

'Thrombocytopenic serum': an artificial factor VIII-deficient reagent

E. DAVIDSON¹ AND S. TOMLIN

From the Department of Medicine, University of Cambridge

SYNOPSIS Thrombocytopenic serum is a simple reagent derived from platelet-poor plasma. The high levels of factor V and 'zero' levels of factor VIII in this reagent make it a reliable alternative to haemophilia A plasma in the qualitative and quantitative assessment of factor VIII in the thromboplastin generation test.

The quantitative and qualitative assessment of factor VIII (A.H.G.) using the thromboplastin generation test (Biggs and Douglas, 1953) requires haemophilia A plasma or an alternative source of factor V, such as processed human citrated plasma (Biggs, Eveling, and Richards, 1955), rabbit serum (Wolf, 1956), or adsorbed precipitated beef serum (Pool and Robinson, 1959). This paper reports on the use of serum derived from thrombocytopenic plasma ('thrombocytopenic serum') as a source of factor V in the assay of factor VIII.

MATERIALS AND METHODS

The methods in general use are those recommended by Biggs and Macfarlane (1962).

PREPARATION OF THROMBOCYTOPENIC SERUM Blood collected from normal donors by clean venepuncture was centrifuged immediately using an M.S.E. major centrifuge in siliconized tubes at 4°C. for 10 minutes at 2,500 r.p.m. The supernatant plasma was recentrifuged at 3,000 r.p.m. for an additional 10 minutes. The thrombocytopenic plasma (platelet range, 2,000 to 8,000 /mm.³), which clotted in three to four hours, was left in siliconized tubes at room temperature (20°C.) for 24 hours to allow complete prothrombin consumption and neutralization of thrombin and antithrombin (Wolf, 1956). The samples of thrombocytopenic serum were stored at -20°C. for periods varying from two to eight weeks. Specimens of citrated plasma (20% disodium citrate-0.2 ml./10 ml. blood) were collected in parallel, centrifuged, and then stored under identical conditions.

ASSAY OF FACTOR VIII Varying concentrations of factor VIII (0%, 10%, 20%, 40%, 60%, 80%, and 100%) were prepared by mixing fresh normal citrated plasma (100%) with plasma from a patient suffering from severe haemo-

philia A (0%). These are the expected concentrations of factor VIII. The mixtures were assayed blind for factor VIII, using the method of Pitney (1956), with aluminium hydroxide-treated stored haemophilia A plasma diluted 1 in 5, or with aluminium hydroxide-treated stored thrombocytopenic serum diluted 1 in 5. These results provide the observed levels of factor VIII. Normal serum diluted 1 in 10 was stored at 4°C. for 18 hours before use (Pool and Robinson, 1959).

ASSAY OF FACTOR V This was performed according to the method of Wolf (1953). Throughout the study one of us (E.D.) acted as the standard (100%) in both factor V and factor VIII assays.

RESULTS

The levels of factor V and factor VIII in thrombocytopenic serum and the control citrated plasma are presented in Table I.

TABLE I

Mean S.D. Range	Thrombocytopenic Serum		Citrated Plasma	
	Factor V	Factor VIII	Factor V	Factor VIII
6 hours at 4°C.	320.0 ± 151.0 76-400	5.4 ± 3.3 3-12	87.4 ± 38.8 40-160	108.7 ± 22.7 50-200
24-72 hours at 4°C.	223.1 ± 104.5 75-400	0.0	100.8 ± 79.2 35-150	
24 hours at room temperature 2-8 weeks at -20°C.	200.2 ± 185.5 32-600	0.0	36.8 ± 10.8 20-52	

The dilution curves of factor VIII assays using thrombocytopenic serum are presented in Figure 1. This represents 13 consecutive curves performed on six preparations of thrombocytopenic serum using

¹Elmore research student.

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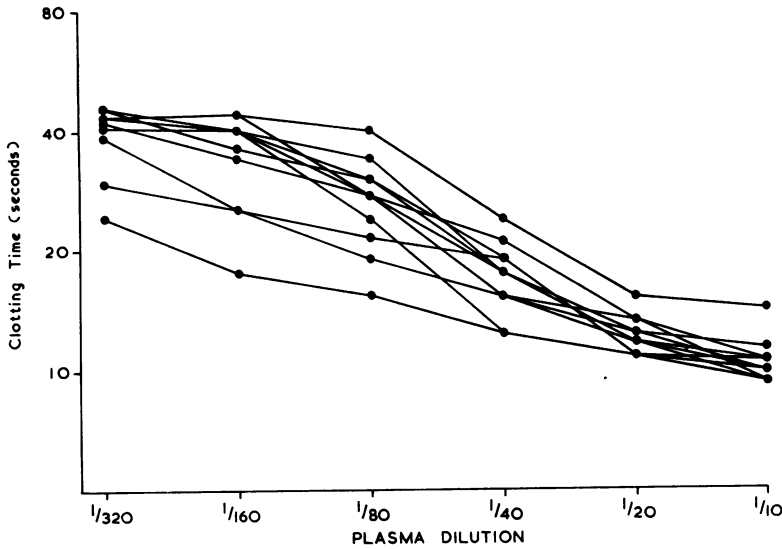


FIG. 1. Dilution curves for factor VIII assays using aluminized thrombocytopenic serum.

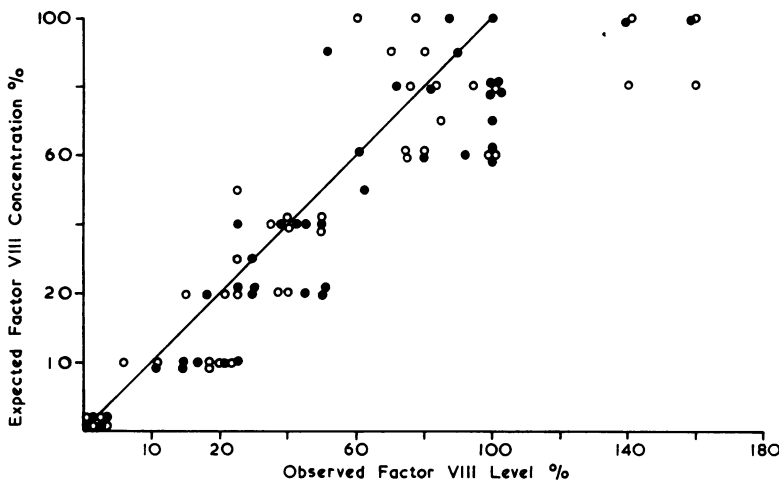


FIG. 2. The diagonal line represents complete agreement between expected and observed levels of factor VIII. ● = using haemophilia A plasma. ○ = using thrombocytopenic serum.

the standard ED-100% on four separate occasions.

The correlation between the expected concentrations of factor VIII and the observed levels of factor VIII using aluminized haemophilia A plasma or aluminized thrombocytopenic serum is presented in

Figure 2. The efficiency of the methods is assessed on the mean differences between the observed and expected levels (Table II).

DISCUSSION

The coagulation of normal blood *in vitro* is associated with a fall to 'zero' in the levels of coagulation factors I (fibrinogen), II (prothrombin), V (labile), and VIII (A.H.G.). The examination of serum derived from thrombocytopenic blood or plasma reveals striking differences, with relatively normal levels of factor II (Patton, Ware, and Seegers, 1948; Quick, 1960) and factor V (Douglas, 1956b; Quick, 1960), diminished factor I (Patton *et al.*, 1948), and 'zero' levels of

TABLE II

Mean Difference in Factor VIII Levels
Expected minus Observed Levels

Mean% ± S.D.	Mean Difference in Factor VIII Levels Expected minus Observed Levels	
	Haemophilia A Plasma	Thrombocytopenic Serum
Assays 60% and over	Minus 10.6 ± 22.4	Minus 11.6 ± 26.7
Assays 40% and under	Minus 6.7 ± 10.1	Minus 5.2 ± 7.3
'Zero' levels	Less than 3%	Less than 3%

factor VIII (Douglas, 1956a; Penick, 1957). A detailed study of these changes and a survey of the literature will be presented in a separate communication.

In the present study trace levels of factor VIII (5%) were present in the specimens of thrombocytopenic serum two hours after coagulation at 4°C. Storage at room temperature for 24 hours or at 4°C for 72 hours reduced the levels of factor VIII to 'zero'.

The supernormal levels of factor V, present in thrombocytopenic serum even after prolonged storage, reflect the effect of adding citrate to normal plasma (Davidson and Tomlin, 1963). This is not surprising in view of the known depressing effect of oxalate, E.D.T.A., and excess citrate on the level of factor V (Zucker, 1954; Mustard, 1958). Investigations on the purification of factor V have emphasized the importance of Ca ions in stabilizing this labile factor (Blomback and Blomback, 1963).

The comparison in the efficiency of the methods (Table II) does not show any advantage in using haemophilia A plasma. In both methods the observed levels are consistently higher than the expected levels, probably due to a lower than average factor VIII content in the standard ED-100%. The wide scatter in the results (Fig. 2) is similar to that reported by Hardisty and Macpherson (1962) using a one-stage

factor VIII assay. The 'zero' levels in the A.H.G. assays using haemophilia A plasma and thrombocytopenic serum are only reported as less than 3% in both methods as a 1 in 10 initial dilution of plasma was adopted as the routine in the blind assays on the plasma specimens. Under normal conditions an initial dilution of 1 in 2 is used in specimens with a suspected low content of factor VIII.

As this reagent is easy to prepare, stable on storage at -20°C., specific and sensitive to changes in the factor VIII levels, it fulfils many of the criteria of an ideal reagent (Pool and Robinson, 1959).

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