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[1]. Although deficiency in this plasma protein is rare, clinical manifestations of this condition can be life-threatening[2]. 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 clotting the samples and Acetic acid or Urea as the lysing agent. Assays were performed based on Chromogenic or Liatest 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%.

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

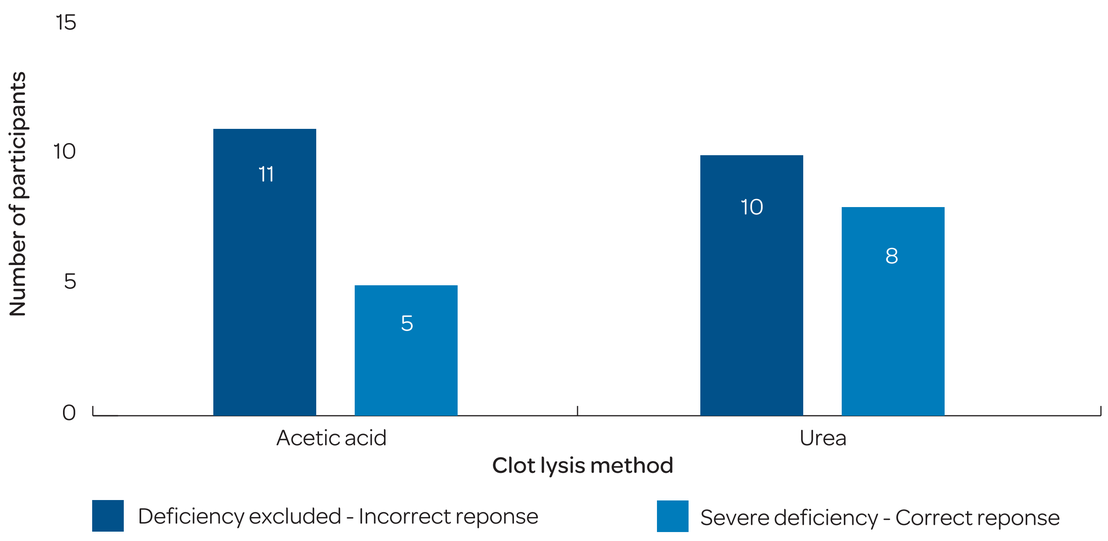

Figure 1: Summary data for FXIII screen results from 3 samples with a target of 1-3% FXIII for the clot lysis methods, acetic acid and urea.

Summary of FXIII Screen results (FXIII target ≥4%)

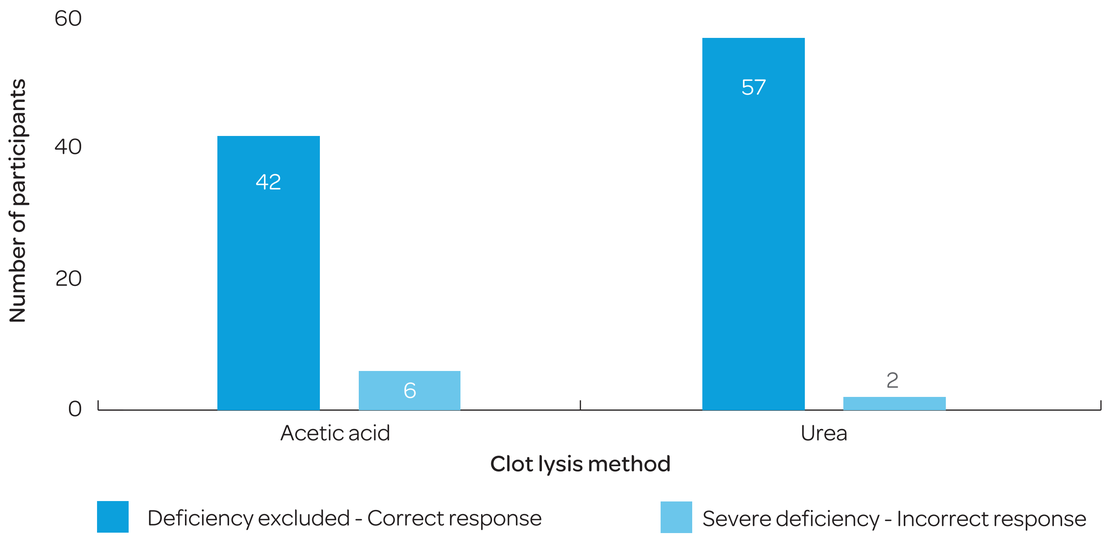

Figure 2: Summary data for FXIII screen results from 9 samples with a target of ≥4% FXIII for the clot lysis methods, acetic acid and urea.

FXIII assay results:

Table 1: FXIII assay result medians of RCPAQAP survey samples with expected FXIII of 1-3%

RCPAQAP Survey number	Expected FXIII results (%)	Range of results	Chromogenic Median with Blank		Chromogenic Median without Blank		Liatest Median	
				Number of participants		Number of participants		Number of participants
FXIII17-08a	1	0 – 9.7	1	6	5.7	7	1	3
FXIII16-08b	3	0 – 17.4	3	3	8.7	5	2.5	4
FXIII15-03b	3	0 – 20	3.9	2	8	5	2	3

Table 2: FXIII assay results medians of RCPAQAP survey samples with expected FXIII of ≥4%

RCPAQAP Survey number	Expected FXIII results (%)	Range of results	Chromogenic Median with Blank		Chromogenic Median without Blank		Liatest Median	
				Number of participants		Number of participants		Number of participants
FXIII17-03b	4	0 - 14.5	1.6	5	6.1	9	1.5	4
FXIII15-08b	4	0 - 13	1.3	3	10	5	3	3
FXIII16-03a	13	4 - 28.9	12.5	4	11.5	6	13	2
FXIII15-08a	36.1	26 - 46	31.1	2	34.5	6	43	3
FXIII16-08a	38	0.4 - 52	35	2	37.2	6	46	4
FXIII15-03a	82	59 - 114	82.7	2	81.5	6	87	3
FXIII17-08b	89.6	74.9 - 109	86	2	89.6	11	109	3
FXIII17-03a	110	76.7 - 195	94.5	2	110	12	143	4
FXIII16-03b	123.5	85-150.1	111	3	122	7	140	2

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% (13/34) of participants incorrectly excluded FXIII deficiency (Figure 1). The clot lysis screening test results when FXIII levels were ≥4%, showed 90% (99/109) 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%.

The detection limit of this qualitative clot solubility method is reported to be between <0.5% and 5% FXIII based on the variables of fibrinogen level, the clotting reagent and lysis reagent[3,4]. As there are 38% of participants incorrectly excluding FXIII deficiency in samples with 1-3% FXIII, the need for a FXIII assay for the definitive exclusion of FXIII deficiency is evident.

For 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 Liatest method produced lower median FXIII results than the Chromogenic method for 2 out of 3 samples (Table 1).

In the group of 9 samples with ≥4% FXIII, the chromogenic 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 chromogenic method. This is essential with low levels of FXIII, where overestimation can cause false negative diagnosis[2]. The Liatest method in these 9 samples produced higher FXIII values than the chromogenic method. Therefore, the chromogenic method does appear to perform with better sensitivity based on these survey sample results.

Conclusion

The experience of the RPCAQAP 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 [5,6]. FXIII lysis tests presented low sensitivity which could cause a FXIII deficiency diagnosis to be missed. Chromogenic FXIII assays have the advantage of measuring functional activity but require a blank procedure to attain optimal sensitivity to detect a severe reduction. A quantitative FXIII assay should be used to exclude FXIII deficiency.

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