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INFLUENCE OF HEPARIN ON FACTOR VIII (FVIII) ASSAY. T.H.Tran (1), U.Zühlke (1), J.Hauert (2), F.Duckert (3), G.A.Marbet (3), R.Wagenwoord (4). Merz+Dade, 3186 Düdingen (1), Hematology Laboratory, CHUV, 1011 Lausanne (2), Coagulation Laboratory, Kantonsspital, 4031 Basel (3), Switzerland and Biochemistry Department, University Limburg, Maastricht, The Netherlands (4).

Thrombin-activated FVIII accelerates the conversion of factor X to activated factor X (FXa) by activated factor IX, phospholipids and calcium ions. In plasma, FVIII is activated by initial traces of thrombin, which, in the presence of heparin, is rapidly inhibited by binding to antithrombin III and heparin cofactor II. To avoid the effect, we have experienced with increasing amounts of exogenous thrombin. We were able to match the heparin cofactors concentration in diluted plasma with thrombin, so that the presence of heparin did not affect the formation of FXa, whose activity was assessed with a chromogenic substrate. Indeed, addition of heparin at any concentration to citrated plasma showed no significant deviation from the FVIII control value. Levels of FVIII in heparinized plasmas similar to those in citrated plasmas further confirmed the finding. Patients plasmas showed comparable FVIII levels before and after heparin infusion, though plasma PTT was clearly prolonged after in vivo heparinization. FVIII chromogenic assay was correlated with the one-stage clotting assay by measuring FVIII levels in 60 hemophiliacs A and carriers, in patients with von Willebrand disease (27) and other congenital deficiencies (4), high risk of thrombosis (15), bleeding tendency (20), disseminated intravascular coagulation (4) and circulating anticoagulants (2), and commercial concentrates. There was a highly significant correlation between both techniques (N=127, r=0.97, Y=0.91X + 4, range 1-380%). Three severe hemophiliacs with <1% were detected with both methods. Data obtained from both techniques were also in good agreement in the range of 1-20% FVIII.
Thrombin was added both to activate instantaneously FVIII and to neutralize heparin cofactors in samples. It thus abolishes the incubation time needed to generate in situ traces of thrombin and the influence of heparin on our FVIII assay. An eventual fibrin formation does not affect the FXa formation and the reading. The technique is also suited for automation.

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EFFECT OF COLD ACTIVATION, $AL(OH)_3$ ADSORBTION, TISSUE FACTOR AND PLATELET ON ONE/TWO STAGE CHROMOGENIC ASSAYS OF F.VIII
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Two stage Coatest assay of F.VIII is reportedly insensitive to pretreatment of F.VIII with thrombin. Since thrombin is formed rapidly, during the incubation step, a one stage method was tried by incorporating S2222, containing thrombin inhibitor (I-2581) in the incubation system, thus making the assay highly specific to F.Xa-induced activation of F.VIII and allowing to monitor directly the formation of paranitroaniline in microtray plate at 2-5 min. intervals. Initial comparative analyses performed on cold activated and/or adsorbed/non-adsorbed samples (FFP, cryoprecipitate and hypo- or hypercoagulable state) revealed that in all cases the lag phase was prolonged (2-3 fold) in the one stage method. Cold activation had little effect on the lag phase/reaction rate, whereas $AL(OH)_3$ decreased up to 50% F.VIII like activity, prolonged the lag phase and dose-response curves become non-parallel. Substituting phospholipid (pL) by tissue factor (TF) or addition of diluted TF (1/500) to reaction mixture increased synergistically the rate of F.Xa generation in both adsorbed and non-adsorbed system. In contrast washed platelets (PLT), up to 3000 x 10^9/l, were less effective on both TF or PL-induced F.Xa generation. The presence of I-2581 in this system prolonged the lag phase to 1h. Substitution of the conventional O.D. reading by the time required to achieve a fixed absorbancy (O.D. = 0.5) make the one stage coatest F.VIII equivalent to the APPT-type assay. Based on these results it is concluded that thrombin is involved in increasing F.VIII catalytic activity. TF and F.VII contribute to the shortening of the lag phase and increased F.a generation. The kinetic property of cell surface-bound F.VIII is not the same in the presence or absence of thrombin. The reported insensitivity coatest F.VIII to thrombin is probably due to the fact that thrombin activated F.VIII is a good substrate for F.Xa and is cleaved by F.Xa which is produced in abundance in the two-stage chromogenic assays. A new procedure for monitoring various pathways of F.Xa generation, based on the coatest reagent is provided, which is particularly suitable for large scale screening of blood donors and blood products.

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ENZYME LINKED IMMUNOASSAY (ELISA) FOR FACTOR VIII ANTIGEN
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A two site ELISA test was developed for measuring factor VIII antigen (FVIII:Ag). The assay is based on two antibodies developed in a non-haemophilic and in a severe haemophilia-A patient, respectively. The IgG fraction prepared from the non-haemophilic plasma was used for coating, and the IgG isolated from the haemophilia-A plasma was labelled with horse-radish peroxidase.

FVIII:Ag and FVIII activity was measured in 28 healthy blood donors and in 41 haemophilia-A patients. The normal range for FVIII:Ag was 40 - 180 %, the correlation coefficient between FVIII:Ag and FVIII activity assays was 0.8. The sensitivity of the assay ranges between 0.005 - 0.2 U/ml FVIII:Ag. In 18 cases of severe haemophilia-A FVIII:Ag was not detectable. In 3 out of 23 mild haemophilia-A cases FVIII:Ag was significantly higher, then FVIII activity, indicating CRM^+ variants of the disease. Due to the high sensitivity of FVIII:Ag detection, the assay appears to be suitable for prenatal diagnosis.

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DEVELOPMENT OF A SIMPLE FACTOR VIII-ASSAY FOR CLINICAL USE.
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We have developed an assay for the determination of factor VIII in human plasma. The criteria that such an assay must fulfil are: the method should be simple, the reagents should be stable for several hours at room temperature, the method should be sensitive and linear in the amount of factor VIII. The assay we have developed fulfils all these criteria.

The working procedure is simple. Both a lyophilized factor VIII assay (containing factor IXa, thrombin, phospholipids and Ca^{++}) and lyophilized factor X are reconstituted with water. A reaction tube is filled with 100 µl factor VIII assay, prewarmed at 25° or 37 °C, then 100 µl of a diluted (10-20 times) plasma sample is added (t = 0) and after 30 seconds activation time the reaction is started with 100 µl factor X. After 1-2 minutes a sample is taken and diluted in an EDTA-containing buffer to stop the reaction. The formed factor Xa is meausured with a FXa-substrate from which p-nitroaniline will be split, causing an increase of the A405nm. The lyophilized reagents are stable for several months (at least) and after reconstitution they do not loose activity during a whole working day. The sensitivity of the method is high. A plasma containing 1% factor VIII gives an increase in absorption of three to four times of a fully factor VIII deficient plasma. Extensive studies have shown that a complete linearity excists between 0 - 200% factor VIII in the plasma and the increase of the A405nm.