The determination of INR in stored whole blood

D R Leeming, S Craig, K J Stevenson, D A Taberner

Abstract

**Aims**—To examine the reliability of international normalised ratio (INR) determination on samples stored as whole blood for up to two days at room temperature.

**Methods**—The INR of 40 patients receiving oral anticoagulants was determined on fresh blood and on samples stored for 24 and 48 hours, using five locally calibrated prothrombin time systems. These incorporated Manchester reagent, Recombiplastin, IL PT Fibrinogen HS Plus, Manchester combined capillary prothrombin time reagent, and a freeze dried in-house reference rabbit brain thromboplastin, RBT 1010. In addition, factors II, V, VII, and X were determined on samples obtained from 18 of these patients before and after incubation at room temperature.

**Results**—The INR of the samples changed by differing amounts during storage, depending on which system was employed. Although the mean change after 24 hours storage was relatively small, there were individual samples that changed by >0.5 INR with all systems. These changes would lead to adjustment in dosage of certain patients. After 48 hours these effects were greater with all systems except that employing Recombiplastin. There were only small reductions in the measured factors by 48 hours.

**Conclusions**—After storage of samples for only 24 hours, some patients’ INR changed sufficiently to affect dosage. In view of these observations, the practice of storing whole blood samples for INR determination cannot be recommended.

(J Clin Pathol 1998;51:360–363)

Keywords: warfarin; prothrombin time; international normalised ratio

The demand for laboratory monitoring of oral anticoagulant treatment is increasing as more patients are treated. One important reason for the increase is that the value of warfarin in reducing the risk of ischaemic stroke in high risk patients with atrial fibrillation is now being prescribed anticoagulants. This has increased the pressure on those responsible for anticoagulant management to consider monitoring systems which are suitable for use in the community or in primary care, thus avoiding transporting non-ambulant patients to hospital outpatient anticoagulant clinics.

Expansion of anticoagulant clinics and the extension of access through “drop in” clinics have been employed with some success to cope with increased demand. Some attempts have also been made to shift the service into primary care. This has not been widespread, however. Taylor et al reported that general practitioners were concerned about resources and lack of expertise. Fitzmaurice et al believed that computerised dosing was of benefit in guiding the management of long term anticoagulation in primary care.

Baglin et al have considered an alternative approach. They have shown that a system based on taking blood samples from patients and transporting these to the laboratory for testing resulted in dose control at least equal to that in a completely hospital based clinic. An advantage of this technique is that the normal laboratory quality assurance may be maintained. Such systems, however, depend on the reliability of international normalised ratio (INR) determination on samples that may be many hours old.

In this study we examined the changes in INR and in factors II, V, VII, and X in samples stored as whole blood for up to 48 hours.

**Methods**

Blood obtained by venepuncture from 40 patients receiving oral anticoagulant treatment was stored as whole blood for up to 48 hours.

**Table 2** Mean international normalised ratio (INR) obtained with each of the reagents with samples at baseline and those stored for 24 and 48 hours at room temperature. The value in parenthesis is the mean change in INR.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Mean INR baseline</th>
<th>Mean INR sample stored for 24 hours</th>
<th>Mean INR sample stored for 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBT 1010</td>
<td>2.31</td>
<td>2.45 (0.14)</td>
<td>2.70 (0.39)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>CCR</td>
<td>2.29</td>
<td>2.41 (0.12)</td>
<td>2.59 (0.30)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>MR</td>
<td>2.27</td>
<td>2.48 (0.21)</td>
<td>2.64 (0.57)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>RP</td>
<td>2.31</td>
<td>2.38 (0.07)</td>
<td>2.42 (0.11)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.02</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0005</td>
</tr>
<tr>
<td>IL</td>
<td>2.28</td>
<td>2.36 (0.08)</td>
<td>2.50 (0.22)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

CCR, combined capillary reagent; IL, IL PT Fibrinogen HS Plus; MR, Manchester reagent; RBT, rabbit brain thromboplastin; RP, Recombiplastin.
was collected into Vacutainer tubes (Becton and Dickinson, HM&S Ltd, Northampton, UK) containing 0.105 M trisodium citrate. Three tubes were taken from each patient.

Prothrombin times were determined fresh (tube 1) and on samples stored at room temperature for 24 hours (tube 2) and for 48 hours (tube 3). Various system combinations of thromboplastin reagent were used with manual or coagulometer technique. These were:

1. Freeze dried rabbit brain thromboplastin (RBT 1010, Thrombosis Reference Centre, Manchester, UK) and manual method.
2. Manchester combined capillary pro-thrombin time reagent (CCR10, Thrombosis Reference Centre, Manchester, UK) and Thrombotrack (Nycomed, Birmingham, UK).
3. Manchester reagent (Helena Laboratories, Sunderland, UK) and KC 10 (Amelung, Brownes (UK) Ltd, Reading, UK).

Figure 1  Bland and Altman plot of mean international normalised ratio (INR) against difference in INR at 24 hours and 48 hours. The left hand panels show the effect of storage for 24 hours; the right hand panels give the results for 48 hours. MR, Manchester reagent.
Table 3  Numbers of patients with international normalised ratio (INR) values in each therapeutic interval at baseline, 24, and 48 hours

<table>
<thead>
<tr>
<th>System</th>
<th>INR range</th>
<th>Baseline</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBT1010/manual</td>
<td>&lt;2</td>
<td>17</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.0—2.5</td>
<td>11</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.0—3.0</td>
<td>16</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>3.0—4.5</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CCR/Thrombotrack</td>
<td>&lt;2</td>
<td>19</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2.0—2.5</td>
<td>9</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2.0—3.0</td>
<td>14</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>3.0—4.5</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MR/KC 10</td>
<td>&lt;2</td>
<td>20</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.0—2.5</td>
<td>9</td>
<td>17</td>
<td>14</td>
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<td>2.0—3.0</td>
<td>14</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>3.0—4.5</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Recombiplastin/KC 10</td>
<td>&lt;2</td>
<td>17</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.0—2.5</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.0—3.0</td>
<td>17</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>3.0—4.5</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>PT Fibrinogen HS Plus/ACL</td>
<td>&lt;2</td>
<td>20</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.0—2.5</td>
<td>8</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.0—3.0</td>
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<td>17</td>
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<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;4.5</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(4) Recombiplastin (Ortho-Clinical Diagnostics, Amersham, UK) and KC 10
(5) PT Fibrinogen HS Plus reagent performed on the ACL 300R coagulometer (both from Instrumentation Laboratories (IL), Warrington, UK)

System 2 used whole blood in the prothrombin time test. For the other combinations, plasma was obtained by centrifuging the stored whole blood at 2000 g for 15 minutes. International sensitivity indices (ISI) were assigned to each of the systems using fresh plasma obtained from the 40 anticoagulated patients and 20 freshly collected plasmas from 20 normal volunteers. RBT 1010 was used as the reference. This preparation was calibrated previously in a 20 centre international exercise against the existing WHO international reference. Each system was controlled by testing normal and abnormal control plasmas (Thrombosis Reference Centre, Manchester, UK) at each time of testing.

Assays of factors II, V, VII, and X were performed by standard one stage methods on fresh plasma samples and on those obtained by centrifugation of the whole blood stored for 24 and 48 hours.

**Discussion**

At first glance, this study suggests that delay between blood collection and testing the prothrombin time is probably not of concern in anticoagulant dose adjustment. This is the likely conclusion based on comparison of the mean change in INR between freshly drawn samples and those obtained after 24 hours of storage.
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Figure 2 Mean percentage change in each therapeutic interval after storage for 24 and 48 hours. The white bar represents change at 24 hours and the black bar change after 48 hours.

Unfortunately, there are some patients whose INR falls outside our arbitrary acceptable difference of 0.5 INR and who are overlooked when only the mean results are considered. This represents 2.5% of patients with RBT 1010, 5% with CCR 10, 7.5% with Manchester reagent, and 5% with recombinplastin. By 48 hours, this had risen to 30% of patients with RBT 1010, 20% with CCR 10 and Manchester reagent, and 10% with the IL system. With recombinplastin the numbers were unchanged at 48 hours.

Interestingly the thromboplastin employed seems to affect the degree by which the INR is increased on storage. Recombiplastin and PT Fibrinogen HS Plus showing smaller change than Manchester reagent, Manchester combined capillary reagent, and RBT 1010.

Baglin and Luddington concluded that there was no clinically significant change in INR when analysis was delayed for up to three days. Their conclusions, however, were based on mean INR differences and may have missed the occasional patient who behaved differently.

Another possible reason for the differences between Baglin and Luddington’s study and ours is that blood was collected by different methods, Sarstedt Monovet tubes being preferred by Baglin, while we used Vacutainers. Hernandez et al have also reported on this subject, but, like Baglin and Luddington, they only gave mean INR values, making complete interpretation of their results difficult.

There were only small decreases in the levels of factors II, V, VII, and X and it is unlikely that these would provide the reason for the changes in INR seen with some of the patients.

In conclusion after storing samples for only 24 hours, some patients’ INR values may have changed sufficiently to result in anticoagulant dose alteration. In view of these observations, we cannot support this practice.

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J Clin Pathol 1998 51: 360-363
doi: 10.1136/jcp.51.5.360

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