TECH TIP:
Standardized Determination of FVIII Inhibitors with the Improved FVIII INH kit

By L. Rosenmayr, L. Wagner, N. B. Binder, Technoclone GmbH

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INTRODUCTION

Introduction

The development of antibodies to Factor VIII:C in the treatment of patients with haemophilia A is still a problem. The majority of laboratories continue to perform FVIII inhibitor assays using their own in-house method causing high variability of results..

Aim of this study was to investigate the performance of the FVIII INH kit, which provides all reagents and instructions and in addition a positive and a negative control, and to correlate the results with those obtained by the classical Bethesda and by the Nijmegen assay.

MATERIALS AND METHODS

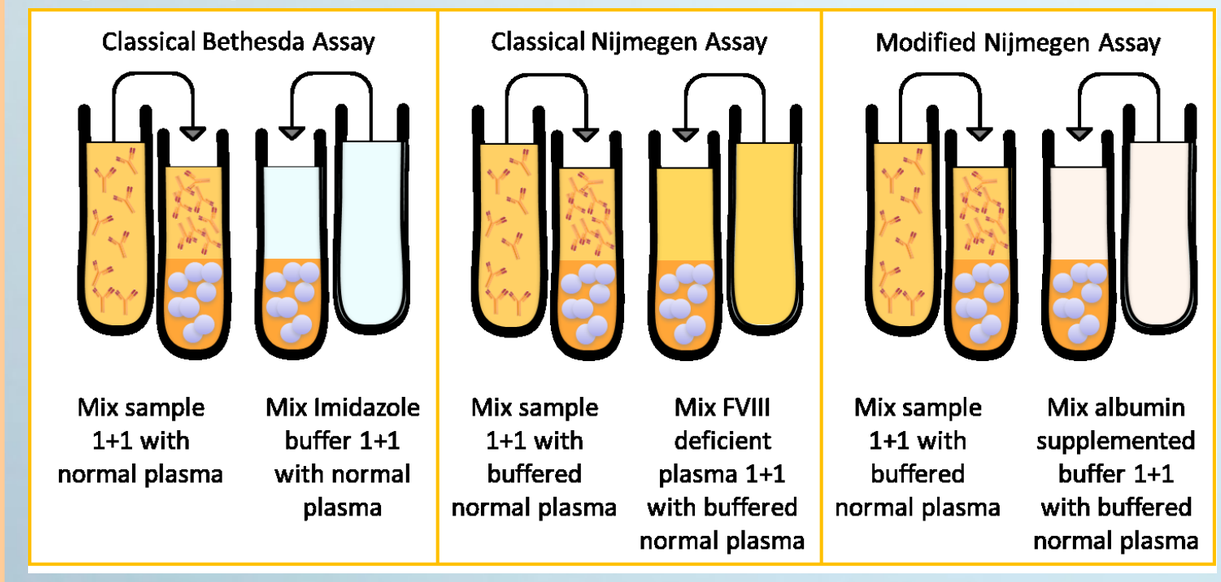
Method:

Haemophilia A plasma samples with and without inhibitors were tested in the classical Bethesda assay, the Nijmegen assay and with the FVIII INH assay kit**.** The FVIII INH kit follows the modified Nijmegen assay principal and uses buffered normal plasma in a 1+1 mixture with sample plasma. The control mix is a 1+1 mixture of imidazole buffer with BSA and buffered normal plasma.

Plasma sample dilutions are made with imidazole buffer with BSA. After incubation, the residual FVIII activity was determined with the chromogenic assay Technochrom FVIII:C and Siron LS for FVIII one stage clotting assay.

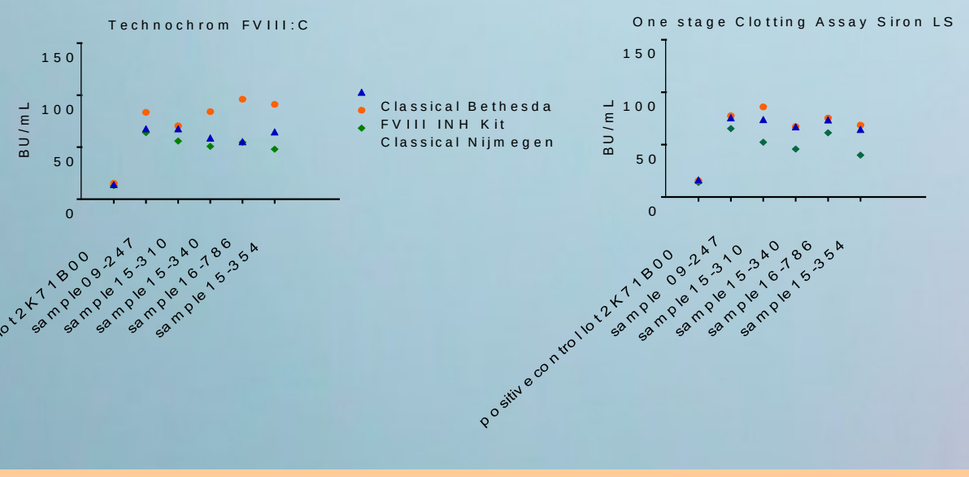
RESULTS

Fig. 1 Assay Principal



Mixtures are incubated at 37°C for 2 hours, reaction is stopped on ice and FVIII activity is determined.

Fig. 2 The 3 Haemophilia A plasma samples without inhibitors were tested negative with all assays. Results for positive control plasma 2K71B00 gives results within confidence range for all assays with an overall CV of 8.7%. The 6 FVIII INH positive plasma samples had highest BU results in modified Nijmegen assay setting, confirming it's highest sensitivity towards the FVIII INH



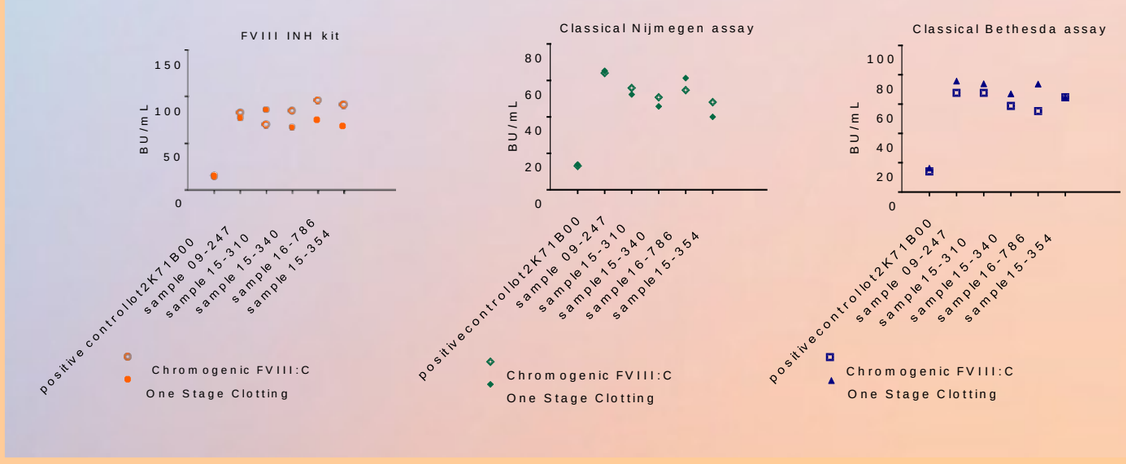
Tab. 1 Results for positive FVIII INH control lot 2K52B00. FVIII activity was determined with the chromogenic assay Technochrom FVIII:C and one stage clotting assays using different aPTT reagents and shows big differences in determined BUs depending on method used.

2K52B00	Technochrom FVIII:C	Siron LS	DAPTTIN TC	Pathromtin SL	STA CK-Prest	SythASil
Mean BU	26.8	27.4	19.4	31.7	27.0	32.0
SD	1.83	1.95	1.42	2.96	5.05	2.55
CV %	0.68	0.71	0.73	9.33	8.71	7.97

Tab. 2 Positive control lot 2K71B00 with a lower target value has lower differences between the 3 used methods. CVs for all methods is with <2% very good

2K71B00	Technochrom FVIII:C	Siron LS	DAPTTIN TC
Mean BU	16.4	16.7	14.5
SD	1.79	2.74	2.84
CV %	1.09	1.64	1.96

Fig. 3 Comparison of results of one stage clotting assay with those of chromogenic FVIII determination shows sample specific differences between results for each FVIII INH method.



As the samples are treated with rFVIII preparations, the developed antibodies may interfere with phospholipid binding of FVIII. This is reflected in different BUs, using reagents with different phospholipid composition/concentration.

CONCLUSIONS

The FVIII INH kit (5152005) can be used with good performance for determination of FVIII INH in plasma samples, assay performance is controlled by use of the provided controls. There is a trend towards higher BUs with FVIII INH kit demonstrating high sensitivity of the assay.

Technoclone GmbH ♦ Brunner Str. 67 1230 Vienna Austria

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