
A simple method for measurement of von Willebrand's factor (VWF) suitable for the clinical coagulation laboratory would substantially aid in the differential diagnosis of bleeding disorders. We have developed a simple microscopic titration assay of plasma VWF using the Ristocetin induced aggregation of washed, fixed platelets. The assay employs arithmetic dilutions of test plasma in buffer, Ristocetin in buffer, and a final volume of 0.4 ml per well. After 20 minutes' agitation on a rotary shaker at room temperature, an endpoint of visible aggregation can be determined. Fifty normal plasmas tested had a mean titer of 1/500 (range 1/240 to 1/1501) whereas 8 plasmas from patients with known von Willebrand's disease (VWD) had a mean titer of 1/21 (range 1/10 to 1/186). Deviation from the normal range correlated with the severity of the VWD. Reproducibility of results was within ±10.

Platelets washed before fixation with paraformaldehyde (method modified from Allans et al., J. Lab. Clin. Med. 83:318, 1973) appear almost twice as sensitive to VWF in this assay as those fixed in platelet rich plasma and washed subsequently. Fresh and frozen plasmas give equivalent results, permitting storage of specimens. This microtiter assay thus offers a sensitive measure of VWF Ristocetin cofactor activity, utilizes stable and standardizable reagents, requires only small amounts of patient plasma (or other biological material) and permits rapid testing of different specimens simultaneously.

THE ASPIRIN BLEEDING TIME – A SCREENING TEST FOR EVALUATION OF VON WILLEBRAND'S DISEASE. Marie J. Stum, Merrill Miller, Joel Selig and Frederick Harway. Dept. of Peds. and Path., SUNY, Upstate Med. Ctr., Syracuse, N.Y.

In an attempt to elucidate the usefulness of the Bleeding Time (BT) post aspirin (ASA) ingestion this test was done with other tests of coagulation in 20 controls and 77 patients evaluated for a possible bleeding diathesis. Coagulation studies included PT, APTT, TT, VIII AHP & AGN, IX, XI, XII platelet retention and aggregation, and modified Ivy BTs pre and 2 hrs. post 600 mgmes ASA. The mean control BT in 25 normals was 2.6 ± 1.2 (SD), P.05, 6.0 ± 3.5 (SD), P.01, 7.0 ± 4.1 (SD), P.01, 5/30 "controls" without bleeding history had abnormal BTs post ASA. In this group, 4 were proven to have unrecognized von Willebrand's disease (VWD) and one a platelet defect. Of the 74 patients studied, 26 had Ristocetin and washed, fixed platelets and a final volume of 0.4 ml per well. After 20 minutes' agitation on a rotary shaker at room temperature, an endpoint of visible aggregation can be determined. Fifty normal plasmas tested had a mean titer of 1/500 (range 1/240 to 1/1501) whereas 8 plasmas from patients with known von Willebrand's disease (VWD) had a mean titer of 1/21 (range 1/10 to 1/186). Deviation from the normal range correlated with the severity of the VWD. Reproducibility of results was within ±10.

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COMPARISON OF FACTOR VIII LEVELS AFTER ADRENALIN IN HEMOPHILIA A AND VON WILLEBRAND'S DISEASE (VWD). M. E. Eykoff, J. O. Ballard and B. Prager - Pennsylvania State University School of Medicine, Hershey, Pa., and the Allentown General Hospital, Allentown, Pa., U.S.A.

Two-fold or greater increases of Factor VIII procoagulant activity (VIII AHP) have been described following the administration of adrenalin in normal individuals and in some patients with VWD. VIII AHP and Factor VII-like antigen (VII ACH) levels were measured pre and one hour post the s. q. injection of adrenalin 0.35 cc. In 4 adult VWD patients and in 10 adult hemophiliacs with VIII AHP levels ranging from 1-29%, normal VIII ACH levels and normal VIII AHP levels were measured by the washed platelet ristocetin assay. Eighteen normal adults served as controls. The four patients with VWD showed two-fold or greater increases of VIII AHP with variable VIII ACH responses. All 10 hemophiliacs showed no change in VIII AHP with significant increases in VIII ACH when analyzed by the paired t-test.

The fixed rate of VIII AHP production, release or activation observed in hemophiliacs may help to explain the constancy of VIII AHP levels in individual hemophiliacs and their affected family members. Furthermore, this simple provocative test may be helpful in distinguishing certain VWD patients from those with hemophilia.