Medicines & Healthcare products Regulatory Agency

South Mimms Inhibitor Assay (SMIA): An affordable and improved method for measurement of FVIII inhibitors

Sanj Raut, Alan Heath

National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, EN6 3QG, UK.

NIBSC Confidence in Biological Medicines

INTRODUCTION

Development of a neutralising Factor VIII (FVIII) inhibitor is the most significant treatment complication in patients with haemophilia A. It can be life threatening (morbidity rates 70% higher in inhibitor patients), it can decrease effectiveness of treatment (ITI, FEIBA, FVIIa, Porcine FVIII etc.) and it has significant cost (diagnostic, treatment & haemophilia care) implications. Laboratory plays an important role as it is required to provide a reliable and reproducible assays for the detection and quantitation of neutralising inhibitors, where accurate diagnosis of inhibitor patients is essential. It is also important for monitoring & management of haemophilia care and evaluation of novel factor product safety. However, current inhibitor assays (Bethesda assay & Nijmegen Modification Assay) have consistently shown high inter-laboratory variability with coefficients of variation (CVs) often greater than 30%, and where the percentage of false positive & negative results are unacceptably high.

HYPOTHESIS

FVIII deficient plasma: Introduction of a critical variant in the assays ?

In the Nijmegen Modification assay (current gold-standard), patient's inhibitor titres are measured relative to a Reference ("Control") mixture consisting of equal volumes of buffered-normal-pooled plasma (BNP) and FVIII-deficient plasma (FDP), the latter being an expensive reagent. We questioned the need for FDP, which was introduced into the assay as a like-for-like diluent for the Reference and we hypothesised that this would actually introduce a variant in the assay whose variability can be exacerbated by the many different FDPs now commercially available (see Fig.1). Furthermore, FDPs require normal levels of VWF; Immunodepleted plasma may be contaminated with capture antibody; Congenital deficient plasmas may contain inhibitors; Chemically depleted plasma may result in activation of FV. More importantly, as the inhibitor titre is based on % of FVIII in the Reference in the Nijmegen assay (Figs 2a & 3a), we should be able to substitute the FDP with a more like-for-like diluent such as BNP. The unknown inhibitor titre in SMIA (Figs 2b & 3b) would now be expressed relative to 200% FVIII in the Reference (rather than 100% FVIII previously). Furthermore, this approach would remove the critical variant and, in addition, significantly reduce the cost of an inhibitor assay.

Fig 1: Variability in different commercial FDPs using Thrombin Generation assays

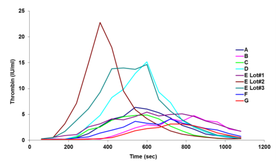


Fig 2a: Nijmegen-Modification Assay

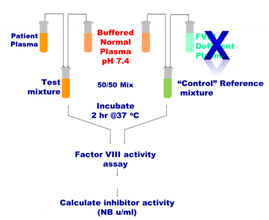


Fig 2b: South Mimms Inhibitor Assay (SMIA)

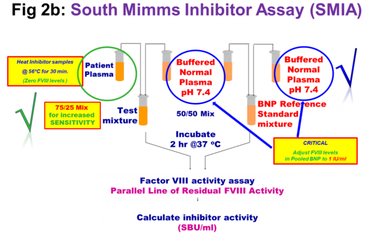


Fig 3a: Nijmegen-Modification Assay

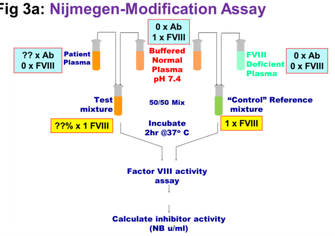
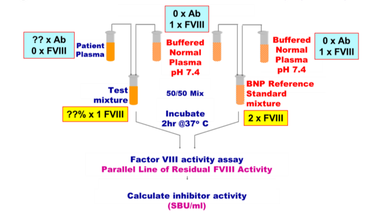


Fig 3b: South Mimms Inhibitor Assay (SMIA)



MATERIALS AND METHODS

Test Materials: Monoclonal and Polyclonal FVIII neutralising antibodies "inhibitors" were used to develop surrogate patient samples by spiking antibodies in FVIII deficient plasma at various dilutions. Inhibitor Patients' samples were also used.

Methods: FVIII antibodies and clinical inhibitor samples, over a wide range of concentrations, were used to test the above hypothesis by comparing Nijmegen inhibitor assay (Control/Reference mixture: BNP + **FVIII deficient Plasma**) with South Mimms Inhibitor Assay, SMIA (Control/Reference mixture: **BNP Only** (No FVIII deficient Plasma). For the FVIII assay stage, both one-stage clotting (OSCA) & chromogenic assays were used.

RESULTS

Fig 4a: Comparison of South Mimms Inhibitor Assay (SMIA) vs Nijmegen Assay: **High Titre** (OSCA)

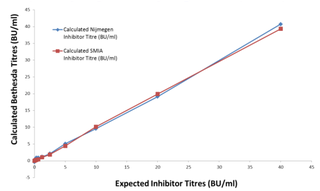
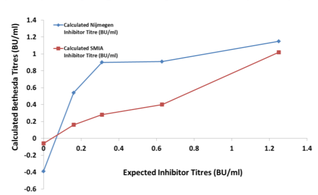


Fig 4b: Comparison of South Mimms Inhibitor Assay (SMIA) vs Nijmegen Assay: **Low Titre** (OSCA)



Data showed that, for high titre inhibitor samples (5 - 40 BU/ml), comparable inhibitor titres were obtained using the two inhibitor methods. This was the case when using either OSCA (Fig 4a) or chromogenic assay (Fig 5a).

For low titre (1.0 - 0.15 BU/ml) inhibitor samples, Nijmegen assay detected inhibitor titres down to ~0.6 BU/ml (Figs 4b, 5b & 6), whilst SMIA could detect inhibitor titres down to ~0.2 BU/ml. This was observed for both OSCA and chromogenic assay. Furthermore, below inhibitor titres of 0.6 BU/ml, data from SMIA was found to be more linear than data from the Nijmegen assay (Figs 4b & 6).

Assessment of clinical inhibitor samples from haemophilia A inhibitor patients showed that there was a good correlation in inhibitor titres between SMIA and Nijmegen assays (see Fig 7).

Fig 5a: Comparison of South Mimms Inhibitor Assay (SMIA) vs Nijmegen Assay: **High Titre** (Chrom)

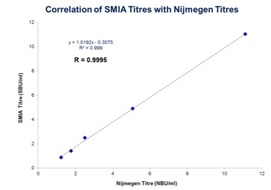


Fig 5b: Comparison of South Mimms Inhibitor Assay (SMIA) vs Nijmegen Assay: **Low Titre** (Chrom)

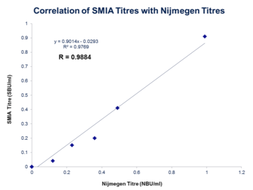


Fig 6: Comparison of Nijmegen & SMIA Assays vs Labelled Titre: **Low Titre** - Chromogenic

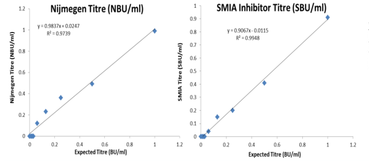
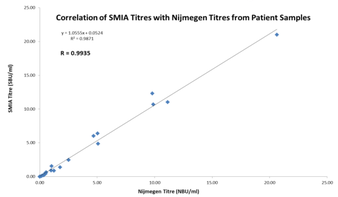


Fig 7: Comparison of SMIA vs Nijmegen Assay: **Patient Inhibitor Samples** - Chromogenic



CONCLUSIONS

- SMIA can obtain equivalent results compared to the Nijmegen Inhibitor Assay (1-stage clotting & chromogenic assays).
- SMIA is sensitive to lower levels of inhibitor titres ~0.2 BU/ml (sensitivity can be further refined - different dilution/mix of Test)
- SMIA has a significant step reduced in inhibitor assay (FVIII-deficient plasma not required) - Critical Variant Removed
- SMIA will significantly reduce the cost of inhibitor assays (FVIII-deficient plasma not required; BNP already available)
- A very simple, easy & welcome modification for clinical Laboratories
- This assay will be accessible (financially) to all laboratories, including those in developing countries

FUTURE ASPECTS

Can SMIA improve inter-laboratory variability?

In order to address the above question an international collaborative study to evaluate the South Mimms Inhibitor Assay (SMIA) has been initiated, where: Bethesda titres of inhibitor samples, inter-laboratory variability and the sensitivity of this assay will be assessed in comparison to the Nijmegen assay.

If you are interested in participating in this study or for further information, please contact: **Sanj Raut, NIBSC, UK**
e-mail: sanj.raut@nibsc.org

ACKNOWLEDGEMENTS

We would like to thank Dr Pratima Chowdary & Anne Riddell from Haemophilia Centre, Royal Free Hospital, for providing patient plasma samples for analysis.

PosterSessionOnline.com
Poster Presented at:
DOI: 10.3252/pso.au.WFH2016.2016
Inhibitors Sanj Raut
78-PP-W
WFH2016