, clot lysis methods could not exclude a moderate to mild deficiency from 5% to 40%.

In the 3 samples with target FXIII of 1-3%, the median FXIII result with the use of a blank was lower with all 3 samples as shown in Table 1. The latest method produced lower median FXIII results than the Chromometric method for 2 out of 3 samples (Table 1).

In the group of 9 samples with 44% FXIII, the chromometric FXIII result median when the blanking procedure is used is lower than the when the blanking procedure is not used 78% of the time. The blanking procedure accounts for the ‘background’ reaction in the chromometric method. This is essential with low levels of FXIII, where overestimation can cause false negative diagnosis. The latest method in these 9 samples produced higher FXIII values than the chromometric method. Therefore, the chromometric method does appear to perform with better sensitivity based on these survey sample results.

Conclusion
The experience of the RCPAQAP survey confirms the findings of other international surveys to demonstrate that FXIII assay results showed better sensitivity to detect a severe deficiency compared to clot lysis screening tests. FXIII lysis tests presented low sensitivity which could cause a FXIII deficiency diagnosis to be missed. Chromometric FXIII assays have the advantage of measuring functional activity but require a blank procedure to attain optimal sensitivity to detect a severe deficiency. A quantitative FXIII assay should be used to exclude FXIII deficiency.

References
4. NCT01883594, A double-blind, randomised, placebo-controlled, single arm phase IIa study to evaluate the safety and efficacy of FXIII16-03a, FXIII16-03b, FXIII17-03a, and FXIII17-03b in patients with congenital FXIII deficiency. ClinicalTrials.gov Identifier: NCT01883594.