Abstract

The objective of this study was to evaluate the feasibility of implementing mixing study and factor assay protocols for the assessment of Factor VIII (FVIII) activity in human plasma using the STart®4 Analyzer for use in the student laboratory at Virginia Commonwealth University (VCU). Specifically, this project aimed to establish protocols that can be used to demonstrate the benefits and practical use of advanced hemostasis testing to junior level Clinical Laboratory Sciences students at VCU. In this study, the activated partial thromboplastin time (APTT) assay was initially employed in concert with a mixing study protocol. Next, a factor assay protocol was utilized to assess FVIII activity in human plasma. The student researcher’s results demonstrate that the STart®4 Analyzer at VCU can be used to test for FVIII deficiency using mixing study and factor assay protocols. In the future, the instructor plans to implement these protocols as part of the Hematology student laboratory activities in order to enhance students’ knowledge of hemostasis testing while serving to better prepare them for clinical rotations. Moreover, future studies will involve assessing whether implementation of these protocols in the student laboratory has a positive impact on student grades and clinical rotation performance.

Method/Materials

In this study, the APTT assay was employed in order to evaluate the activity of FVIII in human plasma. Assay controls, provided by Diagnostica Stago, Inc. (Parsippany, NJ), were used in the initial part of this study in order to determine the suitability of the STart®4 Analyzer (Diagnostica Stago, Inc.) for performing APTT assays. Next, a 1:1 ratio mixing study protocol was evaluated in the presence assay controls, pooled normal human plasma (PNP), and FVIII deficient plasma. Lastly, a factor assay protocol was assessed to determine the level of FVIII activity in human plasma.

Reagents used for APTT test, Factor Assays and Mixing Study:
- System Controls: Lot # 112022
- APTT reagent: Lot # 10985
- CaCl₂ 0.025 M: Lot # 112162

Reagents used for Factor Assays and Mixing Study:
- Owren-Koller Buffer: Lot # 114622
- PNP: Lot # A118
- FVIII Deficient Plasma: Draw # 15-368
- Uncalibrator: Lot # 105649

Introduction

Hemophilia A (HA) is an X-linked recessive inherited hemorrhagic disorder in which a coagulation cofactor known as Factor VIII (FVIII) is deficient. Due to the inheritance pattern of HA, males are affected more often than females; however, females can be carriers of the genetic abnormality that leads to HA. Specifically, HA affects approximately 1 in 5,000 male births (2, 3). In conjunction with the activated partial thromboplastin time (APTT), mixing studies and specific factor assays are two coagulation tests that are used to aid in the diagnosis of bleeding disorders such as HA.

Within the clinical laboratory, the reason for prolonged prothrombin time (PT) and/or APTT results can be further investigated via the performance of a mixing study test. Performing a mixing study can help distinguish whether a prolonged PT and/or APTT result is due to a factor deficiency or a factor inhibitor (e.g., a factor specific alloantibody). In a mixing study, the patient’s plasma may be mixed in a 1:1 ratio with pooled normal plasma (PNP). If the PT and/or APTT results correct towards normal range, it is concluded that the patient has a factor deficiency; however, the specific factor deficiency cannot be determined via a mixing study. If the patient’s PT and/or APTT results are not corrected during the mixing study, then the presence of an inhibitor is indicated (1, 3). If the results of the mixing study indicate a factor deficiency, then a specific factor assay can be performed to determine the level of specific coagulation factor activity within the patient’s plasma. Specific factor assays involve mixing diluted patient plasma (or control plasma) with factor deficient plasma and comparing the patient (or control) results to a factor activity curve that is prepared using normal reference plasma (1, 3). It is important to carefully evaluate a patient’s FVIII assay results before diagnosing a patient with HA.

Results

- Intra-assay Precision (n = 4):

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>33.3 sec</td>
<td>0.19 sec</td>
<td>0.6%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>55.2 sec</td>
<td>0.78 sec</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

- Inter-assay Precision (n = 5):

<table>
<thead>
<tr>
<th></th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>33.6 sec</td>
<td>0.61 sec</td>
<td>1.8%</td>
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<tr>
<td>Positive Control</td>
<td>56.2 sec</td>
<td>1.30 sec</td>
<td>2.3%</td>
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</tbody>
</table>

- Mixing Study results*:

<table>
<thead>
<tr>
<th>APTT</th>
<th>APTT Result Interpretation</th>
<th>1:1 Mixing Study</th>
<th>Rosner Index (RI)</th>
<th>1:1 Mixing Study Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>121.2 sec</td>
<td>Prolonged (Abnormal)</td>
<td>41.5 sec</td>
<td>3.55 %</td>
<td>Corrected</td>
</tr>
</tbody>
</table>

FVIII Activity

< 1.48% Severe deficiency

* Sample: FVIII deficient plasma

Formula for RI:

\[ RI = \frac{\text{APTT}_{\text{mixed}} - \text{APTT}_{\text{normal}}}{\text{APTT}_{\text{patient}}} \times 100 \]

Future Plan

In the future, the Clinical Hematology instructor at VCU plans to implement these protocols as part of the Hematology student laboratory activities in order to enhance students’ knowledge of special coagulation testing while serving to better prepare them for clinical rotations. Furthermore, a survey will be conducted to assess the effectiveness of this particular laboratory as part of the student experience.

Works Cited


Picture sources:

[A link to an image on ResearchGate](https://www.researchgate.net/profile/Sara_Pourshahrastani/publication/282423019/figure/fg1/AS_288721590730752/1445847999702/Fig-1-Schematic-of-the-coagulation-cascade-activation-of-both-intrinsic-and-extrinsic.png)

[A link to an image on dotmed.com](https://images.dotmed.com/images/listings/1048415.png)

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