Implementation of a Mixing Study Protocol and a Factor Assay Protocol for the Assessment of Factor VIII Activity in Human Plasma Using the STart®4 Analyzer

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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.

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.

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.

<u>Reagents used for APTT test, Factor Assays and Mixing Study:</u>
- System Controls: Lot # 112022
- APTT reagent: Lot # 10985
- $CaCl_2$ 0.025 M: Lot # 112162

<u>Reagents used for Factor Assays and Mixing Study:</u>
- Owren-Koller Buffer: Lot # 114622
- PNP: Lot # A1177
- FVIII Deficient Plasma: Draw # 15-368
- Unicalibrator: Lot # 105649

Results

- Intra-assay Precision (n = 4):

	Average	Standard Deviation	Coefficient of Variation
Normal Control	33.3 sec	0.19 sec	0.6%
Positive Control	55.2 sec	0.78 sec	1.4%

- Inter-assay Precision (n = 5):

	Average	Standard Deviation	Coefficient of Variation
Normal Control	33.6 sec	0.61 sec	1.8%
Positive Control	56.2 sec	1.30 sec	2.3%

- Mixing Study results*:

APTT	APTT Result Interpretation	1:1 Mixing Study	Rosner Index (RI)	1:1 Mixing Study Result Interpretation
121.2 sec	Prolonged (Abnormal)	41.5 sec	3.55 %	Corrected

FVIII Activity	FVIII Activity Interpretation
< 1.48%	Severe deficiency

* Sample: FVIII deficient plasma

•Formula for RI: $$RI = \frac{APTT_{mixed} - APTT_{standard}}{APTT_{patient}} \times 100$$

Conclusions

- Intra- and inter-assay precision data demonstrated the performance characteristics of assay controls and showed that they were accurate and consistent within a run and between runs.
- The mixing study demonstrated that there was sufficient FVIII activity in the PNP to compensate for the FVIII deficiency in the known FVIII deficient plasma sample.
- FVIII activity assay demonstrated that the FVIII deficient plasma was severely deficient in FVIII activity.

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

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Picture sources:
A.https://www.researchgate.net/profile/Sara_Pourshahrestani/publication/282423019/figure/fig1/AS:288721590730752@1445847999702/Fig-1-Schematic-of-the-coagulation-cascade-Activation-of-both-intrinsic-and-extrinsic.png
B.https://images.dotmed.com/images/listingpics/1048815.jpg

Acknowledgements

I would like to thank my mentor, Dr. Jenica Harrison, Sheila Pai (VCU Health), and the Department of Clinical Laboratory Sciences for their guidance and assistance with this project.

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