

[Haemostasis](#). 1990;20(5):276-88.

**Development of a sensitive and rapid chromogenic factor IX assay for clinical use.**

[Wagenvoord R](#), [Hendrix H](#), [Tran T](#), [Hemker HC](#).

Department of Biochemistry, University of Limburg, Maastricht, The Netherlands.

A chromogenic factor IX assay is developed which requires only two time-dependent steps. Diluted plasma is mixed with a reagent containing factors VIII and X. The reaction is started by addition of a reagent containing factor XIa, thrombin, CaCl<sub>2</sub>, and phospholipids. Then factor XIa activates factor IX if present, thrombin activates factor VIII, and subsequently the complete factor X activating complex (factor IXa, factor VIIIa, Ca ions, and phospholipids) rapidly activates factor X. Finally, ethylenediaminetetraacetic acid plus a chromogenic substrate are added to stop the reaction and to measure formed factor Xa. Factor Xa formation is proportional to the plasma factor IX concentration (from 0 to 140%). The two reagents needed for the assay are stable at room temperature during a whole working day and for 3 h at 37 degrees C. A new isolation procedure for factor VIII is described. Factor VIII is purified from bovine plasma in a few steps with a yield of 20% and a 8,000-fold purification.