A simple method for the preparation of labile factor deficient plasma

Department of Medicine (Dr. H. Alessandri), University of Chile Medical School, Hospital del Salvador, Santiago, Chile

Ricardo Katz, M.D. and Héctor Ducci, M.D.

Labile factor (1) also known as factor V (Owren [2]), plasma Ac. globulin (Ware [3]), plasmatic cofactor of thromboplastin (Honorato [4]) and accelerin (Owren [5]) is a component of the prothrombin complex, produced in the liver independently of the presence of vitamin K.

Its determination has proven very useful in the study of the clotting mechanism specially in patients with biliary and hepatic diseases. It has been shown that labile factor concentration is not reduced by the prothrombopenic drugs (6, 7) or vitamin K deficiency (8); contrarywise it is readily decreased in severe liver damage (8, 9). Moreover reports have appeared on congenital (10) and acquired (8) deficiency of this factor.

The method commonly used for the determination of labile factor is that of Stefanini (8) a modification of that previously described by Quick and Stefanini (11).

Stefanini's method is based on the addition of the plasma under study to the labile factor-deficient plasma in the proportion of 1 to 9. The one stage prothrombin time of the mixture is proportional to the labile factor concentration of the former. This accounts for the need of using as reagent, a labile factor-deficient plasma prepared by storage of oxalated human plasma at 4° C for 15 to 25 days.

The storage of plasma is an inconvenient method for the routine performance of the labile factor determination as Wolf (12) has recently emphasized, and our efforts have been directed towards a more rapid procedure for rendering a plasma deficient in labile factor.

Owren (2) showed that heating of normal oxalated human plasma to 56—57° C produced a rapid inactivation of labile factor as well as coagulation of fibrinogen. By heating to 50° C a slower inactivation occurred which was complete in 15 minutes and at 37° the concentration of labile factor was unchanged up to 15 minutes.

Wolf (12) has advocated the preparation of labile factor deficient plasma by incubation of fresh normal oxalated human plasma at 37° C for 24 hours. According to him the one-stage prothrombin time of this plasma is extended to between 29 and 35 seconds which is a more satisfactory result than that obtained

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by storage. Nevertheless in one of his examples the incubated plasma shows a one stage prothrombin time of 74 seconds.

Wolf's modification of Stefanini's method involving the use of equal volumes of incubated and fresh plasmas, is not to be recommended. It reduces the range of the clotting times of the mixture to 10—11 seconds for the concentration of labile factor between 10 and 100 per cent and the difference between 100 and 50 per cent is of only 1 to 4 seconds. This is probably due to the excess of labile factor added with the unknown plasma.

Beaumont and Bernard (13) obtained labile factor deficient plasma by storage at 5° C for 15 to 20 days or by incubation at 37° C during 24 to 48 hours.

Vetter and Vinazzer (14) prepared their labile factor reagent by heating 10 ml of normal oxalated human plasma in a water bath at exactly 50° C during 30 minutes. This plasma is free of labile factor but also deficient in fibrinogen.

In an attempt to prepare in a short period of time labile factor deficient plasma to be used for the quantitative estimation of this factor it soon became apparent that the amount of normal oxalated human plasma to be incubated at 37° C and the relative size of the container were of the utmost importance for obtaining consistent results. In order to standardize the technique, the following experiments were carried out.

Experimental

Effect of incubation at 50° C on normal oxalated human plasma (1 : 9 sodium oxalate 1,84 gm% : plasma). After incubation for 10 minutes the one stage prothrombin time of the plasma is prolonged to 40 seconds. The addition of 0,01 ml of normal human plasma to 0,09 ml of incubated plasma, gives a one stage prothrombin time of 21 seconds. This corresponds to a 100% concentration of labile factor when stored plasma is used instead of incubated plasma. Nevertheless the coagulation is of the granular type and no real clot is formed.

Effect of incubation for 24 hours at 37° C on normal oxalated human plasma. As already said the amount of plasma and the relative size of the container serving for its incubation are factors which produce widely variable results. This has been confirmed in many experiments which can be summarized as follows:

(One stage prothrombin time of the plasma prior to incubation 12 ")*).

*) Dried human brain thromboplastin prepared according to Quick (15) has been used throughout.

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1 : 9 normal oxalated human plasma (ml)	*Container*	*One stage prothrombin time (seconds)*	*Designation*
1	13 × 100 mm pyrex tube	44	A
3	13 × 100 mm pyrex tube	30	B
5	13 × 100 mm pyrex tube	30	C
5	50 ml Erlenmeyer pyrex flask	120	D
10	50 ml Erlenmeyer pyrex flask	75	E
55	50 ml Erlenmeyer pyrex flask	27	F
55	250 ml Erlenmeyer pyrex flask	49	G

The endpoints of the one stage prothrombin times of plasmas A, D, E and G were poorly defined and no real clot was formed; on the contrary those of plasma B, C and F were clearcut.

When these plasmas are stored in the refrigerator at 4° C the one-stage prothrombin time becomes prolonged in the case of plasma B, C and F to 45—90 seconds. With further storage under these conditions small fluctuations of the one stage-prothrombin time of these plasmas do not interfere with their behaviour in the estimation of labile factor. When by unknown reasons the one-stage prothrombin time becomes shortened below 45 seconds or prolonged over 90 seconds the reagent is not suitable.

It appears that the deterioration of plasmas A, D, E and G which renders them unsuitable for the determination of labile factor is due to the relative surface in contact with air as it is avoided by layering the plasmas with mineral oil.

The addition of 0,01 ml of fresh normal oxalated human plasma to 0,09 ml of the different incubated plasmas stored in the refrigerator gives the following results:

0,01 ml normal human plasma added to 0,09 ml incubated stored plasma	One stage prothrombin time of the mixture (seconds)
A	25
B	22
C	21
D	46
E	33
F	21
G	27

It is clearly seen that the only plasmas which can be conveniently used for the determination of labile factor are B, C and F.

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These results are not explained by different degrees of inactivation of the labile factor. When 0,01 ml of oxalated rabbit plasma is added to 0,09 ml of the incubated human plasmas the one stage prothrombin time is rendered normal only in the case of plasmas B, C and F. This shows that fibrinogen and/or other coagulation factors are affected when the exact amount of plasma and size of container are not strictly adhered to.

The different behaviour of the incubated plasmas to the addition of normal plasma is not explained by changes in prothrombin or stable factors, as their concentrations were normal in all these plasmas.

Method

Depending on the availability of normal human plasma, 5 ml in a 13 × 100 mm pyrex test tube or 55 ml in a 50 ml Erlenmeyer pyrex flask are placed in the incubator at 37° C for exactly 24 hours. The plasmas which have a one-stage prothrombin time of around 30 seconds are then stored in the refrigerator at 4° C in 5 ml amounts. It is advisable to check daily the reactivity of the stored plasma by a one-stage prothrombin time; it has to give values between 45 and 90 seconds. When the values are over 90 seconds, the plasma is discarded; when they are below 45 seconds the plasma may be further incubated at 37° C and stored to get the desired values.

Summary and conclusions

A method is presented which permits the easy preparation of a stable reagent for labile factor determination.

The reagent is obtained in 48 hours by incubation of normal oxalated human plasma in definite amounts and specified containers and further storage in the refrigerator.

This labile factor-deficient plasma behaves similarly to the plasma stored at 4° C for 15 to 25 days.

This method simplifies the routine performance of labile factor determinations.

Zusammenfassung

Es wird eine einfache Methode für die Herstellung von Reagens zur Bestimmung von Labilem Faktor angegeben.

Man benötigt für die Herstellung des Reagens aus menschlichem Oxalatplasma nur 48 Stunden.

Dieses so gewonnene, an labilem Faktor arme Plasma, hat dieselben Eigenschaften wie das Plasma, welches 15 bis 25 Tage bei 4° C aufbewahrt worden ist.

Diese Methode vereinfacht in der Praxis die Bestimmung des labilen Faktors.

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Résumé

On présente une méthode qui permet la préparation facile d'un réactif stable pour la détermination du facteur labile.

Le réactif est obtenu en 48 heures par incubation du plasma humain normal oxalaté placé en quantités déterminées dans des récipients spécifiés, avec conservation ultérieure à 4° C.

Ce plasma avec un déficit de facteur labile réagit comme le plasma conservé à 4° C pendant 15 à 25 jours.

Cette méthode simplifie la détermination du facteur labile.

Bibliography

(1) Quick, A. J.: On the constitution of prothrombin. Am. J. Physiol. **146**: 212 (1943).
(2) Owren, P. A.: The coagulation of blood. Investigations on a new clotting factor, Gundersen, Oslo, 1947.
(3) Ware, A. G., Guest, M. M. and Seegers, W. H.: A factor in plasma which accelerates the activation of prothrombin. J. Biol. Chem. **169**: 231 (1947).
(4) Honorato, R.: The plasmatic cofactor of thromboplastin: its absorption, with prothrombin and fibrinogen, by alumina and tricalcium phosphate gels. Am. J. Physiol. **150**: 381 (1947).
(5) Owren, P. A.: Prothrombin and accesory factors; clinical significance. Am. J. Med. **14**: 201 (1953).
(6) Quick, A. J. and Stefanini, M.: Effect of Dicumarol on concentration of the labile factor. Proc. Soc. Exp. Biol. Med. **72**: 232 (1949).
(7) Owren, P. A. and Aas, K.: The control of Dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scand. J. Clin. Lab. Invest. **3**: 201 (1951).
(8) Stefanini, M.: Activity of plasma labile factor in disease. Lancet **1**: 606 (1951).
(9) Sykes, E. M. jr., Seegers, W. H. and Ware, A. G.: Effect of acute liver damage on Ac-globulin activity of plasma. Proc. Soc. Exp. Biol. Med. **67**: 506 (1948).
(10) Owren, P. A.: Parahemofilia: hemorrhagic diathesis, due to absence of a previously unknown clotting factor. Lancet **1**: 446 (1947).
(11) Quick, A. J. and Stefanini, M.: The concentration of the labile factor of the prothrombin complex in human, dog and rabbit blood; its significance in the determination of prothrombin activity. J. Lab. Clin. Med. **33**: 819 (1948).
(12) Wolf, P.: A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. J. Clin. Path. **6**: 34 (1953).
(13) Beaumont, J. L. and Bernard, J.: Syndrome hémorrhagique congenital dû au défaut du facteur de coagulation récemment isolé sous le nom de Facteur VII, Convertine, S.P.C.A. (Hypoconvertinemie congénitale hémorragipare). Acta Med. Scand. **145**: 198 (1953).
(14) Vetter, H. and Vinazzer, H.: Proaccelerin deficiency following irradiation with therapeutic doses of radiophosphorus. Blood **9**: 163 (1954).
(15) Quick, A. J.: The physiology and pathology of hemostasis, Lea & Febiger, Philadelphia, 1951, page 120.

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