### Background

- Antibody-based inhibitor response to Factor VIII replacement therapy is one of the most common complications in the treatment of persons with hemophilia A (PwHA).

- International proficiency studies on FVIII inhibitor testing revealed coefficients of variation as high as 50% between laboratories.

- Quantification of FVIII inhibitors is not standardized. Laboratory-developed tests (LDTs) often vary between laboratories in:
  - processing of the patient plasma before testing
  - reagents used as the diluant and FVIII source in the assay
  - FVIII activity measurement at the assay endpoint

- Different LDTs have different specificities. Some methods fail to detect FVIII inhibitor in the presence of emicizumab (EMIZUMAB®).

- This work describes the characterization of a chromogenic modified Nijmegen-Bethesda assay (MNBA) using a standardized set of reagents.

### Materials and Methods

- **Repeatability**
  - The chromcheck™ FVIII Inhibitor Kit used to perform the assay consists of five steps, which can be completed using as little as 500 μL of 3% 0.2% 0.2% diluted human plasma.
  - **Performing the FVIII inhibitor assay with a chromcheck FVIII Inhibitor Kit consists of five steps:**
    1. Heat Inactivation: The patient plasma and controls are incubated in a 56 °C water bath for 30 minutes to minimize residual FVIII activity in the samples.
    2. Centrifugation: The inactivated plasma is spun at 1700 × g for 5 minutes to remove precipitate from the solution.
    3. Dilution: Serial twofold dilutions of the supernatant are made with IB-BSA.
    4. Mixing: the neat plasma and dilutions are mixed with an equal volume of IB-PNP. The resulting mix is incubated at 37 °C for two hours in a water bath after two hours, the reaction is halted by placing the samples in an ice bath for 10 minutes.
    5. Measuring: The FVIII activity of the dilutions is measured, and the inhibitor titre is calculated relative to a control mix containing equal volumes of IB-PNP and IB-BSA.

- In this study, all FVIII inhibitor measurements were performed on a Siemens BCS® XP analyzer, using the bovine-based Siemens FVIII Chromogenic Assay.

- **Reproducibility**
  - In this study, a standardized chromogenic MNBA showed excellent within-laboratory and across-site precision.
  - Within-laboratory precision was < 10% for inhibitor-positive patient samples, and < 0.1 BU/mL for inhibitor-negative samples.
  - Reproducibility of the assay was < 15% for inhibitor-positive patient samples, and < 0.1 BU/mL for inhibitor-negative samples.

- The limit of quantification (LoQ) of the assay was found to be 0.2 BU/mL, well below the medical decision level of 0.8 BU/mL.

- The chromcheck FVIII Inhibitor Kit shows potential for labs seeking repeatable and reproducible FVIII inhibitor measurement that can otherwise vary significantly within or between labs.

- Standardization of reagents and protocol yields consistent results and is suitable for multi-center clinical studies of PwHA.

### References


### Conclusions

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