

# **GENERATION ASSAYS IN COAGULATION AND FIBRINOLYSIS: PRINCIPLES AND POSSIBILITIES**

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Both coagulation and fibrinolysis are cascade processes with multiple steps.

The study of separate components and single steps in the cascade, has been complemented with various complex tests to study multiple steps. The end-point of clotting or lysis has been supplemented with end-points recording enzyme activity on synthetic substrates. An important role of fibrin as a co-factor in the cascade remains of significance, however.

The well-known example in coagulation is the “thrombin generation test” which starts with tissue factor or contact activation and records thrombin on a chromogenic or fluorogenic substrate. Several variants have been developed with different characteristics and including also the use of platelet-rich plasma and whole blood.

An advantage of such complex tests in plasma or blood is that they include influences of multiple cofactors and inhibitors and can be used to study therapeutic inhibitors in a complex environment.

The kinetic analysis of complex tests has been refined by using kinetic models. Its read out variables includes now various items such as lag time, maximal rate of generation of the final enzyme, its peak activity, time to peak, decline of activity and area under the curve. Each variable has a different set of determinants in the coagulation system and is differently sensitive to variations in patients and to added inhibitors. Tests are also differently sensitive to the influence of microparticles.

Specific data will be presented about the difference in characteristics of the currently prominent thrombin generation tests. New tests recording factor Xa generation or kallikrein generation and old tests recording plasmin formation will be discussed. In particular the use of these test in evaluation of inhibitors such as thrombin inhibitors, factor Xa inhibitors and TAFI-inhibitors will be discussed.

It is concluded that there are many interesting applications of generations tests, but also that it is important to acknowledge differences between tests.