Preanalytical Recommendations of the 'Groupe d'Etude sur l'Hémostase et la Thrombose' (GEHT) for Venous Blood Testing in Hemostasis Laboratories

Benoît Polack Jean-François Schved Bernard Boneu

on behalf of the 'Groupe d'Etude sur l'Hémostase et la Thrombose' (GEHT)

Hématologie Biologique, CHU de Grenoble, France

Key Words

Blood sampling · Coagulation · Laboratory, hemostasis · Preanalytical testing

Introduction

Clinical laboratory investigation of hemostasis has considerably progressed during the last decade with the use of high-quality instruments and reagents. In order to transfer these improvements to the quality of patient care and to increase inter- and intra-laboratory reproducibility, more stringent requirements in the management of samples from blood collection to the validation of results are needed. The outcome of even the best clinical test in hemostasis is critically dependent upon the quality of the sample used. Therefore, the quality of blood sampling, the transport to the laboratory, sample preparation and storage are factors determining the test results.

To minimize this significant source of variation, the implementation of good biological practice rules is compulsory. Both the National Committee for Clinical Laboratory Standards (NCCLS) and the European Concerted Action on Thrombosis have established and/or reinforced such guidelines [1–5]. In addition, at least in France, the clinical pathologist is responsible for the whole preanalytical phase, including blood collection, even if it is carried out by a third party [6].

In this study, the criteria approved by the 'Groupe d'Etude sur l'Hémostase et la Thrombose' (GEHT) are summarized and updated [7].

Material for Venous Blood Sampling: Tube and Closure

Materials used in the manufacturing of blood collection tubes are as diverse as their

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
0301-0147/01/0311-0061\$17.50/0

Accessible online at:
www.karger.com/journals/hae

Benoît Polack, MD, PhD
Hématologie Biologique, CHU de Grenoble, BP 217
F-38043 Grenoble Cedex 9 (France)
Tel. +33 476 765 487, Fax +33 476 765 935
E-Mail bpolack@chu-grenoble.fr

impact on coagulation tests. The use of evacuated blood collection tubes has been recommended by the European Concerted Action on Thrombosis since 1987 [4]. Tubes must be sterile and the sterility guaranteed by the manufacturer. The volume evacuated should represent at least 80% of the total tube volume in order to reduce platelet activation caused by the decrease in pH observed in partially evacuated tubes [8].

Material and General Characteristics

The tube components should neither activate the coagulation factors nor adsorb them [9]. The inside of glass tubes must be siliconized and excessive silicone removed, since some silicones (e.g. SilwetTM Copolymer L-720) have been shown to activate platelets. Although siliconized glass remains the standard material, evacuated 'plasticware' tubes may also be used keeping in mind that plastics comprise different materials with different characteristics and therefore different performances: prolongation of clotting times [10–12], heparin adsorption, interference with fibrinogen assays, modification of thromboplastin sensitivities [13] or calibration [14], and activation of contact phase of coagulation [15–17] have been reported. 'Plasticware' tubes may require strict storage temperature conditions. In addition, their evacuation is usually incomplete, therefore their performances should be rigorously established.

For safety reasons, tube closure must be designed to decrease the risk of aerosol formation and blood spillage when opening. In addition, the stopper itself must be free of any substance that could interfere with the anticoagulant added, the blood clotting factors and drugs such as heparin.

Labeling should be in accordance with international regulatory standards, i.e. on each tube, the batch number, the date of expiry under optimal storage conditions (tempera-

ture around 20°C, absence of light exposure), the volume drawn, the volume of additives and composition must be clearly indicated. The expiry date should indicate the last day of possible use. The phlebotomist must check this information before using any tube.

Influence of the Collection Tube Size

The size of the collection tube must be adapted to the volume of blood drawn and not to the ease of use. Comparison of results obtained from tubes of identical sizes but partial filling (i.e. reduced volume of anticoagulant and reduced evacuation) shows significant differences in clotting times, especially for APTT [8, 18] and platelet counts. The variations in the ratio of blood volume/surface area have not been fully investigated. It certainly has an impact on blood flow in the tube and on platelet activation that may be increased by centrifugation. It also influences the sample pH through gas exchange.

Anticoagulants

The type, the volume, the concentration of divalent cations, the pH of the anticoagulant, its ratio to the blood volume and the hematocrit are preanalytical variables which influence blood coagulation tests.

Type and Concentration

The recommended anticoagulant is trisodium citrate. It can be mixed with other additives if necessary.

The recommended concentration range is 0.105–0.109 M (usually 3.2%). The 0.129 M (3.8%) citrate, which is still available, should be abandoned because its citrate concentration modifies the internal sensitivity index of some thromboplastins [19]. In addition, one must keep in mind that the calibration of the reference thromboplastin is performed with

plasmas from blood drawn on 0.109 M citrate [20]. Citrate concentration has little or no effect on coagulation tests from normal subjects; conversely, a high citrate concentration prolongs coagulation time of anticoagulated patients (heparin or anti-vitamin K) in a reagent-dependent manner [21].

Anticoagulant/Blood Ratio

The recommended ratio is 1 volume of anticoagulant to 9 volumes of blood. Therefore, in an underfilled tube, the final citrate concentration is increased. The APTT test is the most sensitive to variations in the final citrate concentration. It lengthens by an average of 3% for 10% underfilling and 10% for a 20% underfilling. The PT test lengthens by an average of 5% for 20% underfilling and by 15% for 30% underfilling [22, 23].

Overfilling denotes that the evacuated tubes had not been used according to the manufacturer's specifications. Such conditions would happen if the tube was filled either after opening it or with a syringe previously used for blood drawing.

pH Effect

Citrate must be buffered with citric acid to a pH of 5.1–5.3 [4] in order to maintain the pH of the plasma sample between 7.3 and 7.45. Outside this range, a spurious prolongation of abnormal PT is possible. In unbuffered citrate, the pH of a plasma sample will be rapidly outside these ranges since the pH of trisodium citrate is slightly alkaline and because the plasma has lost the buffering capacity of red cells that are removed by centrifugation.

Hematocrit Effect

The hematocrit directly influences the ratio of plasma to anticoagulant. Any concentration of plasma protein (e.g. fibrinogen) is influenced by this dilution effect. For hematocrit values >55% or <30%, it is recommended

to modify the volume of anticoagulant. It is possible to use abacuses or the equation of McGann [24]: Anticoagulant volume (ml) = $0.00185 \times \text{final volume (ml)} \times [100 - \text{hematocrit (\%)}]$ or, as recommended by the NCCLS, the one of Ingram [4]: Anticoagulant volume (ml) = $\text{blood volume (ml)} \times [100 - \text{hematocrit (\%)}] / [595 - \text{hematocrit (\%)}]$.

The hematocrit also modifies PT and APTT through the concentration of Ca^{2+} in the test medium after recalcification. In daily practice, the use of evacuated tubes prevents any adjustment of the anticoagulant volume to the hematocrit value.

Additives

Citrate and Antiplatelet Mixture

The citrate-theophylline-adenine-dipyridamole buffer (CTAD) mixture is made of trisodium citrate/citric acid (0.105 M); theophylline (15 mM); adenosine (3.7 mM) and dipyridamole (0.198 M) with a pH between 5.32 and 5.38. This mixture is well suited for monitoring heparin therapy either by APTT or by anti-Xa assays [25, 26] since it reduces heparin neutralization by platelet factor IV very significantly. Recently, the use of the CTAD has been proposed for flow-cytometric analysis of platelets [27–30]. On the other hand, its use is proscribed for any type of platelet function testing as it inhibits platelet function. In addition, this anticoagulant is very sensitive to light; tubes must therefore be stored in their original light-proof package until assayed [31].

Citrate and Fibrinolysis Inhibitor

This category of additive may be of interest for monitoring fibrinolytic therapy since, in the absence of a fibrinolysis inhibitor, blood-borne plasmin will continue to proteolyze fibrinogen in the sampling tube. Therefore,

Table I. General recommendations for hemostasis testing

	Good	Fair	Poor
Tube	siliconized glass 4.5-ml evacuated tubes	plastic, after validation	poorly siliconized glass
Anticoagulant	buffered trisodium citrate 0.105–0.109 <i>M</i> or CTAD	buffered trisodium citrate 0.129 <i>M</i>	any others
pH of anticoagulated plasma	7.1–7.35		>7.35 <7.1
Filling	100%	90%	<90%
Hematocrit	0.30–0.55 l/l	must be corrected if <0.30 or >0.55 l/l	<0.30 or >0.55 l/l and uncorrected
Needle diameter	0.7–1 mm (19–22 gauge)		<0.7 or >1 mm for an adult
Catheter blood sampling	to avoid	after rejecting 5–10 ml	using the first 5 ml
Tourniquet	<1 min		>1 min
Order of sampling	2nd tube	1st tube	after a tube containing heparin
Transport temperature	ambient temperature		<4 or >30 °C
Time to analysis	<2 h	4 h if previously centrifuged	>4 h
Centrifugation	2 × 2,000 <i>g</i> , 15 min between 15 and 20 °C	1 × 2,000 <i>g</i> , 15 min refrigerated	<1,000 <i>g</i> , <10 min overheating during centrifugation
Freezing	snap freezing		slow
Preserving	–80 °C –20 °C <8 days	–20 °C (<30 days)	> –20 °C
Thawing	fast, water bath at 37 °C		ambient temperature microwave

the fibrinogen level will be underestimated depending on time to analysis: the longer the delay, the lower the fibrinogen. Addition of aprotinin enables a better evaluation of fibrinogen but modifies the APTT. Because of its protein nature, aprotinin does not store well in a liquid medium. Therefore, the necessity of an extemporaneous preparation hinders the use of citrate tubes containing fibrinolysis inhibitor.

Medical Devices for Venous Sampling

Needle

The needle must be adapted to the vein diameter. A needle with a diameter greater than 1 mm (19 gauge) may be traumatic for the vein, leading to the release of vascular fragments which, in the tube, could modify hemostasis [3, 32]. Needles with a diameter

under 0.7 mm (22 gauge) prolong blood collection and the pressure gradient in the needle which could lead to hemolysis and platelet activation.

Collection sets ('butterfly') are routinely used, especially in patients with difficult venous access. They consist of a small-diameter winged needle prolonged by plastic tubing. This system reduces the blood flow and consequently increases the risk of platelet activation. In addition, the void volume, which could reach 500–700 µl, modifies the anticoagulant/blood ratio, especially when low volumes are sampled, as for example in children. Therefore, collection sets with void volume <10% of the draw volume must be used (i.e. <500 µl for a 4.5-ml collection tube).

Catheter

Although not recommended, it is sometimes unavoidable to draw blood from a catheter. Published studies agree that there is a good correlation between blood from a catheter and blood obtained by direct venous puncture for the PT test and fibrinogen level [33, 34]. Conversely, results of APTT and thrombin time tests are discordant [35], especially because of the presence of heparin in the catheter. It is therefore recommended to rinse the catheter, and several techniques have been proposed: to discard the first 5 ml [36, 37], or the catheter volume plus 2 ml [25, 37], or to discard five times the void volume [38]. For some authors not even this is enough [33] and recommendations to discard volumes as high as 10 or 20 ml of blood have been found [3, 39].

Venous Blood Collection

The first step is the identification of the patient and the labeling of the tubes. In addition, clinical and therapeutic data and patient

characteristics should be mentioned on the test request form.

Posture

It is well known that body posture influences the concentration of blood constituents [40]. For example, changing from the supine to the upright position reduces the plasma volume by about 12% [41]. Therefore, blood is preferably taken with the patient in the supine position. A rest of up to 30 min has been recommended for fibrinolysis tests [42].

Vein Selection

The large veins of the elbow (ulnar, cephalic) are to be preferred. A small diameter vein can collapse, which may cause blood coagulation and/or underfilling of the tube. The choice of the arm is also important. Therefore, blood should preferably be taken from the arm without infusion or arteriovenous fistula in hemodialyzed patients. Contamination of laboratory samples by infusion solutions (especially those containing heparin) is the most common preanalytical error in hospital [41].

Tourniquet

The only use of the tourniquet is to help locating the vein. If tourniquet application lasts >1 min, the levels of factor VIII, von Willebrand factor and t-PA are increased, and fibrinolysis is activated. In addition, there is a time-dependent hemoconcentration reaching its plateau after 5–6 min, which increases the level of all blood proteins [41]. Therefore, the tourniquet must not be too tight and must be kept in place less than 1 min; as soon as blood begins to flow in the tube, it must be removed.

Procedure

Diurnal variations and food intake can modify some parameters. Therefore, blood should be collected between 7 and 9 a.m., 12 h

after the last meal, and from an unstressed recumbent patient [41], except if time-dependent drug effects are to be monitored (e.g. heparin). The venipuncture must be as atraumatic as possible. The necessity of well-trained and qualified phlebotomists has been recently highlighted. Prothrombin fragments 1 + 2 in plasma collected by an untrained technologist was increased by an average of 21% compared to the corresponding reference specimen. For thrombin-antithrombin complexes, the increase was 277% [43].

Order of Blood Collection

The order of blood collection of the various collection tubes is still the subject of controversy. The NCCLS recommend either the removal of the first 5 ml or their use for another purpose than hemostasis testing [5]. Indeed the 1st ml of blood can be contaminated by tissue factor. McPhedran et al. [44] showed that APTT was more sensitive than PT. More recently, Yawn et al. [45] suggested that PT could be performed on the first tube, and finally the main point is the qualification of the operator, as proposed by Gottfried and Adachi [46].

However, the characteristics of the first tube are of paramount importance, and blood sampling for hemostasis should be avoided after sampling in either a tube containing an activator or a tube containing any other anticoagulant such as heparin or EDTA.

Practically, the tube determined for hemostasis assays may be the first one if blood is drawn under strict conditions: a 4.5-ml hemostasis tube drawn by a well-trained operator. In any other conditions, we recommend to draw the sample for hemostasis secondly, but never following any other anticoagulant or a tube containing an activator. These recommendations should be reinforced when blood is collected from a catheter or with a blood collection set.

The tube must be correctly filled. For an optimal homogenization of blood and anticoagulant, the tube must be gently mixed by 5–6 inversions but never shaken as documented by Iversen [43].

Sample Transport

Before sending the blood sample to the hemostasis laboratory, it must be kept at room temperature to avoid the cold activation of factor VII and platelet disruption, especially in the monitoring of anticoagulants. When several hemostasis tubes for the same patient are sent to the laboratory, it is necessary to indicate the order of sampling. Furthermore, the identification of the operator must also be clearly noted in order to be able to trace any nonconformity.

Time to Analysis

Since the delay between sampling and centrifugation is of prime importance, the precise time of blood sampling must be indicated on the label. Whether the delay before centrifugation is acceptable depends on the type of the coagulation test. For the PT test, a delay of up to 4 h is acceptable. For monitoring heparin therapy, because of the variable heparin neutralization by platelet factor IV, the delay before centrifugation should not exceed 1 h for a sample collected in citrate and 4 h in CTAD. This applies also to fibrinolysis parameters.

Transport Conditions

Tube handling must be smooth. If not protected, in a pneumatic system, protein denaturation through foaming and platelet activation may occur.

Concluding Remarks

Blood sampling is the first step for routine and specialized hemostasis evaluation. The quality of the results depends entirely upon this easy and often neglected crucial stage. Simple precautions, special training of operators and the choice of evacuated blood collection tubes and the medical devices for blood sampling will facilitate to obtain the best analytical quality.

Acknowledgment

This paper was originally published in February 1998 in a special issue of '*Sang Thrombose et Vaisseaux*', therefore we would like to thank: P. Aguilar-Martinez, M.-C. Alessi, C. Barro, B. Bouttière, C. Biron, C. Droullé, M. Giansili, N. Hézard, M.-F. Hurtaud-Roux, I. Juhan-Vague, L. Maisoneuve, P. Nguyen, P. Nurden, G. Potron, and N. Schlegel for their contribution.

References

- 1 National Committee for Clinical Laboratory Standards: Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, ed 3. Approved Standards NCCLS Documents. H3-A3. Villanova, NCCLS, 1991.
- 2 National Committee for Clinical Laboratory Standards. Collection, Transport and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation, ed 2. Approved Guideline, NCCLS Document H21-A2. Villanova, NCCLS, 1991.
- 3 National Committee for Clinical Laboratory Standards: Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, ed 4. Approved Standards, NCCLS Document H3-A3. Villanova, NCCLS, 1999.
- 4 Walker ID: Blood collection and sample preparation: Pre-analytical variations; in Jespersen J, Bertina RM, Haverkate F (eds): Laboratory Techniques in Thrombosis: A Manual, 2nd revised edition of ECAT assay procedures. Dordrecht, Kluwer, 1999, pp 21–28.
- 5 Thompson JM: Blood collection and preparation: Preanalytical variation; in Jespersen J, Bertina RM, Haverkate F (eds): ECAT Assay Procedures. Dordrecht, Kluwer, 1992, pp 13–20.
- 6 Arrêté du 26 novembre 1999 relatif à la bonne exécution des analyses de biologie médicale. J Off Rép Fr, 11 décembre 1999, pp 18441–18452.
- 7 Les variables préanalytiques en hémostase: recommandations du groupe d'études sur l'hémostase et la thrombose (GEHT). Sang Thromb Vaisseaux 1998;10:1–40.
- 8 Ray MJ: An artefact related to the ratio of sample volume to the blood collection vial size which effects the APTTs of specimens taken to monitor heparin therapy. Thromb Haemost 1991;66:387–388.
- 9 Polack B, Barro C, Pernod G, Bourgeat P, Vigier JP: Impact of the blood collection tube on the activation of coagulation. Thromb Haemost 1997;77:217–218.
- 10 D'Angelo G, Villa C: Measurement of prothrombin time in patients on oral anticoagulant therapy: Effect of 2 different evacuated tubes. Haematologica 1999;84:656–672.
- 11 Biron-Andréani C, Mallol C, Séguiret F, Schved J-F: Plastic versus siliconized glass tubes: Evaluation in current laboratory practice. Thromb Haemost 2000;83:801.
- 12 Van Den Besselaar AMPH: Different sensitivities of various thromboplastins to two blood collection systems for monitoring oral anticoagulant therapy. Thromb Haemost 1999;82:153–154.
- 13 van den Besselaar AMHP, Chantarangkul V, Tripodi A: A comparison of two sodium citrate concentrations in two evacuated blood collection systems for prothrombin time and ISI determination. Thromb Haemost 2000;84:664–667.
- 14 Van Den Besselaar AMPH: Effect of evacuated blood collection tube on thromboplastins calibration. Thromb Haemost 1998;79:1062–1063.
- 15 Sundaram S, Irvine L, Courtney JM, Taggart DP, Wheatley DJ, Lowe GDO: Patterns of blood response during cardiopulmonary bypass. Int J Artif Organs 1992;15:243–248.
- 16 Boisclair MD, Lane DA, Philippou H: Mechanisms of thrombin generation during surgery and cardiopulmonary bypass. Blood 1993;82:3350–3357.
- 17 Burman JF, Chung HI, Lane DA, Philippou H, Adami A, Lincoln JCR: Role of factor XII in thrombin generation and fibrinolysis during cardiopulmonary bypass. Lancet 1994;344:1192–1193.
- 18 Siegel JE, Bernard DW, Swami VK, Sazama K: Monitoring heparin therapy: APTT results from partial-full-draw tubes. Am J Clin Pathol 1998;110:184–187.
- 19 Duncan EM, Casey CR, Duncan BM, Lloyd JV: Effect of concentration of trisodium citrate anticoagulant on calculation of the international normalized ratio and the internal sensitivity index of thromboplastin. Thromb Haemost 1994;72:84–88.

- 20 Thomson JM, Tomenson JA, Poller L: The calibration of the second primary international reference preparation for thromboplastin (thromboplastin, human, plain, coded BCT/253). *Thromb Haemost* 1984;52:336–342.
- 21 Adcock DM, Kressin DC, Marlar RA: Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. *Am J Clin Pathol* 1997;107:105–110.
- 22 Adcock DM, Kressin DC, Marlar RA: Minimum specimen volume requirements for routine coagulation testing – Dependence on citrate concentration. *Am J Clin Pathol* 1998;109:595–599.
- 23 Musgrave KA, Triplett DA: Internal quality insurance in blood coagulation; in Poller L (ed): *Recent Advances in Blood Coagulation*. Edinburgh, Churchill Livingstone, 1985, pp 235–250.
- 24 Musgrave KA, Triplett DA: Internal quality insurance in blood coagulation; in Poller L (ed): *Recent Advances in Blood Coagulation*. Edinburgh, Churchill Livingstone, 1985, pp 235–250.
- 25 Contant G, Gouault-Heilmann M, Martinoli JL: Heparin inactivation during blood storage: Its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole-C.T.A.D. mixture. *Thromb Res* 1983;31:365–374.
- 26 Gouault-Heilmann M, Leroy-Matheron C: Surveillance biologique des patients sous HBPM. Amélioration des conditions préanalytiques par prélèvement sur Diatube®-H. GEHT. Geneva, 1994.
- 27 Freedman J: Preanalytical requirement for platelet activation tests. *Proceedings of the First Symposium on the Impact of the Preanalytical Phase on the Quality of Laboratory Results*. Montpellier, 1996.
- 28 Neufeld M, Nowak-Göttl U, Junker R: Citrate-theophylline-adenine-dipyridamole buffer is preferable to citrate buffer as an anticoagulant for flow cytometric measurement of platelet activation. *Clin Chem* 1999;45:2030–2033.
- 29 Mody M, Lazarus AH, Semple JW, Freedman J: Preanalytical requirements for flow cytometric evaluation of platelet activation: Choice of anticoagulant. *Transfus Med* 1999;9:147–154.
- 30 Ahnadi CE, Lepine M, Lambert G, Chapman ES, Grant AM: Comparison of in-vitro platelet activation in blood collected into sodium citrate vs Diatube-H. AACC. St Louis, poster 196.
- 31 Polack B, Barro C, Mossuz P, Pernod G: Inadequate quality of a blood collection tube containing an anticoagulant platelet inhibitor mixture. *Thromb Haemost* 1997;77:1035–1036.
- 32 Thomson JM: Blood collection and preparation: Pre-analytical variation; in Jespersen J, Bertina RM, Haverkate F: *ECAT Assay Procedure*. Dordrecht, Kluwer, 1992, pp 13–20.
- 33 Haynes SR, Allardyce W, Cowan B, Tansey P: Accuracy of coagulation studies performed on blood samples obtained from arterial cannulae. *Br J Anaesth* 1992;69:599–601.
- 34 Van Genderen PJ, Gomes M, Stibbe J: The reliability of Hickman catheter blood for the assessment of activation markers of coagulation and fibrinolysis in patients with hematological malignancies. *Thromb Res* 1994;73:247–254.
- 35 Templin K, Shively M, Riley J: Accuracy of drawing coagulation samples from heparinized arterial lines. *Am J Crit Care* 1993;2:88–95.
- 36 Konopad E, Grace M, Johnston R, Noseworthy T, Shustack A: Comparison of PT and aPTT values drawn by venipuncture and arterial line using three discard volumes. *Am J Crit Care* 1992;1:94–101.
- 37 Gregersen RA, Underhill SL, Detter JC, Schmer G, Lax K: Accurate coagulation studies from heparinized radial artery catheters. *Heart Lung* 1987;16:686–693.
- 38 Krueger KE, Carrico CJ, Detter JC, Raisys VA, Underhill SL: The reliability of laboratory data from blood samples collected through pulmonary artery catheters. *Arch Pathol Lab Med* 1981;105:343–344.
- 39 Reinhardt AC, Tonneson AS, Bracey A, Goodnough SK: Minimum discard volume from arterial catheters to obtain coagulation studies free of heparin effect. *Heart Lung* 1987;16:699–705.
- 40 Felding P, Tryding N, Hyltoft Petersen P, Holder M: Effects of posture on concentrations of blood constituents in healthy adults: Practical application of blood specimens collection procedure recommended by the Scandinavian Committee on Reference Values. *Scand J Clin Lab Invest* 1980;40:615–621.
- 41 Guder WG, Narayanan S, Wisser H, Zawta B: *Samples: From the Patient to the Laboratory. The Impact of Preanalytical Variables to the Quality of Laboratory Results*. Darmstadt, GIT Verlag, 1996.
- 42 Depasse F, Samama MM: Conditions pré-analytiques en hémostase. *Spectra Biol* 1999;18:27–31.
- 43 Iversen LH: Pre-analytical variation in the measurement of sensitive markers of coagulation and fibrinolysis: The influence of venipuncture and mixing of blood. *Haemostasis* 1997;27:119–124.
- 44 McPhedran P, Clyne LP, Ortolini NA, Gagnon PG, Sanders FJ: Prolongation of the activated partial thromboplastin time associated with poor venipuncture technique. *Am J Clin Pathol* 1974;62:20.
- 45 Yawn BP, Loge C, Dale J: Prothrombin time – One tube or two. *Am J Clin Pathol* 1996;105:794–797.
- 46 Gottfried EL, Adachi MM: Prothrombin time and activated partial thromboplastin time can be performed on the first tube. *Am J Clin Pathol* 1997;107:681–683.