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CHROMOGENIC SUBSTRATE METHODS FOR THE DETERMINATION OF FVIIIc, ENDOTOXIN AND PLASMINOGEN ACTIVATOR. E. Eriksson, S. Rosén, M. Knös and P. Friberger. Kabi AB, Peptide Research, Mölndal, Sweden.

FVIII clotting activity in plasma and concentrates can be assayed with good reproducibility in the range 20-120% (C.V. approximately 3%) using purified FIX_a and FX in excess. By incubating the sample with above factors, Ca^{2+} and phospholipid, FX is activated in proportion to the amount of FVIIIc. Generated FX_a is measured with the substrate S-2222. FVIIIc in the range below 20% can be determined by modifying the reaction conditions.

Endotoxin in water solutions can be accurately determined down to 1 pg/ml by incubating the sample with an excess of Limulus lysate. The generated enzymatic activity which is linear in proportion to the amount of endotoxin, is then measured with the substrate S-2423 (Ac-Ile-Glu-Gly-Arg-pNA). Accurate endotoxin determinations in plasma and cerebrospinal fluid are also possible after destroying interfering inhibitors by heat treatment.

Plasminogen activator activities can be measured via plasminogen and the plasmin substrate S-2251 or directly by substrate S-2288 (H-D-Ile-Pro-Arg-pNA). Interfering activities can be minimized by strategic selection of various protease inhibitors.

In all the methods the assay conditions, reagents and procedure have been optimized.

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MEASUREMENT OF PLATELET FACTOR 3 AVAILABILITY BY USING CHROMOGENIC SUBSTRATE. J. Hársfalvi, J. Chmielewska*, Z.S. Latallo* and L. Muszbek. Department of Clinical Chemistry, University School of Medicine, Debrecen, Hungary, and *Institute of Nuclear Research, Warsaw, Poland.

The measurement of platelet factor 3 (Pf 3) availability (the prothrombin converting procoagulant activity of platelet membran phospholipids) induced by collagen, ristocetin, arachidonic acid or other activating agents is a potentially valuable test in the diagnosis of platelet disorders. Its diagnostic potential, however, is greatly impaired by the fact that RVV clotting time method usually used for Pf 3 determination is influenced by many factors, its reproducibility is rather poor, and results are difficult to express in quantitative well defined biochemical terms.

In the present paper a new method measuring Pf 3 activity by using a chromogenic substrate was developed. Platelet rich plasma (PRP) was incubated by various activating agents, then prothrombin thrombin conversion was started by the addition of purified factor Xa. After a certain interval the reaction was stopped by soybean trypsin inhibitor and thrombin formed during the reaction was quantified by the chromogenic substrate S-2238 (Kabi). By varying incubation time, prothrombin, factor V concentrations, the optimal conditions, where platelet phospholipid was the rate limiting factor, were determined. In these conditions Pf 3 activity was a linear function of the number of activated platelets. The results are expressed as the ratio of thrombin generated in activated and unactivated PRP. The method is a rather simple quantitative photometric technique, has an improved reproducibility, and results showing the increase in thrombin generation have well defined biochemical meaning.