Comparison of liquid and dried sodium citrate as the anticoagulant for Thrombotest and prothrombin time estimations

D HOCTOR, J BOTTOMLEY, K HYDE,* AH GOWENLOCK, JE MACIVER

From the Departments of Haematology and *Biochemistry, Clinical Laboratories, Manchester Royal Infirmary, Oxford Road, Manchester

SUMMARY Samples which are taken for the Quick one-stage prothrombin time estimation for the control of patients receiving oral anticoagulant treatment are by convention taken into liquid sodium citrate anticoagulant. Dried sodium citrate has been rejected on the grounds that excessive haemolysis causes activation of clotting factors and therefore falsely short clotting times. Tests were performed with both liquid and dried sodium citrate as an anticoagulant for prothrombin time and Thrombotest. No clinically significant difference between the values given by the two anticoagulants was observed.

The control of oral anticoagulant treatment is normally effected either by measuring the prothrombin time (PT) and then calculating the prothrombin ratio (PR) or by using the Thrombotest technique. The preferred anticoagulant for the blood samples has usually been a sodium citrate solution as this, combined with good venepuncture technique, produces negligible haemolysis of red cells. The use of dried sodium citrate has been criticised by Mibashan on the grounds that this produces a greater risk of haemolysis. It has been suggested that haemolysis could be associated with activation of clotting factors and consequently falsely short clotting times. This effect has not been properly documented.

The use of a liquid anticoagulant has certain potential disadvantages. Evaporation of the liquid during storage will alter the anticoagulant concentration after mixing with blood. In contrast, loss of anticoagulant solution from spillage, cracks in the container, or unauthorised removal before the blood is added will reduce the anticoagulant concentration. None of these problems arises with commercially available bottles containing dried citrate.

In view of the uncertainty of the magnitude of the effect of dried citrate, this was compared to a liquid citrate anticoagulant using blood from normal volunteers and from patients receiving oral anticoagulant treatment. The degree of haemolysis and the effect on the Thrombotest and the Quick one-stage PT estimation were studied.

Material and methods

Material

1 Owren’s veronal buffer,2 pH 7-35.
2 Trisodium citrate, Na3C6H5O7.2H2O dissolved in distilled water to give a 31-0 g/l solution.
3 British Comparative Thromboplastin (National (UK) Reference Laboratory, Withington Hospital, Manchester).
4 Plastic tubes (13 × 90 mm) containing 0-5 ml of citrate solution were prepared freshly as required.
5 Commercial sodium citrate tubes (Sherwood Medical Industries, Crawley, West Sussex) prepared by drying 0-25 ml of a 31-0 g/l solution of Na3C6H5O7.2H2O.
6 Thrombotest reagent (Nyegaard and Co A/S Oslo).
7 Calcium chloride solution (0-025 mol/l).
8 A haemolysate of washed red cells from normal blood was prepared with a final haemoglobin concentration of 10-0 g/l.

Methods

Venous blood was collected by careful venepuncture using a 20 gauge needle from 10 normal volunteers and from 50 patients receiving oral anticoagulant treatment. Nine volumes of blood were added to one volume of liquid citrate and 2-5 ml of blood were added to the tubes containing the dried citrate. This
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gave a final concentration in both of 3.1 g/l.
Thrombotests were performed on both types of sample immediately after collection. The residual whole blood samples were then centrifuged at 3000 rpm for 10 min. Their resulting plasmas were divided into two parts, one for PT estimations and the other for plasma haemoglobin. The PT was estimated on the day of collection. Remaining plasmas were stored at −40°C and plasma haemoglobin concentrations were measured in one batch after all the samples had been collected.

1 Thrombotest
Well-mixed blood (0.05 ml) was added to 0.25 ml of Thrombotest reagent and the clotting times determined according to the manufacturer’s instructions. The percentage activity was obtained from the graphs supplied. In order to correct the results for the volume difference (dilution factor using liquid anticoagulant) the liquid citrate results were read from the venous graph and the dried citrate results read from the capillary graph. All results are means of duplicate estimations.

2 Prothrombin time
The PT was estimated using British Comparative Thromboplastin and the result was expressed as the prothrombin ratio (PR)

\[ \text{PR} = \frac{\text{Test time}}{\text{Mean of 10 normal clotting times}} \]

All results are means of duplicate estimations.

3 Plasma haemoglobin difference (PlHb)
The haemolysate was diluted in sodium citrate solution to give concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 0.0 mg/l. Each solution was further diluted 1/5 in Owren’s veronal buffer. Using the 0.0 mg/l solution as a blank the absorbance of these dilutions were determined on a spectrophotometer at 415 nm using a 1 cm cuvette. A standard curve was plotted of log absorbance against log concentration.

Each test plasma was diluted 1/5 in Owren’s buffer. Since the use of liquid citrate results in minimal haemolysis we decided to use each liquid citrate plasma as a blank. The difference in absorbance between the liquid and dried citrate plasma was recorded and interpolated from the standard curve as the difference in plasma haemoglobin concentration (PlHb).

Results

The results for the normal volunteers are collected in Table 1. The PR figures are the results for each person expressed as a ratio to the relevant mean. The percentage difference refers to the difference of the ratio using dried citrate to that using liquid citrate. The results for the patients receiving anticoagulant treatment are arranged similarly in Table 2.

For the normals the results for the PR are not significantly different for the two anticoagulants and the two mean times have been used to derive the PR figures in Table 2. There is no significant difference in the Thrombotest results. The use of dried citrate produces some haemolysis, the PlHb average figure being 236.5 mg/l (SE 51.5). The corresponding mean figures for PlHb in the patients is 399.1 mg/l (SE 35.7) but this is insignificantly different as judged by Student’s t test (t = 1.95, p > 0.05). Anticoagulant treatment does not seem to influence the degree of haemolysis produced by using dried citrate.

When the patients on anticoagulant treatment are considered, there is a small but very significant difference in the PR figures. The ratio using dried citrate is on average 2.7% ± 0.39 (SE) lower than the ratio with the conventional liquid citrate technique. The difference is also significant using the Wilcoxon matched-pairs signed-ranks test (z = 4.96, p < 0.001). This difference is also apparent in the Thrombotest results where the mean

<p>| Table 1 Prothrombin time and Thrombotest measurements for normal samples |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>No (normal)</th>
<th>Liquid time (s)</th>
<th>Dried time (s)</th>
<th>Liquid PR</th>
<th>Dried PR</th>
<th>Difference (%)</th>
<th>Liquid (%) normal</th>
<th>Dried (%) normal</th>
<th>Plasma Hb difference (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13-90</td>
<td>13-20</td>
<td>1-033</td>
<td>1-011</td>
<td>−2.13</td>
<td>71</td>
<td>66</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>13-05</td>
<td>12-95</td>
<td>0-970</td>
<td>0-992</td>
<td>2.27</td>
<td>70</td>
<td>70</td>
<td>230</td>
</tr>
<tr>
<td>3</td>
<td>13-00</td>
<td>12-50</td>
<td>0-966</td>
<td>0-957</td>
<td>−0.90</td>
<td>66</td>
<td>60</td>
<td>196</td>
</tr>
<tr>
<td>4</td>
<td>13-95</td>
<td>13-35</td>
<td>0-037</td>
<td>1-023</td>
<td>1.37</td>
<td>67</td>
<td>78</td>
<td>192</td>
</tr>
<tr>
<td>5</td>
<td>14-50</td>
<td>14-10</td>
<td>1-078</td>
<td>1-080</td>
<td>0.22</td>
<td>&gt;100</td>
<td>97</td>
<td>165</td>
</tr>
<tr>
<td>6</td>
<td>12-70</td>
<td>12-95</td>
<td>0-944</td>
<td>0-992</td>
<td>5.09</td>
<td>&gt;100</td>
<td>86</td>
<td>236</td>
</tr>
<tr>
<td>7</td>
<td>12-50</td>
<td>12-00</td>
<td>0-936</td>
<td>0-996</td>
<td>6.34</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>520</td>
</tr>
<tr>
<td>8</td>
<td>12-75</td>
<td>12-25</td>
<td>0-948</td>
<td>0-938</td>
<td>−0.98</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>530</td>
</tr>
<tr>
<td>9</td>
<td>13-50</td>
<td>12-40</td>
<td>1-003</td>
<td>0-920</td>
<td>−5.33</td>
<td>61</td>
<td>57</td>
<td>145</td>
</tr>
<tr>
<td>10</td>
<td>14-60</td>
<td>13-85</td>
<td>1-085</td>
<td>1-061</td>
<td>−2.23</td>
<td>70</td>
<td>58</td>
<td>94</td>
</tr>
<tr>
<td>Mean value</td>
<td>13-455</td>
<td>13-055</td>
<td>1-098</td>
<td>0-998</td>
<td>−2.23</td>
<td>236.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4**
The patients’ group embraces a range of PR figures from 1.29 to 5.70 for liquid citrate. As might be expected there is a good correlation between the ratios for the two anticoagulants (r = 0.996, p < 0.001) and the relation between the two ratios is expressed by the regression equation:

\[
PR_{\text{dried}} = 0.985 \times PR_{\text{liquid}} - 0.029
\]

If haemolysis has an important effect on the difference between the two PRs this may be obscured by a possible increase in discrepancy between the two ratios as the liquid ratio becomes larger. This was investigated using multiple regression coefficients and the model:

\[
PR_{\text{liquid}} - PR_{\text{dried}} = A \times PR_{\text{liquid}} + B (\text{PIHb}) + C
\]

The best fit is given by A = 0.0142, B = 0.0001, C = 0.0087. The values of A and B are not significantly different from zero (p > 0.05) and the overall correlation (r = 0.267) is also insignificant. The difference in PRs is therefore not attributable to different degrees of haemolysis or different degrees of prolongation of PT by anticoagulant treatment.

For the Thrombotest results, the findings are...
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The amount of plasma haemolysis produced by dried citrate although variable is no different in the normal controls than in the patients, indicating that haemolysis is unrelated to anticoagulant treatment. Such haemolysis could, as Mibashan suggests, be due to incomplete dissolution of anticoagulant but other factors may be operating. Variations in haemolysis caused by differences in venepuncture techniques and sample mixing were minimised by having all sample collections performed by one experienced person.

It seems unlikely that activation of clotting factors coincident with increased haemolysis would account for the difference in PRs and Thrombotest figures which occur on changing from liquid citrate to dried citrate. For the PR the average fall of 2-70% means that the samples are less anticoagulated while the fall of 0-49% in Thrombotest implies that they are more anticoagulated.

These differences, although statistically significant, are small and their practical importance in the clinical management of anticoagulant treatment is minimal. For example a change of PR from 1-5 to 1-45 or from 4-0 to 3-9 is of little importance in clinical management. Likewise for Thrombotest values between 5 and 20% of normal, any difference attributable to changing from liquid to dried citrate is less than 0-1% which again is insignificant in clinical management. These predictable differences are small compared with the unpredictable effects of changes due to variations in liquid citrate.

In view of these findings it is concluded that the convenience of using containers prepared with dried citrate makes them preferable for the collection of blood samples used to control oral anticoagulant treatment.

Discussion

The amount of plasma haemolysis produced by dried citrate although variable is no different in the normal controls than in the patients, indicating that haemolysis is unrelated to anticoagulant treatment. Such haemolysis could, as Mibashan suggests, be due to incomplete dissolution of anticoagulant but other factors may be operating. Variations in haemolysis caused by differences in venepuncture techniques and sample mixing were minimised by having all sample collections performed by one experienced person.

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References


Requests for reprints to: Miss J Bottomley, Department of Haematology, Clinical Laboratories, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL, England.
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D Hoctor, J Bottomley, K Hyde, A H Gowenlock and J E Maciver

*J Clin Pathol* 1981 34: 902-905
doi: 10.1136/jcp.34.8.902

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