

A NATIONAL REFERENCE CURVE FOR ASSAYING FACTOR VIII INHIBITORS. G. Mariani, F. Serbanescu, Z. M. Ruggeri P. M. Mannucci. Working Party of the Fondazione dell'Emofilia, Italy.

The results of FVIII inhibitor assays obtained in different laboratories are of difficult comparison, and the dose-response curve from which the inhibitor potency is read in units appears to be one of the main variables. Differences in inhibitor kinetic behaviour make difficult to adopt as a reference the curve of a single patient. On the other hand, the use of individual curves for each patient is laborious and time consuming. We have evaluated whether the adoption of a single reference curve obtained from the mathematical elaboration of the individual experimental points of the curves of 33 inhibitors was statically acceptable and could be proposed as a national reference curve for factor VIII inhibitor assay. The experimental data have been used to make statistical series from which theoretical points were calculated by the least square method resulting in a second degree equation curve. Observed experimental values corresponded to the theoretical points of the calculated curve within a variation of  $\pm 50\%$ , resulting in a variation of the calculated inhibitor potency of  $\pm 20\%$  compared with those read on each individual inhibitor curve. A national trial to confirm the applicability of such reference curve is presently in progress.

AN ARTIFICIAL 'HAEMOPHILIC' PLASMA FOR ONE-STAGE FACTOR VIII ASSAY. V. Chantarangkul, G. I. C. Ingram and S. Darby. St. Thomas' Hospital Medical School, London, England.

An 'artificial' haemophilic plasma for one-stage factor-VIII assays is made by incubating human plasma with EDTA,  $\text{Na}_2$  to destroy factor VIII, and afterwards removing the anticoagulant by dialysis. Bovine factor V is then added to a given level. In the assay, contact activation is controlled by adding contact product.

It was confirmed that factor-VIII activity was destroyed and that the EDTA,  $\text{Na}_2$  was subsequently removed. The fibrinogen in the treated plasma clotted normally with thrombin. Likely variation in factor-V activity would not be critical. The concentration of fibrinogen and other factors was adequate. Variation between batches was small. The artificial plasma yielded assay results closely comparable to haemophilic plasma in samples with factor-VIII activities in the range 0.01-20.0 iu/ml.

DEGRADATION OF FACTOR VIII-RELATED PROTEIN IN FACTOR VIII CONCENTRATES. T. Jakab, R. Pflugshaupt, M. Furlan and E. A. Beck. Hematology Laboratory, Inselspital, and Central Laboratory, Transfusion Service, Swiss Red Cross, Berne, Switzerland.

Lipolytic and/or proteolytic treatment of highly purified human factor VIII results in a progressive disappearance of high-molecular weight protein as shown by electrophoresis on 3 % polyacrylamide gels in the presence of sodium dodecyl sulphate. Partial degradation of factor VIII is typically accompanied by a relative increase of factor VIII-related antigen (VIII R:AG) whereas coagulant activity (VIII:C) or von Willebrand activity (VIII R:WF) may still appear intact. VIII R:AG was measured by crossed immunoelectrophoresis against specific heterologous antibody in agarose gels. VIII R:WF was expressed as ristocetin cofactor activity in comparison with a normal plasma pool. Assuming a VIII R:AG/VIII R:WF ratio of 1.0 in our normal plasma, we found a similar ratio using intact purified factor VIII. This ratio increased regularly following proteolytic degradation of factor VIII concentrate by contaminating proteases or plasmin. Our findings suggest that a high VIII R:AG/VIII R:WF ratio could indicate undesirable alterations of VIII-related protein(s) during plasma fractionation.