Influence of Specific Antibody, Normal Pool, and Detection Methodology on Von Willebrand Factor (VWF) Multimer Analysis

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Plasma Von Willebrand factor (VWF) multimer analysis is used to type or subtype von Willebrand disease (VWD). Earlier methods utilizing radiolabeled antibody have largely been replaced with luminescent or colorimetric visualization techniques which rely on quantitatively electroblotting VWF multimers. We describe the effects of differing anti-VWF antibodies, normal pooled plasma controls and detection methods on quality and interpretation of VWF multimer analysis.
Methods: Plasma VWF multimers were separated in 0.8/2.0% agarose gels on a flat-bed electrophoresis unit. Multimers were imaged in the gel using either in-house radiolabeled specific antibody and autoradiography, or unlabelled primary antibody, near infrared dye-labeled secondary antibody and infrared fluorescence imaging (Odyssey, Li-Cor Biosciences). Primary antibodies and normal pooled plasmas were compared for multimer molecular weight distribution and infrastructure imaging.
Results: Both imaging techniques correctly showed the full distribution of VWF multimers for normal and type 1 VWD plasmas, and missing or decreased high-molecular-weight (HMW) multimers for types 2A and 2B plasmas. The full range of normal multimers was present in in-house and commercial normal pooled plasmas, but the largest molecular weight multimers were relatively decreased in the commercial preparation. This would influence attempts to quantitatively compare patient multimer distribution with normal. While two commercially-available primary antibodies correctly identified type 2 VWD patients, one antibody showed stronger binding to a very fast-moving electrophoretic band. This intense band would also influence efforts to quantitatively compare relative abundance of low molecular weight to HMW multimers.
Conclusions: The use of infrared fluorescent imaging technology allows in-gel detection of VWF multimers, eliminating the need for radioisotopes or blotting. While qualitative interpretation was not affected, choice of antibody and normal control influence quantitative expression of VWF multimer distribution in this method.