Unsuitability of evacuated tubes for monitoring heparin therapy by activated partial thromboplastin time

A du P HEYNS, DJ van den BERG, PHT KLEYNHANS, AND PW du TOIT

From the Departments of Haematology, Cardiology, and Critical Care, University of the Orange Free State, Bloemfontein 9300, South Africa

SUMMARY Activated partial thromboplastin times (APTT) for monitoring heparin therapy for venous thromboembolism tended to be inappropriately short if blood was collected in commercially available evacuated glass tubes. Five types of evacuated tubes marketed under the trade names Vacutainer and Venoject were examined. The APTT of heparinised blood collected in these tubes correlated poorly (r = 0.04 to r = 0.25) with that of blood samples from the same patients collected in plastic tubes. Most of the evacuated tube APTT were shorter than that of blood collected in plastic or siliconised glass tubes, but the results were unpredictable and varied from tube to tube and from batch to batch. This effect on heparin is apparently due to an unidentified substance which is eluted from the rubber stoppers of the tubes. Heparin control according to the APTT blood collected in these evacuated tubes is hazardous.

Evacuated glass tubes are used extensively for the collection of blood specimens for haematological, biochemical, and other analyses; some of these tubes, containing liquid citrate, are labelled ‘for coagulation purposes’. We noticed that activated partial thromboplastin times (APTT) for monitoring heparin therapy tended to be inappropriately short if blood was collected in these evacuated tubes.

Material and methods

SILICONISED GLASS TUBES
Glass-stoppered 10 ml glass tubes (Quickfit) were cleaned for 48 hours in dichromate-sulphuric acid, washed repeatedly with distilled water, and siliconised by rinsing twice with Repelcote (Hopkin and Williams). These will be referred to as ‘reference glass tubes’.

POLYSTYRENE TUBES
Supplied by Medispo, Johannesburg.

COMMERCIAL EVACUATED GLASS TUBES
Five different types of tubes from two major manufacturers were investigated in this study:

Vacutainer (Becton and Dickinson; BD 3206) Black stopper with 4.5 ml draw (VacBla): a siliconised tube containing 0.5 ml 0.1 mmol/l sodium citrate and 0.1 mg potassium sorbate (an antimycotic agent).

Vacutainer (BD 6464) Blue stopper with 4.5 ml draw (VacBlu): a siliconised tube, labelled ‘for coagulation procedures’, containing 0.5 ml buffered 0.105 mol/l citrate solution (12.35 mg trisodium citrate dihydrate, 2.21 mg citric acid monohydrate) and 0.1 mg potassium sorbate.

Vacutainer (BD 6505) Blue stopper with 2.5 ml draw (VacSmallBlu): a siliconised tube containing 0.3 ml 0.105 mol/l buffered citrate solution and 0.1 mg potassium sorbate.

Venoject (Terumo Corporation; T-206SW) Black stopper with 4.5 ml draw (VenBlu): a siliconised tube containing 0.5 ml 0.1 mol/l sodium citrate solution.

Venoject (T-206W) Blue stopper with 4.5 ml draw (venBlu): a siliconised tube containing 0.5 ml 0.1 mol/l sodium citrate.

LAbORATORY CITRATE SOLUTION
0.109 mol/l liquid trisodium citrate.

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HEPARIN
Pularin (Allen and Hanbury's, Wadeville, Transvaal) 10 000 IU/ml, approximately diluted with 0.15 mol/l sodium chloride. Heparin was added to blood with a Hamilton microlitre syringe.

PREPARATION OF CITRATED PLASMA
After discarding the first 2-3 ml collected in a separate syringe, venous blood was obtained by clean venepuncture with an 18 g needle and a disposable polystyrene syringe. Nine volumes of blood was added to one volume of laboratory citrate in a siliconised glass tube. This method of blood collection will be referred to as the reference method. In one group of experiments (see results), blood was added to citrate in polystyrene tubes. The blood was centrifuged at 2000 g for 20 minutes and platelet poor plasma (PPP) was removed 0.5-1 cm above the erythrocytes. The PPP was transferred to stoppered siliconised tubes, kept at 0.4°C, and tested within 60 minutes of venesection.

Blood was drawn into evacuated glass tubes by standard techniques by clean venepuncture with 18 g needles and equipment supplied by the manufacturers. PPP was prepared as described above.

PLASMA APTT TEST
This was performed with Platelin plus Activator (General Diagnostics, Morris Plains, New Jersey, USA). The incubation time was 5 minutes before recalcification and addition of phospholipid. The coagulation end-point was detected with a Clotek instrument (Hyland Division of Travenol Laboratories, California, USA). Results are expressed as the mean of two determinations. The APTT of pooled normal plasma in our laboratory is 37 to 39 seconds.

HOMOGENISATION OF RUBBER STOPPERS
The stopper was cut into 2-3 mm fragments which were placed into 10 ml laboratory citrate and homogenised for 10 minutes at 4°C with an Edmund Bühler blender. The homogenate was centrifuged at 3000 g for 15 minutes, and the clear supernatant stopper extract was removed for use.

Results

HEPARIN CONCENTRATION-APTT RESPONSE CURVE
Blood from the same donor was collected in reference glass tubes and in different makes of evacuated tubes, and PPP was prepared. Heparin was added in final concentrations varying from 0.05 to 0.8 IU/ml PPP, and the APTT was determined. Heparin concentration-APTT response curves were constructed (Fig. 1).

Fig. 1 Heparin concentration-APTT response curves. Blood was collected in reference tubes (●●●), VacSmallBlu (□□□), VacBlu (○○○), VenBlu (■■■), VenBlu (△△△), and VacBlu (▲▲▲). Blood collected in the evacuated tubes has a shorter APTT compared to that of the reference tube at all heparin concentrations.

The blood collected in siliconised tubes had a sigmoid concentration-response curve, APTT showing sensitivity to as little as 0.05 IU/ml heparin/ml plasma. Similar curves constructed with blood collected in evacuated tubes were relatively flat and showed no or slight prolongation of the APTT with increasing heparin concentrations.

APTT ON BLOOD COLLECTED IN EVACUATED GLASS TUBES AND POLYSTYRENE TUBES
Blood specimens were collected in duplicate from patients receiving heparin for the treatment of venous thromboembolism, myocardial infarction, or the prevention of venous thrombosis. The patients received an initial intravenous bolus dose of 5000 IU of heparin followed by a maintenance dose of approximately 24 000 IU/24 hours, given by continuous intravenous infusion. One set of specimens
was collected in polystyrene tubes containing laboratory citrate and the other set in the various types of evacuated tubes. The results of a representative set of experiments are given in Figure 2. The correlations between APTT results of the blood collected in plastic tubes and of that collected in the different evacuated glass tubes were poor in all instances: VacBla, \( r = 0.0425 \), \( n = 68 \); VacBlu, \( r = 0.1687 \), \( n = 52 \); VacSmallBlu, \( r = 0.0979 \), \( n = 72 \); VenBla, \( r = 0.2512 \), \( n = 67 \); and VenBlu, \( r = 0.0712 \), \( n = 57 \).

![Fig. 2 Correlation between APTT performed on the same heparinised patients' blood collected in polystyrene and in Vacutainer (BD 3206) tubes: \( r = 0.0425 \); \( n = 68 \). Results on blood collected in the other evacuated tubes were similar.](image)

**APTT of heparinised plasma from the same subject**

Blood was collected from a normal volunteer by the reference method and also in six tubes randomly selected from different batches of Vacutainer and Venoject supplies. Heparin was added to whole blood in all the specimens to a final concentration of 0.5 IU/ml. This concentration gave an APTT of approximately 100 seconds on blood collected by the reference method. The APTT was then performed on PPP of all the specimens.

The results are given in Table 1. Blood collected in reference glass tubes had APTT with a mean (±1 SD) of 104±4 and a range of 98-110 seconds. The APTT of specimens collected in the evacuated tubes varied widely and unpredictably; APTT of specimens in tubes from the same batch varied (eg, VenBla A and VacSmallBlu B). The mean APTT also varied considerably from batch to batch (eg, VacBla B and C and VacBla A and B). Several evacuated tubes gave APTT within or close to the normal range despite prolonged APTT of blood collected in reference tubes (eg, VenBla B and C and VenBlu B). Some of the evacuated tubes gave APTT reasonably in accord with that obtained by the reference method (VacBla A and B and VenBla A).

**APTT of heparinised plasma from different subjects**

Blood was collected from six normal volunteers by the reference method and in evacuated tubes. Heparin was added at a final concentration of 0.5 IU/ml whole blood, PPP was prepared, and the APTT was determined.

Parts of the experiments were performed on separate days, and thus results include those of two sets of reference methods as controls.

The results are given in Table 2. The APTT of specimens collected in evacuated tubes varied widely and differed from tube to tube and from batch to batch. In some cases APTT were consistently shorter than the control (VenBla B and C and VenBla A), and in others only some tubes from the batch had a shorter APTT (VacBla C and VenBlu B).

**Influence of citrate in evacuated tubes on heparin-APTT response**

Blood from one donor was used in each set of these experiments.
Table 2  *APTT* on blood specimens from different subjects collected in reference tubes and in evacuated tubes

<table>
<thead>
<tr>
<th>Collection tube</th>
<th>Batch (No.)</th>
<th><em>APTT</em> (seconds)</th>
<th></th>
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<th></th>
<th>Mean ± 1 SD</th>
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<tr>
<td></td>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
<td>Subject 3</td>
<td>Subject 4</td>
<td>Subject 5</td>
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<tr>
<td>VenBla A(750311)</td>
<td></td>
<td>125</td>
<td>120</td>
<td>142</td>
<td>115</td>
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<tr>
<td>VenBla B(781113)</td>
<td></td>
<td>39</td>
<td>52</td>
<td>45</td>
<td>46</td>
<td>40</td>
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<tr>
<td>VenBla C(740710)</td>
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<td>40</td>
<td>38</td>
<td>45</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>VenBla A(740625)</td>
<td></td>
<td>40</td>
<td>38</td>
<td>46</td>
<td>41</td>
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</tr>
<tr>
<td>Reference glass</td>
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<td>37</td>
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<td>(Venobject control)</td>
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<td>121</td>
<td>118</td>
<td>172</td>
<td>129</td>
<td>97</td>
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<tr>
<td>VacBla A(7L071)</td>
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<td>240</td>
<td>140</td>
<td>161</td>
<td>80</td>
<td>182</td>
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<tr>
<td>VacBla B(8A063)</td>
<td></td>
<td>240</td>
<td>143</td>
<td>140</td>
<td>118</td>
<td>150</td>
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<tr>
<td>VacBla C(814L022)</td>
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<td>41</td>
<td>39</td>
<td>41</td>
<td>101</td>
<td>49</td>
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<tr>
<td>VacBlu A(9B052)</td>
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<td>159</td>
<td>137</td>
<td>155</td>
<td>105</td>
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<td>VacBlu B(9C055)</td>
<td></td>
<td>140</td>
<td>139</td>
<td>146</td>
<td>110</td>
<td>148</td>
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<tr>
<td>VacBlu C(9D149)</td>
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<td>139</td>
<td>130</td>
<td>103</td>
<td>162</td>
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<tr>
<td>VacSmallU Blu A(9B127)</td>
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<td>117</td>
<td>100</td>
<td>96</td>
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<td>117</td>
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<tr>
<td>VacSmallU Blu B(8B622)</td>
<td></td>
<td>128</td>
<td>112</td>
<td>125</td>
<td>88</td>
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</table>

In experiments, each with its own control. Blood was obtained in a polystyrene syringe and transferred to (a) reference tubes with laboratory citrate, and (b) reference tubes containing citrate transferred from evacuated tubes of different types. Heparin 0·5 IU/ml whole blood was added. In each instance 10 tubes were used, and the mean (± 1 SD) *APTT* was determined.

The results are given in Table 3. The mean *APTT* of the reference method varied from 113 to 245 seconds. The mean *APTT* of the VacBlu specimens was significantly (P < 0·001, Student *t* test for independent means) longer, that of the VacBla also significantly (P < 0·001) longer; that of the VenBla was significantly (P < 0·001) shorter and that of the VenBla significantly (P < 0·001) longer than their corresponding control reference APTT. The citrate of the evacuated tubes thus had an adverse influence on the APTT of heparinised plasma. The effect varied and resulted in either a shortening or prolongation of the APTT.

**Influence of rubber stopper extract on the heparin-APTT response**

Blood from one donor was used in each of these experiments, each with its own control. Blood was obtained in a polystyrene syringe and transferred to (a) reference siliconised tubes with laboratory citrate, and (b) siliconised tubes containing citrate with rubber stopper extract from a specific type of evacuated tube. Heparin 0·5 IU/ml whole blood was added. In each instance 10 tubes were used and the mean (± 1 SD) APTT was determined.

The results are given in Table 3. In all instances the mean APTT of heparinised blood added to citrate containing a rubber stopper extract was significantly (P < 0·001; Student *t* test for independent means) shorter than that of the reference method. The mean APTT of blood anticoagulated with stopper extract was also significantly (P < 0·001) shorter than that of blood collected in citrate obtained from the same batches of VacBla and VenBla. With the VenBlu tube the mean APTT of blood collected in evacuated tube citrate as well as of that collected in a stopper extract were both significantly (P < 0·001) shorter than that of the reference method.

**Discussion**

Heparin is the drug of choice in the treatment of patients with venous thromboembolic disease. The effect of heparin on the coagulation mechanism classically has been monitored with the whole blood clotting time, but in recent years the APTT has been regarded as more accurate and convenient.1-4 In a prospective study, it was shown that recurrence of

Table 3  *APTT* on blood specimens collected in tubes containing citrate from evacuated tubes, and in tubes containing an extract from evacuated tube rubber stoppers (mean ± 1 SD; n = 10)

<table>
<thead>
<tr>
<th>Collection method</th>
<th><em>APTT</em> (seconds)</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>VacBla</td>
<td>VacBla</td>
<td>VenBla</td>
<td>VenBla</td>
<td></td>
</tr>
<tr>
<td>Evacuated tube citrate in reference tube</td>
<td>287 ± 14</td>
<td>162 ± 4</td>
<td>36 ± 2</td>
<td>136 ± 4</td>
<td></td>
</tr>
<tr>
<td>Stopper extract</td>
<td>54 ± 4</td>
<td>38 ± 2</td>
<td>39 ± 2</td>
<td>41 ± 1</td>
<td></td>
</tr>
<tr>
<td>Reference method</td>
<td>254 ± 11</td>
<td>121 ± 3</td>
<td>192 ± 11</td>
<td>113 ± 3</td>
<td></td>
</tr>
</tbody>
</table>
venous thromboembolism was rare if the APTT was prolonged to 1 1/2 times or more control values but did recur in patients with a shorter APTT.4 Bleeding occurs in about 8% of patients on heparin therapy,4,5 but the incidence may be as high as 20 to 25%.6,7 The bleeding episodes seem more likely to occur in patients with excessive prolongation of in vitro coagulation tests,8 but this association has not been confirmed in other series.4,5

The need for the accurate determination of the APTT has thus been clearly established. Commercially available partial thromboplastins unfortunately vary in sensitivity to heparin,9,10 and it is essential that reagents be carefully standardised.11 The reagents and materials we used in the reference method had an acceptable heparin concentration-APTT response curve (Fig. 1).

Other factors also certainly affect the results of coagulation tests. A clean venepuncture, thorough mixing of the sample with anticoagulant, and the use of scrupulously clean glassware and plastic equipment are important. In recent years, the use of evacuated glass tubes for collecting blood samples for a variety of haematological, biochemical, pharmacological, and other laboratory tests has become widespread. Convenience and relatively low cost have led to the introduction of this system in many hospitals. Although only some, such as the Vacutainer with a blue stopper (Beckton-Dickinson Code 6464), have been labelled 'for coagulation purposes', the marketing strategy and format of the tubes with citrate as anticoagulant are clearly aimed at their use for blood coagulation studies. The use of these unsiliconised glass tubes with liquid citrate for prothrombin time determination and oral anticoagulant control has been condemned,12 and it has been suggested that Vacutainer tubes may have an anti-heparin effect.13 Evacuated tubes may be contaminated by trace metals such as Zn, Pb, and Cd, which influence analytical chemistry results.14 Determination of blood propranolol levels may be erroneously low in samples obtained with Vacutainers.15 Similar results occur in the measurement of another basic drug, quinidine.16,17 Protein binding of the basic drug, alpranolol, and imipramine is inhibited by tris-(2-butoxyethyl) phosphate isolated from the stoppers of Vacutainers.18–20

Our results indicate that the monitoring of heparin therapy with an APTT of blood collected in the evacuated glass tubes supplied by two major manufacturers is unreliable; heparinised blood collected in Vacutainer and Venoject tubes showed similar changes. The APTT performed on evacuated tube blood was usually much shorter than that of blood collected in plastic tubes or by our reference method but was sometimes prolonged (Fig. 2; Table 1). The APTT were unpredictable and varied from batch to batch and from tube to tube within the same batch; they also varied from subject to subject if tubes from the same and different batches were used (Table 2). Presumably a contaminant is present in the evacuated tubes. The substance is present in the citrate in the evacuated tubes, and it can be extracted from their rubber stoppers (Table 3); it appears to neutralise heparin (Fig. 1). We have not attempted to identify this substance.

We consider therefore that heparin control by the APTT of blood collected in evacuated tubes is hazardous. It may lead to gross heparin overdosage of patients since the results would indicate that patients are resistant to heparin. It may also cause misinterpretation of results of coagulation studies in patients with postoperative haemorrhage after procedures such as cardiopulmonary bypass surgery. Although it has been suggested that blood coagulation assays may be performed reliably on blood samples collected in evacuated tubes,21 it seems prudent to study the effect of the contaminated citrate of these tubes on coagulation tests more extensively before their use is unequivocally approved.

Our results show clearly that the monitoring of heparin therapy with the APTT is unreliable if blood samples are collected in Vacutainer or Venoject tubes. It is recommended that such laboratory control should be undertaken only on blood samples collected with a polystyrene syringe and transferred to a polystyrene tube containing 0109 mol/l liquid citrate in the proportion of 1 volume to 9 volumes of whole blood.

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References

Heyns, van den Berg, Kleynhans, and du Toit


Requests for reprints to: Professor A duP Heyns, Department of Haematology, University of the Orange Free State, Bloemfontein 9300, South Africa.