Evaluation of a New, Automated Quantitative Factor XIII Assay.
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2Instrumentation Laboratory, Lexington, MA.

Introduction

There is a need for a quantitative, easy-to-perform Factor XIII (FXIII) assay. The most commonly used FXIII assay is a manual, qualitative assay that can only detect severe deficiencies of < 2% FXIII activity. Alternatively, a chromogenic FXIII assay exists that measures FXIII activity. Inherited FXIII deficiencies reported to date have shown decreased antigenic and activity levels, where no known cases have shown decreased activity with normal antigenic levels. In acquired deficiencies, the measured antigenic levels of FXIII (subunit A) is proportional to the activity levels, although they may not be identical, particularly after replacement therapy (Lim 2004). In addition, there is some evidence that heterozygous FXIII deficiency might be associated with bleeding symptoms (Ivaskevich, 2007).

HemosIL® Factor XIII Antigen (Instrumentation Laboratory) is a new, automated latex enhanced immunoassay for the quantitative determination of FXIII antigen (subunit A) in human citrated plasma. It is a liquid, ready-to-use reagent that simplifies the screening of genetic and acquired FXIII deficiency.

Methods

HemosIL Factor XIII Antigen (FXIII Ag) assay was performed on an ACL TOP® Hemostasis Testing System. A normal range study for FXIII Ag was performed by analyzing 45 normal samples to establish a reference range. A patient population of 74 samples was assayed with FXIII Ag, and also with an established quantitative chromogenic FXIII activity assay (Berichrom XIII, Dade Behring) on an AMAX 400 (Trinity Biotech).

Objective

• Evaluate a new, automated quantitative FXIII antigen assay by comparing it against an established, chromogenic method.
• Test the ability of the assay to diagnose low FXIII to explain positive bleeding history in patients with no other bleeding disorders
• Validate the FXIII Ag assay on the ACL TOP System

Patients

• Twenty-three patients undergoing FXIII testing, one of whom had congenital FXIII deficiency
• Twenty patients with a personal or family history of bleeding and normal von Willebrand factor results
• Eleven patients with normal FXIII levels
• Twenty unselected inpatients

Results

The mean FXIII value was 91.6 % using the new antigen assay and 93.6% using the activity assay, with no significant difference using a paired, two-tailed T-test (p = 0.36). A Bland-Altman plot revealed no discernible bias between the two methods (Figure 1). Linear regression revealed an R value of 0.8 (Figure 2).

Low FXIII activity (≤70%, range 28-70) was present in seventeen of the seventy-four patients. If considering the activity results to be the ‘gold standard,’ fifteen of seventeen antigen results were also ≤ 70% for a sensitivity of 88% (15/17) (Table 1). The two discordant pairs were: 77% antigen versus 63% activity, and 82% antigen versus 63% activity. Among the fifty-seven specimens with >70% activity, antigen results were also >70% in fifty-five, for a specificity of 95% (55/57) (Table 1). The two discordant pairs were 55% antigen versus 79% activity, and 62% antigen versus 79% activity.

The normal range for the FXIII Ag assay was calculated to be 70.2 - 146.8%, while the normal range for the FXIII activity assay was 60 - 150%. Based on these ranges, thirteen discordant pairs exist in total. Of the samples that fell within range for the activity assay, one sample surpassed the high limit of the antigen assay normal range. Eleven pairs had disagreement due
to activity values that fell outside the low limit of the
normal range; however seven of the eleven had dif-
fferences of < 10%. Of the samples that fell out of the
normal range for the activity assay, one sample, which
fell out of range on the high end, was within range for
the antigen assay (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Reference Method</th>
<th>Result Category</th>
<th>No. of Cases</th>
<th>Berichrom FXIII Activity</th>
<th>HemosIL FXIII Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt; 70% (n = 57)</td>
<td>&gt; 70%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>&lt; 70%</td>
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<tr>
<td></td>
<td>≤ 70% (n = 17)</td>
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<td>15</td>
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<td>2</td>
<td>&gt; 70%</td>
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<td>Within normal range</td>
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<td></td>
<td>(n = 67)</td>
<td></td>
<td>1</td>
<td>Outside normal range</td>
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<td></td>
<td></td>
<td></td>
<td>11</td>
<td>Outside low limit</td>
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<td></td>
<td>Outside normal range</td>
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<td>Outside normal range</td>
<td>Outside normal range</td>
</tr>
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<td></td>
<td>(n = 7)</td>
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<td>1</td>
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<td></td>
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Conclusions

HemosIL FXIII Ag assay appears to be an improve-
ment over the manual, qualitative assay in that, it is
quantitative and automated.

FXIII Ag performs adequately when compared to a
quantitative activity assay in terms of numerical agree-
ment and clinical classification.

References

Ivaskevius V, Seitz R, Kohler HP et al. International registry on
FXIII deficiency: a basis formed mostly on European data.

Lim W, Moffat K, Hayward CPM. Prophylactic and periopera-
tive replacement therapy for acquired FXIII deficiency.
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Figure 1
Bland-Altman plot showing no discernible bias between the antigen and activity methods.

No difference was observed when comparing the results of the FXIII Ag assay on the ACL TOP versus the FXIII activity assay.

Linear regression analysis demonstrates an r value of 0.8 and a slope of 0.7 when comparing the FXIII Ag assay to the activity assay.

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