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SHORT COMMUNICATION

Verification and Implementation of a Bovine Chromogenic Factor VIII Assay for Hemophilia A Patients on Emicizumab Therapy

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SUMMARY

Backround: **Patients with hemophilia A can develop inhibitors to factor concentrates. Emicizumab, a nonfactor-based therapy, has efficacy despite inhibitors. FVIII activity assessment on emicizumab treatment requires a bovine chromogenic reagent such as TriniCHROM FVIII:C.**
Methods: **FVIII levels were measured in 15 patients with and 35 without hemophilia and 10 patients on emicizumab therapy with a time-to-clot and the TriniCHROM FVIII:C reagents. FVIII inhibitor levels were also determined with both reagents.**
Results: **Acceptable agreement of FVIII and FVIII inhibitor levels were obtained with the 2 reagents (R^2 = 0.92 and 0.96, respectively) in patients not exposed to emicizumab. The time-to-clot FVIII assay overestimated FVIII levels in patients on emicizumab therapy. The chromogenic FVIII assay delivered accurate endogenous FVIII levels in patients on emicizumab therapy.**
Conclusions: **The TriniCHROM FVIII:C assay is compatible with routine automated coagulation analysers and delivers accurate FVIII and FVIII inhibitor levels.**
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KEYWORDS

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INTRODUCTION

Hemophilia A, an X-linked bleeding disorder due to deficient coagulation factor VIII (FVIII), occurs in 1: 4,000 male births [1,2] with patients falling into 3 severity categories: mild, FVIII > 5 - 40, moderate, FVIII 1 - 5 and severe, FVIII < 1 IU/dL [3]. Patients suffer from recurrent, often unprovoked, deep seated bleeding into joints, muscles, and internal organs with joint and muscle damage [2].
The management of hemophilia A patients includes replacement therapy with FVIII concentrates administered as on-demand therapy, during bleeding, or as prophylaxis, at regular intervals, with human or recombinant factor concentrates [2,3]. A complication of factor replacement therapy is development of neutralizing alloantibodies (inhibitors) against infused FVIII rendering

therapy ineffective. This develops in 20 - 30% of patients with severe and 5 - 10% with mild-moderate hemophilia A [2,4].

Options to overcome the effect of inhibitors include nonfactor replacement therapies including pro-coagulants such as emicizumab [2,5,6], a bispecific, recombinant, monoclonal antibody that binds to activated factor IX (FIXa) and factor X (FX) [5]. Emicizumab simulates the action of FVIIIa in bringing these coagulation factors together initiating proteolytic activation of FX. Emicizumab does not require pre-activation by thrombin resulting in more rapid activation of FX versus FVIII. The natural anticoagulants, Proteins C and S, do not have proteolytic action on emicizumab which contribute to its prolonged activity [5,7].

Measurement of FVIII and FVIII inhibitor levels in patients on emicizumab therapy prior to surgery and with breakthrough bleeding is frequently needed. Activated partial thromboplastin time (aPTT) based time-to-clot FVIII assays produce false FVIII and FVIII inhibitor results in patients on emicizumab therapy [8-10]. Emicizumab has a half-life of ~ 28 days. The results of aPTT based assays, including the Bethesda inhibitor assay, will be affected for ~ 5 - 6 months following exposure [7,11]. Chromogenic FVIII assays based on bovine coagulation factors, e.g., the TriniCHROM FVIII:C® (Diagnostica STAGO®, Asnieres-sur-Seine, France) reagent, accurately assesses endogenous FVIII and FVIII inhibitor levels in patients on emicizumab therapy. Chromogenic FVIII assays containing human coagulation factors are sensitive to emicizumab and will result in false FVIII and associated inhibitor levels in patients receiving emicizumab [11,12].

This was a verification study of the performance of the bovine factor based chromogenic TriniCHROM FVIII:C® reagent.

MATERIALS AND METHODS

This study was performed at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in the National Health Laboratory Service (NHLS) laboratory in South Africa in accordance with the International Committee on Standardization in Hematology (ICSH) guidelines [13] with ethics approval from the University of the Witwatersrand (Wits) Human Research Ethics Committee (HREC) (Medical M210830).

Study samples and analysis

Analyses were performed on STAGO® MAX automated coagulation analyzers in use in the NHLS laboratory. The TriniCHROM FVIII:C assay test set-up was performed by STAGO® engineers in accordance with the reagent package insert with the following modifications to improve assay performance:

1) Reconstitution of the FIXa and FX reagents was performed with 2 mL distilled water instead of 1 mL.

2) The reagents were incubated at room temperature (18 - 25°C) for 30 minutes instead of 5 minutes.

Reagents, calibrators, and controls

FVIII and FVIII inhibitor analyses on study samples were performed with both the one-stage time-to-clot STA® FVIII deficient with Automate aPTT® and the chromogenic TriniCHROM FVIII:C® reagents. Calibrations with the respective commercial calibrators and processing of commercial internal quality controls (IQCs) were performed.

Precision (repeatability) study

Normal (N) and pathological (P) TriniCHROM FVIII:C® reagent controls were analyzed 20 consecutive times.

Accuracy study

The accuracy study was performed on FVIII activity levels obtained with the time-to-clot STA® FVIII deficient and the TriniCHROM FVIII:C® chromogenic reagents on samples from:

1) 50 patients not exposed to emicizumab (15 with and 35 without hemophilia A)

2) 10 patients with hemophilia A with known inhibitors of > 0.6 Bethesda units (BUs)

3) 10 patients with hemophilia A on emicizumab therapy

Data analysis

For the precision analysis, the data was presented in tabular form considering mean, standard deviation (SD), and coefficient of variation (CV) in Excel® 2016. The results were compared to known acceptable precision standards from the manufacturer and the ICSH [14]. The accuracy study compared the FVIII results obtained with the 2 reagents assessing the y-intercept and the coefficient of determination (R^2) with linear regression and the standard deviation (SD) and 95% limits of agreement with Bland-Altman analyses.

RESULTS

Precision study

The results of the precision study on TriniCHROM FVIII:C® commercial controls were within the manufacturer's acceptable performance standards and the ICSH recommendations with standard deviations (SDs) below 8%.

Accuracy study

The FVIII results of the 50 patient samples analyzed with the TriniCHROM FVIII:C® reagent (range 1 - 260, median 102, IQR 40 - 154 IU/dL) were in agreement with the results obtained with the STA® FVIII deficient reagent (range 1 - 285, median 107, IQR 28 - 134 IU/dL) with a linearity coefficient of determination (R^2) of 0.92 on linear regression and a bias of 9.08 on Bland Altman (Figure 2). Thirty-five (70%) of the 50 plasma

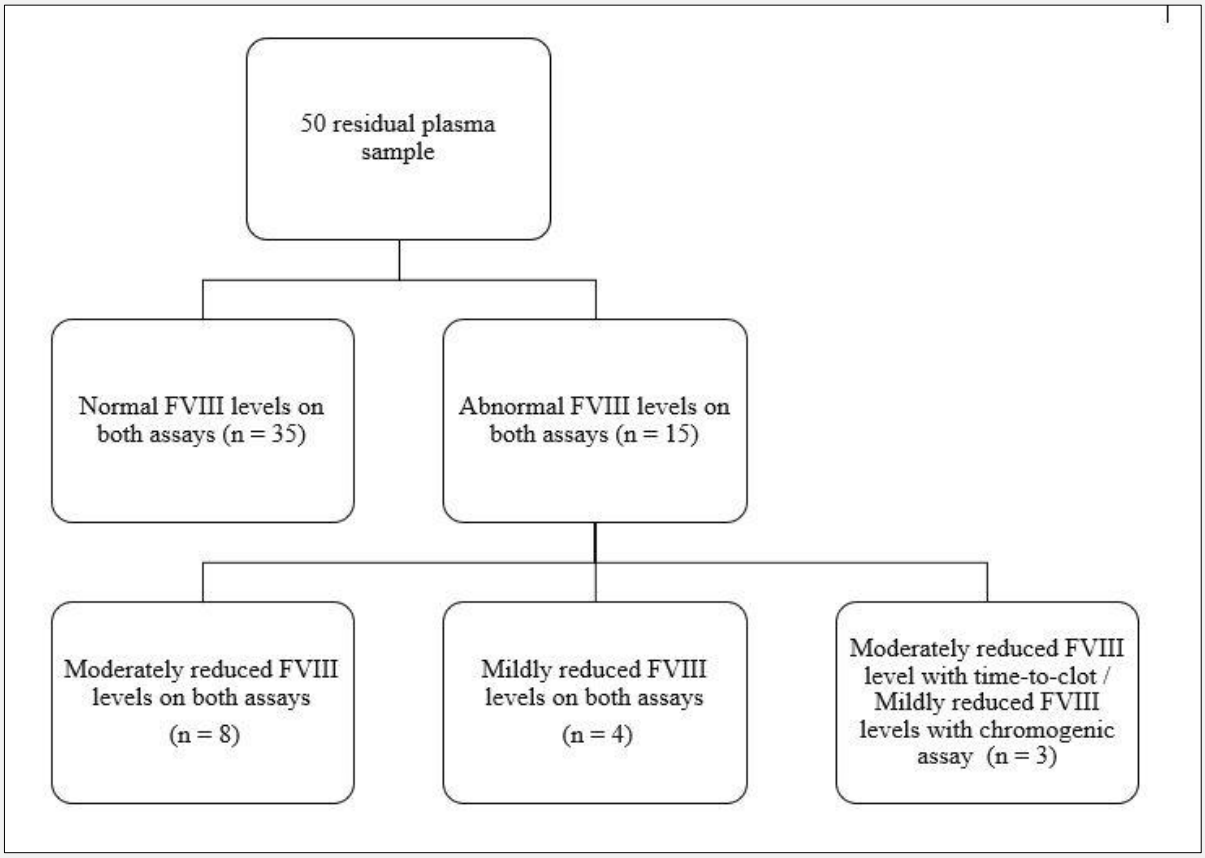

Figure 1. Flow diagram of FVIII results of patient plasma samples (n = 50) anlyzed with a one-stage time-to-clot and the chromogenic TriniCHROM FVIII:C® assays including 15 samples from patients with hemophilia A.

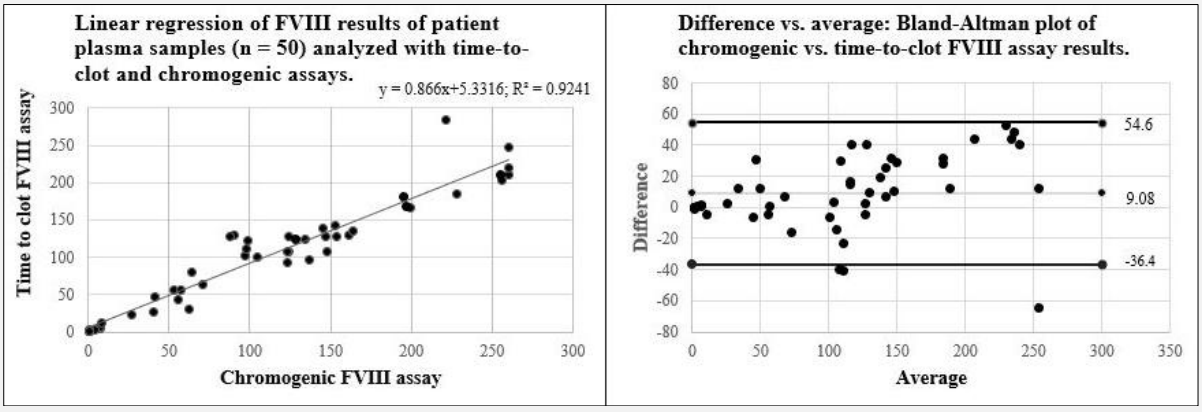

Figure 2. Linear regression and Bland-Altman plot of FVIII results of patient plasma samples (n = 50) analyzed with a one-stage time-to-clot and the chromogenic TriniCHROM FVIII:C® assays including 15 samples from patients with Hemophilia A.

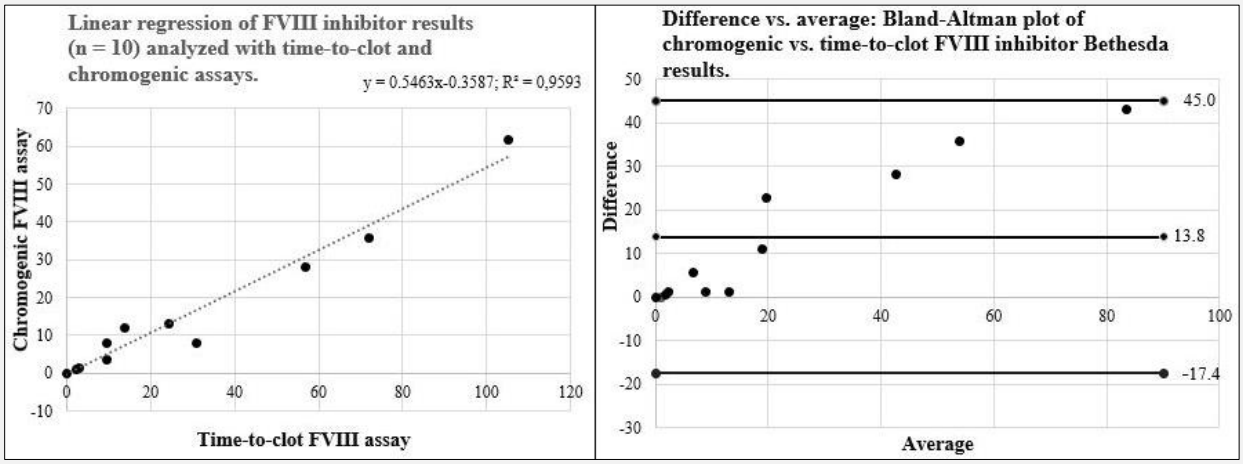

Figure 3. Linear regression and Bland-Altman plot of FVIII inhibitor results of patient samples (n = 10) with known Factor VIII inhibitors analyzed with a one-stage time-to-clot and the chromogenic TriniCHROM FVIII:C[â] assays.

samples were from patients without hemophilia A, and normal FVIII levels of greater than 50 IU/dL were obtained with both reagents. Fifteen (30%) of the 50 plasma samples were from patients with hemophilia A. Eight of the 15 samples (53%) indicated moderately reduced FVIII levels of 2 - 10 IU/dL and 4 (26%) mildly reduced levels of 10 - 40 IU/dL with both reagents. The remaining 3 (20%) samples from patients with hemophilia had discrepant severity levels of FVIII which was moderately reduced with the STAGO® time-to-clot reagent and mildly reduced with the TriniCHROM FVIII: C® reagent (Figure 1).

The results of all 10 plasma samples analyzed for FVIII inhibitor levels with a Bethesda assay based on FVIII levels with both reagents were within the clinical significance category of inhibitors of > 0.6 BUs (R^2 0.96 on linear regression and a bias 13.08) (Figure 3).

The 10 plasma samples from patients with severe hemophilia A on emicizumab therapy demonstrated the effect of emicizumab on the time-to-clot assay delivering detectable FVIII levels. The TriniCHROM FVIII:C® chromogenic assay reflected the correct endogenous FVIII level of < 1 IU/dL which was similar to that prior to therapy.

DISCUSSION

The HAVEN trials demonstrated excellent responses to emicizumab therapy in patients with hemophilia A with and without FVIII inhibitors and the use of this drug will increase especially in patients with FVIII inhibitors [5,15,16]. Measurement of endogenous FVIII and associated inhibitor levels is however challenging in patients on emicizumab therapy as the commonly used time-to-clot assay overestimates the FVIII concentration due to the hemostatic activity of the drug [8,11]. A chromogenic FVIII assay with bovine coagulation factors such as the TriniCHROM FVIII:C® assay is required since emicizumab does not recognize or interact with the factors in this reagent [11].

The current study demonstrated acceptable precision (repeatability) of the TriniCHROM FVIII:C® chromogenic FVIII normal (N) and pathological (P) controls (CVs < 8%). Acceptable accuracy of both FVIII (R^2 0.92; bias 9.08) and FVIII inhibitor (R^2 0.96; bias 13.08) results in comparison with a time-to-clot aPTT assay in patients with and without hemophilia not exposed to emicizumab was obtained with the 2 assays [14]. The overestimation of FVIII levels with the time-to-clot assay was confirmed in patients on emicizumab therapy with the chromogenic assay accurately reflecting endogenous FVIII level in the presence of the drug. Patients with mild or moderate hemophilia A could have discrepant FVIII levels with time-to-clot versus chromogenic FVIII assays with resultant severity misclassification [12]. This discrepancy was demonstrated in the current study in which 3 patients with hemophilia A had higher FVIII results with the chromogenic TriniCHROM FVIII:C® reagent versus the time-to-clot assay with potential misclassification of the hemophilia severity category. Multiple mutations in patients with hemophilia A have been identified to be responsible for the disagreement between different FVIII assays [17]. Further details of the identified mutations and appropriate patient management is outside the scope of the current

research but it is recommended that laboratories attached to hemophilia treatment centers have access to both time-to-clot and chromogenic FVIII assays [18]. Limitations of this study are the inclusion of only 10 samples from patients on emicizumab as well as possible degradation of stored samples from these patients. The interference of hemolysis, icterus, and lipemia on chromogenic assays was assessed during the installation of the STAGO® analyzers but not re-evaluated in this study. Low curve analysis of FVIII levels between 1 - 10 IU/dL and linearity of the TriniCHROM FVIII:C® assay were not performed. These omissions relate to limited availability of samples from patients on emicizumab therapy as well as limited TriniCHROM FVIII:C® reagent and should be evaluated in the future.

Despite these limitations the study indicates that the TriniCHROM FVIII:C® reagent delivers accurate and repeatable FVIII and FVIII inhibitor results in patients with hemophilia A on emicizumab therapy and is compatible with standard automated coagulation analyzers.

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Data Availability:
All study data will be made available on request subject to the ethics guidelines of the University of the Witwatersrand.

Ethics Approval:
Ethics approval was obtained from the University of the Witwatersrand (Wits) Human Research Ethics Committee (HREC) (Medical) (M210830). Individual patient consent was waived for this laboratory verification study.

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Declaration of Interest:
The authors declare no conflict of interest.

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