

Diagnostica di laboratorio in emostasi e trombosi

OSA *VS.* CHA FOR RPFVIII MEASURE: STILL AN ISSUE?

G. Gazzelli, Y. Azzanelli, C. Vernarecci, F. Marotta, F. Giacopelli, B. Tappino, L. Banov, A.C. Molinari

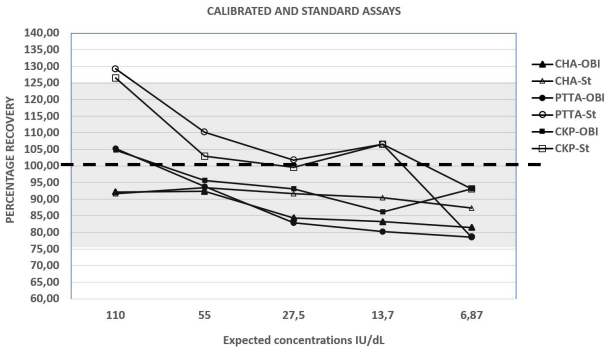
IRCCS Istituto Giannina Gaslini Genova, Italy

Background and Aims. Acquired Hemophilia A (AHA) management requires accurate monitoring of susoctocog alfa (rpFVIII, Obizur®), a recombinant porcine FVIII. However, significant methodological challenges persist: chromogenic assays (CHA) have been reported to underestimate rpFVIII activity compared with one-stage assays (OSA), which have traditionally guided clinical management. In addition, discrepancies between spiked and in vivo studies have been described, further complicating interpretation. Data on the performance of Stago reagents in this setting remain limited. As a result, clinicians relying on Stago platforms may face uncertainty in result interpretation and dose optimization. This study aims to evaluate the performance of Stago OSA and CHA reagents for susoctocog alpha measurement.

Methods. The analytical performance of Stago reagents for the measurement of susoctocog alfa was evaluated using a standardized dilution protocol: The susoctocog alfa calibrator, provided by Takeda, served as the primary standard. The calibrator was analyzed both undiluted and at four serial dilutions (1:2, 1:4, 1:10, 1:20) prepared using FVIII-deficient plasma (Stago). Based on the certified potency of the calibrator, the expected FVIII activity levels were defined as 110, 55, 27.5, 13.75, and 6.87 IU/dL. For standard clotting assays, Unicalibrator® (Stago) was utilized as the reference plasma to establish the standard curves All measurements were performed on the STA R Max3® (Stago) automated coagulation analyzer using three different methodological approaches: two OSAs (CK-Prest®, utilizing kaolin as the surface activator, and PTT-A®, utilizing silica as the surface activator) and one CHA (TriniCHROM®). All reagents and deficient plasmas were stored and handled in strict accordance with the manufacturer's instructions Two distinct calibration strategies were employed: Each dilution level was analyzed in five replicates to ensure statistical robustness and assess intra-assay precision. The same dilution series was analyzed against a dedicated calibration curve established using the susoctocog alfa (Obizur) calibrator itself. We first compared the performance of the OSA against the CHA based solely on measurements derived from the Obizur-calibrated curve. Subsequently, we compared the activity levels measured using the standard calibration curve with those obtained using the Obizur-specific calibration curve to identify potential recovery shifts or method-dependent biases. Results were expressed as percentage recovery relative to the expected theoretical activity to evaluate the accuracy and consistency of each reagent across different calibration models.

Results. All analytical methods and reagents tested demonstrated high repeatability across all concentrations and experimental conditions. When the assays were calibrated specifically using the Obizur (susoctocog alfa) calibrator, all reagents showed high concordance with each other and closely matched the certified expected values. In contrast, when using a standard calibration curve, the two OSAs (kaolin-based and silica-based) consistently yielded activity levels that were slightly higher than those produced by the CHA. Despite this divergence, the measured values remained robust, falling almost entirely within the clinically acceptable range of ±25% of the expected theoretical activity.

Conclusions. Our results demonstrate that OSA and CHA Stago reagents provide high precision and accuracy for the measurement of rpFVIII, also without a product-specific calibration. However, as this study was conducted using spiked samples, these findings must be confirmed in a clinical setting. Further studies on plasma samples from patients with AHA treated with rpFVIII are necessary to validate these analytical performances in the presence of real-world biological variables and inhibitors



Filled symbols: Assays calibrated with the Obizur®-specific calibrator.
Empty symbols: Assays performed using a standard calibration curve (Unicalibrator ®).
CKP: CK Prest® (kaolin-based one-stage assay); PTTA: PTT-A® (silica-based one-stage assay); CHA: TriniChrom® (chromogenic assay)
The dashed line indicates 100% recovery, while the shaded area represents the ±25% clinical acceptability range