# FACTOR VIII – CHROMOGENIC - LOW ACTIVITY SAMPLE ANALYSIS IN A LOW CALIBRATION CURVE MODEL

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**Introduction** : Hemophilia is a bleeding disorder that causes affected people to bleed longer and, in severe cases, spontaneously. A genetic defect in the X chromosome causes decreased activity of circulating factors. Adequate lab. diagnostics is essential and could be done by different methodologies, such as the determination of factor activity by chromogenic and one-stage assays based on APTT. Treatment has improved significantly over the past few decades, and state-of-the-art treatment is more efficient, safe, and convenient than ever. Adverse effects of medications became less severe in recently, but it remains important to be aware of to ensure that treatment is effective and ensure the best possible conditions for affected individuals. The classification of hemophilic patients is divided into 3 categories, according to the activity of FVIII: mild 25% - 5%; moderate, 5% - 1% and severe when the activity is $< 1\%$ of normal FVIII. New substitutive therapies such as emicizumab and some drugs with an extended half-life, the WFH (World Federation of Hemophilia) recommends the use of a chromogenic assay of FVIII containing bovine FX for monitoring factor FVIII activity. This study aimed to evaluate the applicability of a low calibration curve in the chromogenic factor VIII (FVIII-Chr) assay by standardizing the dilution of the standard (calibrator) and thus ensuring the accuracy of the analyses in the lower values like severe hemophilia range. **Materials & Methods** : The samples used in the study were obtained from H&H LAB SAS, which guarantees all requirements. In this study, Sysmex®CS 2100i Siemens Healthineers were used to analyze the samples. FVIII-Chr assay; Standard Human Plasma (SHP); Coagulation FVIII Deficient Plasma; Control Plasma P. Calibration was performed with SHP, together with FVIII-Chr according to the instructions; Internal Quality Control; the accuracy of the calibration curve was checked with appropriate controls. Results: Preparation of the Low Curve: SHP reconstitution according to instructions, followed by 1:10 dilution between the SHP and the plasma deficient in FVIII. The curve was named FVIII-Chr Low. Quality control (QC) was performed through pathological control with 2 dilutions: 1) identical to the formation of the low curve, i.e., dilution 1:10 (2.3 – 3.9%). 2) dilution 1:30 (0.2 - 2%) to accommodate the range of activity comprising severe hemophilia. The values found confirms the curve's quality. **Discussion** : This study sought to develop a way to detect FVIII activities $< 1.5\%$ in a reproductive manner, even with a reduced "N" number of samples for severe state. The detection of chromogenic methodology suggests a limit of detection for the optical density of D.O. 0.0080: they are below the most diluted point of the calibration curve. Suggestion: to report the values of these cases that can be released as an activity $< 0.3\%$ and include the DO. **Conclusion** : Good performance of QC. Dilution 1/10 and 1/30 is checked; reproducibility in the results can be observed. With the protocol FVIII Low and LCC SHP+PDF it is possible to obtain results of FVIII-Chr $< 1.0$ % in severe hemophilia patients compatible with the patient's clinical condition in treatment with emicizumab.