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 plasmas 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 Coamatic Factor VIII and the Aniara Hyphen Biomed chromogenic 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 Coamatic 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 VWD and hemophilic subjects with FVIII:C activities <50% (“low”)

CALIBRATION CURVES

• Both chromogenic methods used high and low FVIII:C calibration curves
  - DP ranges: 0–153% 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

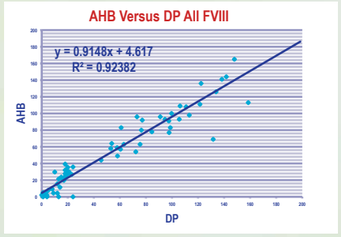
RESULTS

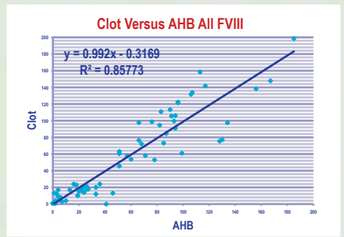
• R values on all calibration curves were >0.985

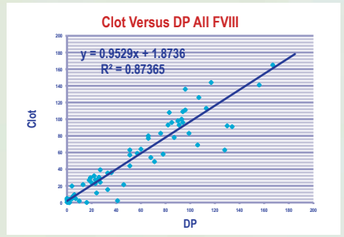
• Using ANOVA, there were no significant differences among the clot-based method and the low chromogenic curves (p=0.19) or the high chromogenic curves (p=0.78)

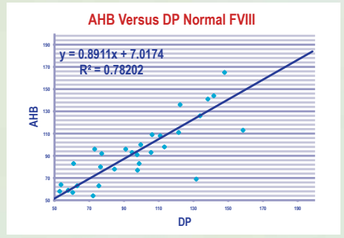
• t-test illustrates significant differences involving low samples tested using AHB ➤

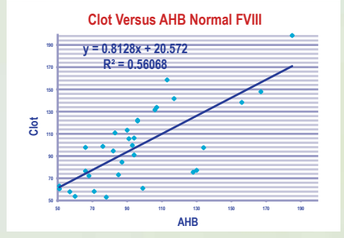
t-test	p =
AHB All to DP All	0.964474
Clot All to AHB All	0.745841
Clot All to DP All	0.747154
AHB Normal to DP Normal	0.237323
Clot Normal to AHB Normal	0.511527
Clot Normal to DP Normal	0.840045
AHB Low to DP Low (Significant—p <.05)	0.019672
Clot Low to AHB Low (Significant—p <.05)	0.033478
Clot Low to DP Low	0.754949

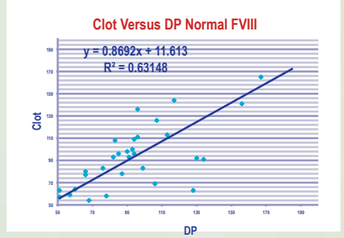


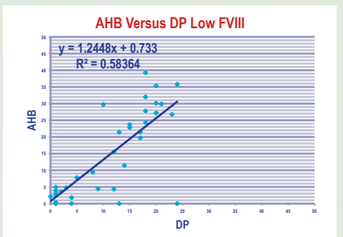


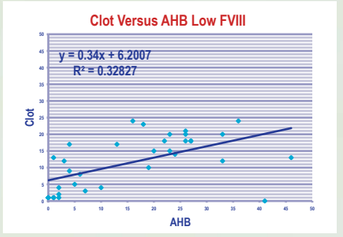


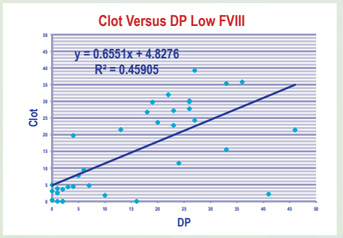












DISCUSSION

• 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

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