A Simple Rapid Method for Estimating Serum Plasmin Activity with Fibrin-Coated Latex Particles

By Humberto Costa Ferreira

Accurate semiquantitative assessment of fibrinolytic activity in blood serum has usually involved the use of complicated technical procedures and specialized apparatus. Alkjaersig1 estimated fibrinolytic activity from the amount of $^{131}$I-labeled fibrin which was released from a standardized clot. Astrup2 measured the zone of lysis produced by plasmin activity on a standardized fibrin plate, produced by coagulation of fibrinogen with thrombin. Blix3 estimated clot lysis from the release of red cells and consequent change in supernatant hemoglobin from a performed standard clot. Other techniques include the photometric method of Grossen,4 using paper fibrinolysis, the thromboelastographic monitoring of clot lysis used by Von Kaulla5 and the coagulated bovine plasma system used by Kontinen.6 The above methods are useful in research work, but are not well suited for the routine clinical laboratory, where plasmin activity is most often estimated by the lysis time of a standard fibrin clot; this is a rather coarse method requiring considerable amounts of time and material.

Recently the author introduced an immunologic test for detecting degraded fibrin products in vivo4,5 which indirectly corresponds to plasmin activity. A method is now proposed for directly estimating this activity in vitro by means of a modified latex fixation test. Latex particles previously coated with human fibrinogen are mixed with the test serum. The addition of thrombin to the mixture causes the fibrinogen to polymerize into fibrin filaments, which aggregate the latex particles into solid clumps. If the serum is actively fibrinolytic the fibrin filaments are rapidly dissolved, releasing free latex particles into suspension into the serum and producing turbidity that can be measured.

Method

(a) Preparation of Fibrinogen-Coated Latex Particles

The method of Singer,9 as originally used for the detection of rheumatoid factor, has been considerably modified for our purposes. One ml. of latex particle suspension (Dow-Corning polystyrene particles of 0.81 μ mean diameter, in a suspension containing 27.6 percent solid) is resuspended in 100 ml. saline buffer. Ten ml. of a solution of commercially obtained human fibrinogen in saline buffer (20 mg./100 ml.) is then added, and the mixture shaken vigorously. After overnight incubation at 4°C., it is centrifuged at 3000 rpm for 15 minutes. Properly coated latex particles are less dense than saline, and they should migrate to the surface as a buffy coat. The fluid under this buffy layer is aspirated as completely as possible in order to remove soluble fibrinogen. The particles are then washed and centrifuged 3 more times with saline buffer, then resuspended in approximately 20 cc. of buffer, the final volume adjusted to obtain a particle concentration of 5,000,000/
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cu. mm. (Different suspensions usually vary slightly from this figure). Latex particles prepared in this manner remain stable at 4 C. for 30 days.

(b) Estimation of Plasmin Activity

(1) The fibrinogen-coated latex suspension is shaken vigorously and 0.1 ml. is added to 0.5 ml. of fresh patient serum. One drop of thrombin (Human or Bovine, 100 N.I.H. units/ml.) is then added and the mixture centrifuged at 1000 rpm for 1-2 minutes. Large clumps of latex particles are now visible on the surface of the serum. The mixture is incubated at 37 C. and turbidity of the serum is read at 10-minute intervals for 60 minutes. Dilution of the specimen with saline is occasionally necessary to facilitate the readings using a spectrophotometer at a setting of 650 m. The turbidity changes are then compared with those induced by normal streptokinase activated serum.

(2) An alternate semiquantitative method using diluted serum is as follows: 0.1 ml. of latex particles and one drop of thrombin are added, as described above, to each of 8 tubes containing 0.5 ml. of serial twofold buffer dilutions of patient serum, from 1/2 to 1/128. After centrifugation and incubation for 60 minutes at 37 C., all tubes are examined for turbidity with the naked eye against a dark background. With strong plasmin activity, turbidity should be observed at 1/8 or 1/16 serum dilution. Tubes with no plasmin activity show only clear serum, with a buffy layer of agglutinated latex particles.

OBSERVATIONS

The above technic was applied to 126 normal control human sera, and to a set of the same sera activated by streptokinase. In the first set, no digestion of fibrin was observed in any of the sera after 24 hours. In the streptokinase-activated sera considerable digestion of fibrin with release of latex particles was observed in every test tube after 10 minutes.

In 3 sera from patients with abruptio placentae increased plasmin activity was noted. In 1 patient this was potent enough to rapidly digest all the fibrin present with sera diluted 1/2 to 1/4. The other two sera only demonstrated partial digestion with undiluted serum. In the streptokinase-activated sera plasmin activation could be observed to a serum dilution of 1/8 or 1/16.

DISCUSSION

The above method possesses certain features that make it particularly suitable for the routine clinical laboratory: the method itself is simple and rapid, and only small amounts of relatively stable reagents are required, and the amount of actual fibrin present during the test is so small that even insignificant amounts of plasmin can produce a positive reaction in minutes. In the absence of plasmin the latex clumps remain intact for several days.

One possible objection to this method is that the commercial preparation of fibrinogen which we use is contaminated with small amounts of plasminogen. Astrup was able to obtain purified fibrinogen free of plasminogen, but this was not available to the author. We were not able to heat the suspension of latex particles to eliminate plasminogen, because of the low melting point of these particles. This plasminogen could conceivably be activated by streptokinase, but even after such activation the amount present is considered to be insignificant by the author.
SUMMARY

A new method is described for rapid determination of plasmin in serum. This method entails the production of clumps of latex particles held together by fine strands of artificially polymerized fibrin followed by the estimation of plasma turbidity as these particles are released from the aggregates by plasmin activity. The advantages and possible objections to this method are discussed.

REFERENCE


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