Assay of Plasminogen Using Latex Flocculation[1] (37598)

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The tanned red cell hemagglutination inhibition technique (TRCHII) is a simple, rapid and highly sensitive method for the assay of plasminogen–plasmin (1). The drawback of this assay system is that the plasminogen-coated red cells can be stored at 4° for only 2 mo. We therefore, developed a new assay of using latex particles coated with human plasminogen, with the hope that these particles might be more stable and still retain similar sensitivity and simplicity.

Materials and Methods. Buffer: glycine–saline buffer, pH 8.6: 1 vol of 0.2 *M* glycine + 1 vol of normal saline. This buffer is used for washing, suspension and storage of the latex particles. Bovine albumin (Miles Laboratory, Chicago) was free of plasminogen as determined by hemagglutination inhibition technique. Blood grouping plates: Transparent plates with rounded wells 15 mm in diameter and 5 mm deep.

Preparation of plasminogen-coated latex solution: The uncoated latex suspension (0.81 u, Difco, Detroit, MI) was washed and centrifuged twice with glycine–saline buffer (pH 8.6) at 15,000*g* for 30 min. Equal volumes of 2% latex solution in glycine saline buffer and 2% bovine albumin, in the same buffer, were mixed at 20° for 5 min. The purpose of pretreating the latex particles with bovine albumin was to enhance the coating of plasminogen and to increase the sensitivity. Like fibrinogen (2), direct coating of latex with plasminogen did not lead to a stable and sensitive reagent. Human plasminogen [Grade A, Kabi, Stockholm, Sweden, final concentration 2.5 casein units (CU)/ml] was added to the mixture at a volume ratio of 1:5. Then the mixture was kept at 4° for 72 hr. After washing and centrifugation, the latex particles, coated with human plasminogen, were suspended as a 2% solution, which was ready for use. For storage, no bacteriocidal agents were added. They could be stored at 4° for at least 18 mo without losing their activity or sensitivity and the end point remained very distinctive.

The preparation of rabbit anti-human plasminogen serum was described previously (1).

Latex clumping inhibition immunoassay: The optimal antiserum titer used in the assay was determined by adding to the blood grouping plates 0.1 ml antiserum, in serial doubling dilutions, 0.1 ml glycine–saline buffer and 0.1 ml of 2% latex solution coated with plasminogen. The samples were mixed and the results were read 30 min later. The optimal antiserum titer was 1–2 doubling dilution less than the maximal clumping; *i.e.*, 1:100 in our studies. The assay was performed by adding 0.1 ml of 1:100 antiserum and 0.1 ml of plasma in the following dilutions, 1:10, 1:20, 1:40, 1:50, 1:60, 1:70, 1:80, 1:100, 1:120 and 1:160 to each well of blood grouping plates. Additional intermediate dilutions were made when necessary. The mixture was kept at 4° for 30 min and then 0.1 ml of 2% plasminogen-coated latex suspension was added. After mixing, the plates were kept at 20° for 60 min before the results were read. The end point resembled that of TRCHII assay (1) and the result was expressed as latex clumping inhibition titer; *i.e.*, the maximum dilution in which the

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clumping was inhibited. A standard reference plasma and a negative control by using washed, uncoated, latex particles were used routinely. The standard reference plasma was obtained from a pool of 20 healthy donors.

The tanned red cell hemagglutination inhibition technique for assay of plasminogen was described previously (1).

A modified caseinolytic method was used for the determination of plasminogen (3). *N*-Ethyl-urethane was used to denature the inhibitors of fibrinolysis.

Thirty healthy subjects and 29 patients were included in this study. Among the 29 patients, neoplastic diseases were in 7, liver cirrhosis in 9, disseminated intravascular coagulation in 6, and women taking oral contraceptive agents in 7.

Citrated plasma was used in the latex clumping inhibition assay. It was prepared by mixing 9 ml of venous blood with 1 ml of 3.8% sodium citrate solution and centrifugation at 2000*g* for 30 min at 4°. The plasma was stored at −20°. No difference could be detected whether fibrinolytic inhibitors were added or not.

Results. Sensitivity. This method is less sensitive than the hemagglutination inhibition technique since the titer of normal pooled plasma by the latex method is only about 1:20 of that by TRCHII method. This was further substantiated by assaying the human plasminogen (initial conc 2.5 CU/ml).

Specificity. The antiserum to human plasminogen was homogeneous and formed only one precipitation line against both human plasminogen and plasma on immunodiffusion, immunoelectrophoresis and crossed immunoelectrophoresis. It did not form any precipitation line with human fibrinogen.

Reproducibility. The test was reproducible at both high and low plasminogen concentrations. The assay of 50 aliquots from a standard reference plasma showed all had a titer of 1:60, except one which had a titer of 1:40. The same procedure done on a known plasminogen deficient plasma (4) revealed 48 had a titer of 1:16, one 1:32 and one 1:8.

Correlation with hemagglutination inhibition technique. The correlation of results obtained with the latex method and the TRCHII assay was good (Fig. 1).

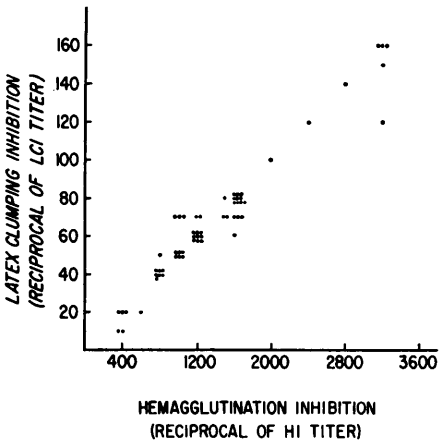

FIG. 1. Correlation of plasma plasminogen values obtained with latex clumping inhibition and tanned red cell hemagglutination inhibition assays.

Correlation with caseinolytic assay. The correlation of results with our method compared with those obtained with the caseinolytic assay is shown in Fig. 2. A good correlation was noted.

The normal range of plasma plasminogen values as determined by the latex flocculation method was 1:40–1:80. Low levels of plasminogen–plasmin antigenic material could be detected as was illustrated by the values obtained for some patients with disseminated intravascular coagulation (DIC) and liver cirrhosis (Fig. 3).

Discussion. This report established that the latex particles when coated with plasminogen can be used for the assay of plasminogen.

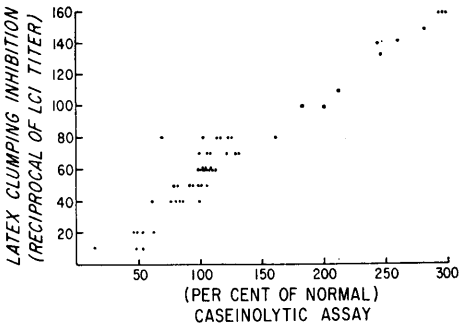

FIG. 2. Correlation of plasma plasminogen values obtained with latex clumping inhibition and caseinolytic assays.

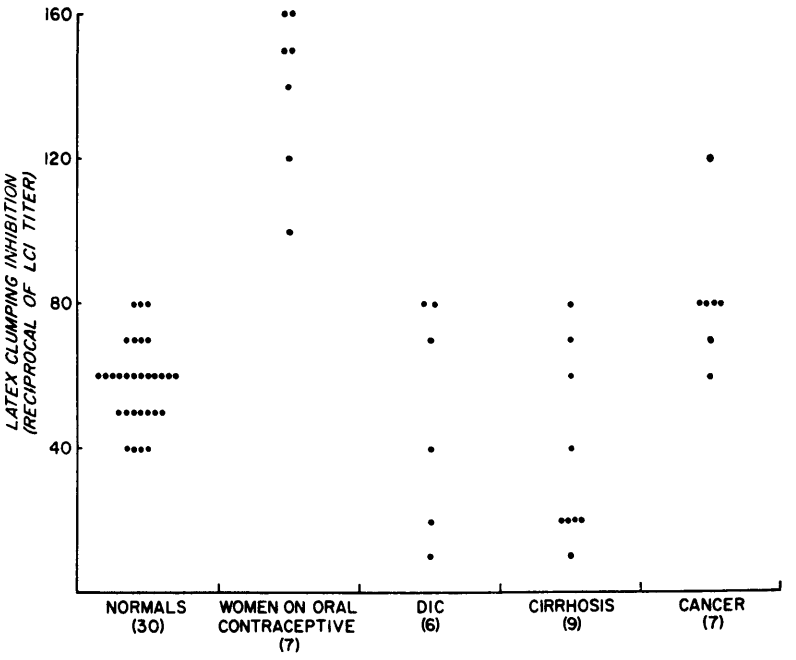

FIG. 3. The plasma plasminogen values in 30 normal subjects and 29 patients with various diseases as measured by the latex clumping inhibition technique. Numbers in parentheses indicate numbers of patients. DIC = disseminated intravascular coagulation.

The technique is simple and rapid. The latex particles coated with plasminogen are more stable than tanned red cells and can be stored at 4° for at least 18 mo. Therefore, it can be used as a substitute for the assay of plasma plasminogen in routine laboratories. However, because of lack of sensitivity, it is not suitable for measuring small amounts of plasminogen in urine, spinal fluid or other body fluids (1, 5).

Summary. An assay of plasminogen by using latex flocculation inhibition technique is described. In comparison with the hemagglutination technique, the method is less sensitive and not suitable for measuring small amounts of plasminogen in human urine or cerebrospinal fluid. However, because of their stability, latex particles may be used to substitute for the tanned red cells in the assay of plasma plasminogen.

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