Comparison of Two Chromogenic FVIII Activity Assays to a Standard Clot-based FVIII Activity Assay

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INTRODUCTION

• Pharmacokinetic FVIII concentrate studies have revealed discrepancies among FVIII assay methods.
• Clot-based assays of post-FVIII Infusion patient plasma yield results 20–50% below chromogenic assay results.
• The choice of phospholipid in clot-based assays is crucial; the use of platelets or liposomes resembling platelet factor 3 instead of traditional PTT reagents raises the factor activity and improves correlation with chromogenic results.
• These and other functional and antigenic assay results, coupled with clinical data, support the chromogenic FVIII assay as an accurate therapeutic monitor.
• We compared results of the Diapharma Group, Inc., chromogenic Coagmatic Factor VIII and the Aniara Hyphen Biomed Biophen FVIII:C kits to the Diagnostica Stago, Inc., clot-based FVIII activity assay using a Diagnostica Stago STA-R Evolution coagulation analyzer.
• We used a single calibration curve for the clot-based assays and compared results to normal-range and low-range calibration curves for both chromogenic assays.

MATERIALS AND METHODS

• Chromogenic kits:
  - Diapharma Group Inc Coagmatic Factor VIII (DP)
  - Aniara Hyphen Biomed Biophen FVIII:C (AHB)
• Clot-based FVIII:C assay used Diagnostica-Stago, Inc., APTT-A and Diagnostica-Stago FVIII-deficient plasma
• Assays were performed on a Diagnostica-Stago STA-R Evolution coagulation analyzer.
• Samples were comprised of specimens from 33 normal subjects ("normal") and 35 WVO and hemophiliacs with FVIII:C activities <50% ("low").

CALIBRATION CURVES

• Both chromogenic methods used high and low FVIII:C calibration curves:
  - DP ranges: 0–135% and 0–23%
  - AHB ranges: 7.8–156% and 1–20%
• The clot-based assay used a single FVIII:C calibration curve with 12–95% activity.
• Specimen were diluted so results were generated from the linear portions of the curves.
• A fresh calibration curve for all methods was prepared daily.

STATISTICS

• We used analysis of variance (ANOVA) to compare results of the three assays (p<0.05).
• We used the t-test to compare results of the three assays (p<0.05).
• We compared normal, low, and combined sample results generated by the three different assays using regression analysis.

RESULTS

• R values on all calibration curves were >0.995
• Using ANOVA, there were no significant differences among the clot-based method and the low chromogenic (p=0.76) or the high chromogenic (p=0.78) assays.
• Table illustrates significant differences involving low samples tested using AHB.

DISCUSSION

• The chromogenic assay kits measuring all FVIII levels correlate favorably.
• Clot-based to chromogenic assays of all FVIII levels (combined normal and low) correlate adequately.
• Correlations of all normal or all low FVIII levels are inadequate.
• Chromogenic FVIII assays may substitute for clot-based VIII assays provided reference intervals compensate for systematic errors.

REFERENCES