# FVIII chromogenic assay and variant method for measuring emicizumab and FVIII inhibitor antibodies in human plasma

J. Amiral, K. Haubry, L. Skrzypczak, I. Cornuejols, C. Dunois
Hyphen BioMed, NEUVILLE SUR OISE, France

**CONTACT INFORMATION: www.hyphen-biomed.com**

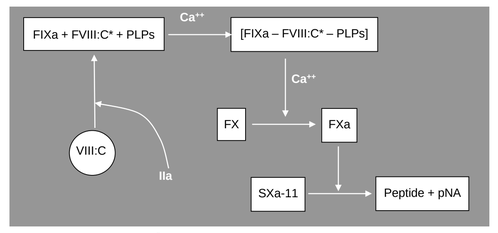
## INTRODUCTION

Emicizumab, a bispecific Anti-FIX/IXa-FX humanized monoclonal antibody, is available for on demand treatment of hemophiliacs A (HA) with inhibitors and is a candidate for prophylaxis of all HA patients. Promising clinical studies provide high expectations for this emerging new therapy. A FVIII chromogenic assay, designed with human FIXa/FX, is available for measuring Emicizumab "FVIII like activity" in plasma. However, it cannot be used for patients with inhibitors, FVIII being also measured. A variant assay designed with bovine FX can be used for complementary testing inhibitor titers, as it is unreactive with Emicizumab.

## AIM

To evaluate a chromogenic FVIII combined assay system (current BIOPHEN™ FVIII, designed with human FIXa and FX, and an assay variant, which is the same with replacement of human FX with bovine FX) for measuring Emicizumab and Anti-FVIII inhibitory antibodies

## METHODS




FVIII chromogenic assay (Biophen™ FVIII) triggered by FIXa, and based on FVIII Dependent FXa generation. It is designed with human FIXa and FX and is sensitive to Emicizumab.
This FVIII chromogenic assay can be used for measuring Emicizumab "FVIII like" activity. However, it cannot be used for titrating FVIII inhibitors in Emicizumab treated patients, as presence of FVIII residual activity is measured and interferes in the method.
Variant designed by replacing human FX with bovine FX, rendering the assay insensitive to Emicizumab.
Using a combination of the original assay (with human proteins) and the variant method (with bovine FX), Emicizumab activity can be measured in citrated plasma, and FVIII inhibitory antibodies can be titrated.
Emicizumab calibrator: R² diagnostics (100 µg/ml in FVIII Def Plasma).
FVIII Deficient plasma and Normal Plasma (Hyphen BioMed).

## RESULTS

BIOPHEN™ FVIII, designed with human FIXa and FX, can quantitatively measure Emicizumab concentrations (FVIII like activity) in plasma (in µg/ml), with high sensitivity and specificity. For Emicizumab activity, expressed in µg/ml, the equivalent FVIII activity (in IU/ml) is much lower when tested with the chromogenic than with the 1-stage clotting assay, explained by the mechanism of action: FVIII is a catalytic cofactor for FIXa in the activation of FX to FXa, and Emicizumab binds to both FIX/FIXa and FX, in a molecule to molecule mode (Figure 1).

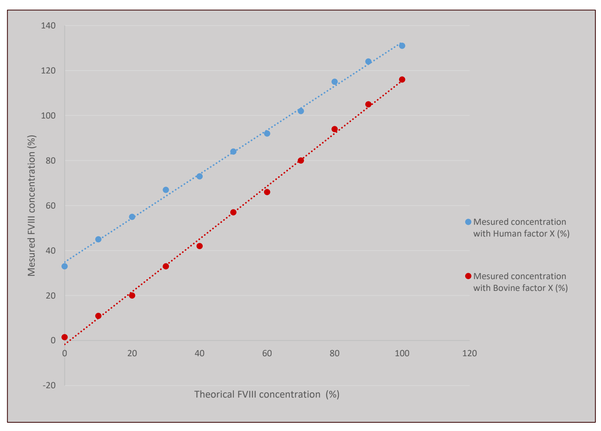



Figure 2: Emicizumab (100 µg/ml) is mixed with citrated human plasmas at different FVIII concentrations and FVIII is tested with the FVIII chromogenic assay using human or bovine FX.

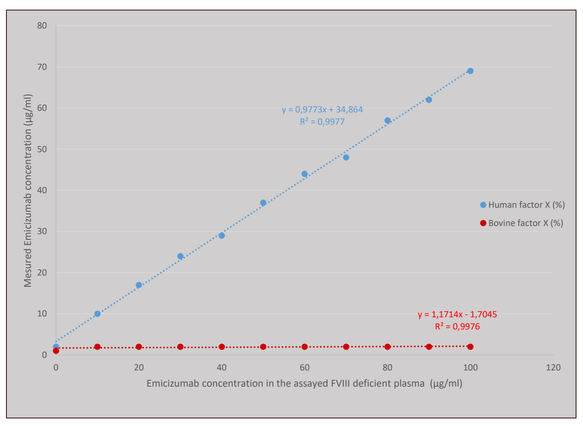
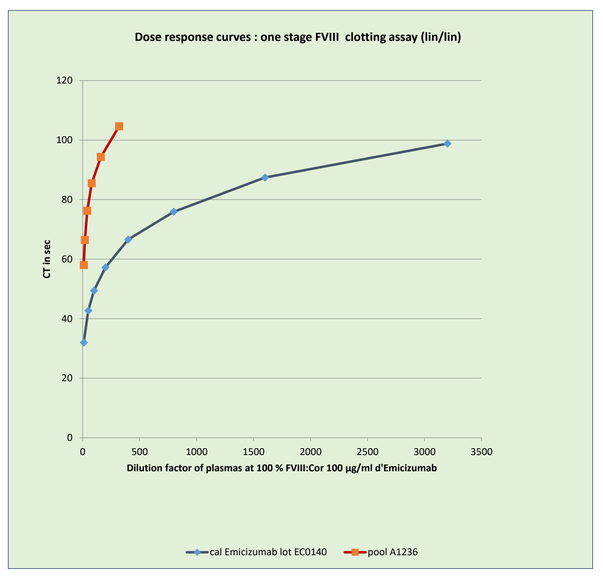
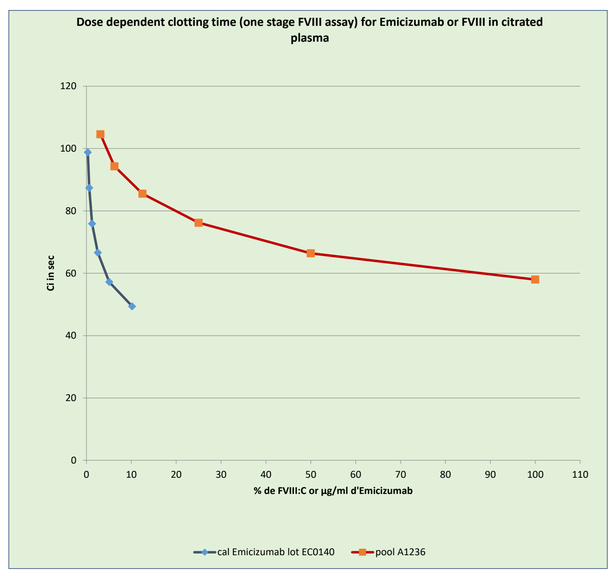


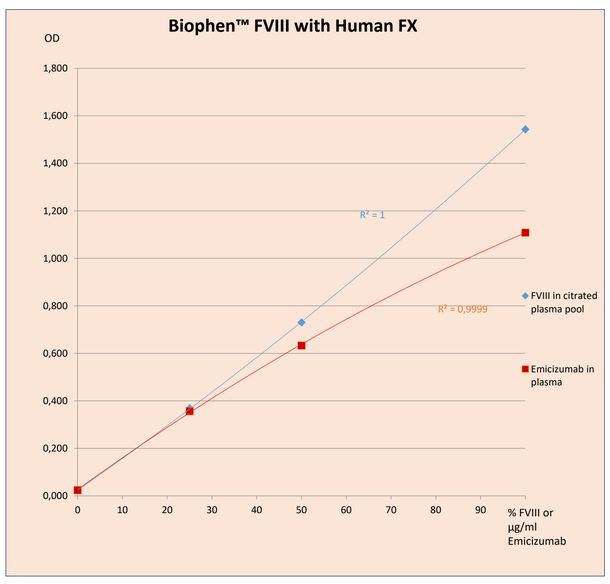

Figure 3: Recovery of Emicizumab spiked in FVIII deficient plasma and measured with the FVIII chromogenic assay using human or bovine FX.

When Emicizumab is spiked in citrated plasma containing a normal FVIII concentration, there is a strong interference of Emicizumab. This interference is abolished with bovine FX is used (Figure 2).

The variant assay with bovine FX is then insensitive to Emicizumab as shown, and can be used for titrating FVIII antibodies in treated patients. Figure 3 shows the recoveries of Emicizumab spiked in FVIII Deficient Plasma and tested with both assays.



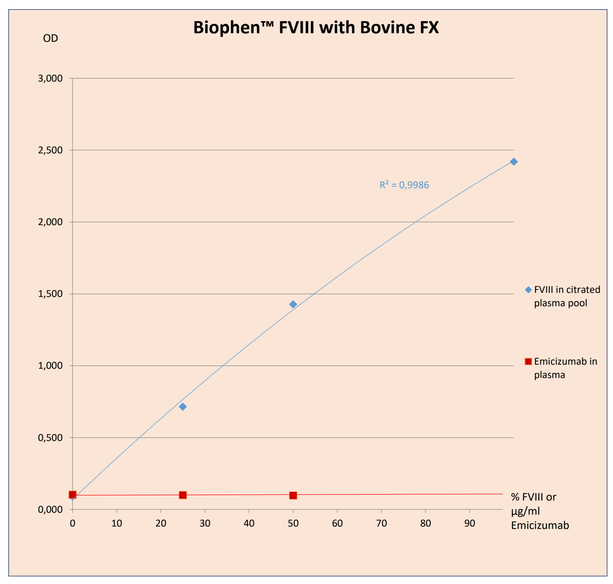




**Figure 1:** Clotting times and dose response curves for FVIII or Emicizumab in citrated plasma, as tested with the one stage clotting assay (upper panels), and with the Biophen FVIII chromogenic assay designed with human factors (FIXa and FX), or after replacement of human FX with bovine FX (lower panels).

## CONCLUSIONS

The combined use of FVIII chromogenic assays designed with human or bovine FX are convenient and practical laboratory tools for testing FVIII, and Emicizumab activities, and titrating FVIII inhibitors. These assays are fully automatable on all coagulation instruments, especially on the major platforms: CS, BCS XP, Atellica, ACL-Top and STA-R.
They allow measuring Emicizumab in treated hemophiliacs (use of human FX), and to evaluate Anti-FVIII inhibitor titres in treated patients (use of bovine FX).

## REFERENCES

1 - Lenting PJ et al. Emicizumab, a bispecific antibody recognizing coagulation factors IX and X: how does it actually compare to factor VIII? Blood. 2017 Dec 7;130(23):2463-2468.
2 - Adamkewicz JI et al. Effects and Interferences of Emicizumab, a Humanised Bispecific Antibody Mimicking Activated Factor VIII Cofactor Function, on Coagulation Assays. Thromb Haemost. 2019 May 7.
3 - Oldenburg J et al. The effect of emicizumab prophylaxis on health-related outcomes in persons with haemophilia A with inhibitors: HAVEN 1 Study.Haemophilia. 2019 Jan;25(1):33-44.

## ACKNOWLEDGEMENTS

R² diagnostics (USA) for the supply of lyophilized citrated plasmas containing Emicizumab.