Preanalytical Variables and Off-Site Blood Collection: Influences on the Results of the Prothrombin Time/International Normalized Ratio Test and Implications for Monitoring of Oral Anticoagulant Therapy

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Background: The quality of oral anticoagulant therapy management with coumarin derivatives requires reliable results for the prothrombin time/International Normalized Ratio (PT/INR). We assessed the effect on PT/INR of preanalytical variables, including ones related to off-site blood collection and transportation to a laboratory.

Methods: Four laboratories with different combinations of blood collection systems, thromboplastin reagents, and coagulation meters participated. The simulated preanalytical variables included time between blood collection and PT/INR determinations on samples stored at room temperature, at 4–6 °C, and at 37 °C; mechanical agitation at room temperature, at 4–6 °C, and at 37 °C; time between centrifugation and PT/INR determination; and times and temperatures of centrifugation. For variables that affected results, the effect of the variable was classified as moderate when <25% of samples showed a change >10% or as large if >25% of samples showed such a change.

Results: During the first 6 h after blood collection, INR changed by >10% in <25% of samples (moderate effect) when blood samples were stored at room temperature, 4–6 °C, or 37 °C with or without mechanical agitation and independent of the time of centrifugation after blood collection. With one combination of materials and preanalytical conditions, a 24-h delay at room temperature or 4–6 °C had a large effect, i.e., changes >10% in >25% of samples. In all laboratories, a 24-h delay at 37 °C or with mechanical agitation had a large effect. We observed no clinically or statistically relevant INR differences among studied centrifugation conditions (centrifugation temperature, 20 °C or no temperature control; centrifugation time, 5 or 10 min).

Conclusions: We recommend a maximum of 6 h between blood collection and PT/INR determination. The impact of a 24-h delay should be investigated for each combination of materials and conditions.

The purposes of effective and safe oral anticoagulation therapy (OAT)4 with coumarin derivatives are to reduce the risk of thromboembolic events and to minimize the incidence of bleeding complications. This can be achieved by maintaining the prothrombin time (PT), transformed to the International Normalized Ratio (INR), within the therapeutic ranges (1). In The Netherlands, the management of OAT for outpatients is performed by specialized anticoagulation clinics, called Thrombosis Services. All Thrombosis Services form part of the network of the Dutch Federation of Thrombosis Services. In 2003, the 62

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4 Nonstandard abbreviations: OAT, oral anticoagulation therapy; PT, prothrombin time; INR, International Normalized Ratio; and ISI, International Sensitivity Index.
Thrombosis Services took care of 327,067 patients with OAT and carried out 4,629,097 PT/INR determinations (The Netherlands has a population of 16.4 million). To reach the appropriate intensity of therapy, the INR must be within narrow therapeutic intervals for each indication (2.0–3.5 or 2.5–4.0, according to the recommendations of the Dutch Federation of Thrombosis Services) (2). In 2003, a mean of 74% of the INRs were within the therapeutic ranges. The quality of OAT management depends on the patient education, knowledge of the variable patient characteristics, prescription of the right dose of the coumarin derivatives, and the reliability of the result of the PT/INR determination. The latter variable depends on preanalytical and analytical influences.

The INR was introduced in 1983 with the intention of harmonizing the results of the PT (3, 4). Since that time, many studies concerning the analytical and preanalytical variables have been carried out to improve the harmonization of the INR, including (local) International Sensitivity Index (ISI) calibration and the study of various thromboplastin reagents, plasma calibrants, blood collection techniques, and citrate concentrations (5, 6). In addition, external quality assessment has been developed and has made a contribution to the harmonization of the INR (7). Although use of the INR substantially reduces the differences in PT results, interlaboratory variation still persists. Possibly some preanalytical variables that have been received less attention may contribute to the variation in PT/INR results.

In The Netherlands, blood collection for determination of the PT/INR in outpatients on OAT takes place primarily at locations remote from the laboratory, including the patients’ homes. Transport of blood samples to the laboratory can take 1–6 h. Furthermore, the samples are transported by car, in which case they experience mechanical agitation and widely varying temperatures. In addition, samples are sometimes centrifuged on arrival at the laboratory and then sit until analyses can be carried out. Whether all of these variables influence the results of the INR is not known.

The aim of our study was to investigate whether the following preanalytical variables influence the PT/INR results and whether any changes seen are clinically relevant: time between blood collection and PT/INR determination on samples stored at room temperature, 4–6 °C, and 37 °C; mechanical agitation at room temperature, 4–6 °C, and 37 °C; time between centrifugation and PT/INR determination; and times and temperatures of centrifugation.

Four laboratories with different combinations of blood collection systems, thromboplastin reagents, and coagulation meters participated.

**Materials and Methods**

**QUESTIONNAIRE**

To collect data about the different procedures and circumstances of off-site blood collection and PT/INR determination, we developed a questionnaire that was submitted to each Thrombosis Service in The Netherlands at the end of 2003. The questionnaire asked for the minimum and maximum time between blood collection and determination of the PT/INR, the positions of samples and method of temperature regulation during transport, the time and methods of centrifugation, and the methods used to determine the PT/INR in plasma or whole blood.

**PARTICIPATING CENTERS**

This comparative study was carried out in the laboratories of three Thrombosis Services respondents, ’s-Hertogenbosch, Etten-Leur, and Veldhoven (Centers 1, 2, and 4), and in the laboratory of the Haemostasis and Thrombosis Research Center in Leiden (Center 3). The centers use different combinations of blood collection systems, thromboplastin reagents, and coagulation meters. Table 1 shows the blood collection systems, sodium citrate concentrations, thromboplastin reagents, automated coagulation meters, and ISI used by the four centers. For one experiment (different times of centrifugation), Center 1 used another blood collection system, Vacutainer instead of Monovette. Blood collection took place by venipuncture according to standard procedures. The PT/INRs were determined according to routine practices in the centers.

**DESIGN OF THE EXPERIMENTS**

We simulated the preanalytical variables in the four laboratories (Table 2). In general, 20 patients participated in each experiment (the exact numbers of patients are noted in the tables). From each patient we collected four or five (experiments at 4–6 and 37°C) tubes instead of the usual one. After blood collection, we subjected the tubes to the several conditions.

The result of the PT/INR determination in tube 1 (stored at room temperature for 0.5–1 h after blood collection) was the reference value. For the experiments at 4–6°C and 37°C, we used one extra tube (1a). Tube 1a was incubated at the tested temperature for 24 h, and the PT/INR was determined at the same times as for tube 1. PT/INR determinations were carried out at fixed times. In conjunction with the PT/INR determinations for tube 2 (incubated for 3 h), 3 (incubated for 6 h), and 4 (incubated for 24 h), we also assayed tubes 1 and 1a. Because of the different centrifugation procedures, as noted in the questionnaire, we carried out two more experiments: one with the centrifuge cooled to 20°C vs no temperature control and the other at different centrifugation times (5 and 10 min). In the last experiment we also counted the platelets.

**STATISTICAL ANALYSIS**

Continuous variables were compared by the t-test for paired PT/INR determinations. A 5% significance level was used. All statistical analyses were performed with SPSS 10 for Windows.
Fifty-eight of the 62 Thrombosis Services responded to the questionnaire. The questions and answers are shown in Table 3. In most cases, blood samples were reported to arrive at the central laboratory within 6 h after blood collection. When blood collection takes place in the laboratory and not at an off-site location, the PT/INR test is typically performed after 0.5 h, but only eight Thrombosis Services perform the test within 0.5 h. Most Thrombosis Services transport the tubes in a vertical position and have some kind of temperature control in the car. The methods of centrifugation (rpm, g force, cooled or not, and duration) differ widely. There is no correlation between the used rpm, g force, and duration of centrifugation. Most Thrombosis Services determine the PT/INR in plasma.

EXPERIMENTS

Shown in Table 4 and Fig. 1 are summaries of the results of experiments 1a, 1b, 1c, 2a, 2b, 2c, and 3a (see Table 2). The mean (SD) INRs, the mean percentage changes (obtained with the formula: \( \% \text{ change} = \left( \frac{INR_t - INR_{t1}}{INR_{t1}} \right) \times 100 \), where \( t_1 \) is the reference value), the ranges of the maximum positive and negative percentage changes, the number of individual INR values that exceeded the limits of 10% change, and the \( P \) values are given in Tables 1–7 of the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol51/issue3/. We considered an INR difference \( >10\% \) as clinically relevant.

For daily practice, the clinically relevant differences are important because they can cause dosage adjustment of the coumarin derivatives. We defined a change in INR as moderate when \( >25\% \) of individual INRs exceeded the 10% limit and as large when \( >25\% \) of individual INRs exceeded the 10% limit. Shown in Fig. 1 are the percentages of individual INRs that exceeded the 10% limit and the moderate and large changes. The data for experiments 3b and 3c are shown in Tables 8 and 9 of the online Data Supplement.

Experiment 1a: Time between blood collection and PT/INR determinations in samples stored at room temperature (Table 1 in the online Data Supplement). For Center 1 (\( n=20 \) patients), the mean percentage changes in INR were negative or zero; all mean changes were \( <10\% \). The percentage of samples with negative changes that exceeded the 10% limit was moderate \((\leq25\% \) of samples) after 24 h (tubes 1 and 4); most of these were from one patient. The differences in INR values for tube 1 were statistically significant after 6 and 24 h. For Center 2 (\( n=20 \) patients), the mean percentage changes were all negative and exceeded 10% after 24 h. The percentages of samples with negative changes that exceeded the 10% limit were large \((>25\% \) of samples) after 6 h (tubes 1 and 3) and 24 h (tubes 1 and 4). All changes were statistically significant.
Experiment 1b: Time between blood collection and PT/INR determinations in samples stored at 4–6 °C (Table 2 of the online Data Supplement). For Center 1 (n = 20 patients), the mean percentage changes in INR were negative or near zero and became positive after 24 h (tubes 1, 1a, and 4); all mean changes were <10%. All maximum changes were within the 10% limit, except for tube 4 (incubation for 24 h), for which the percentage of samples with positive changes that exceeded the 10% limit was moderate. The differences were statistically significant in tubes 1 and 1a after 6 h and in tube 4 after 24 h. For Center 2 (n = 20 patients), the mean percentage changes in INR were negative for all tubes; all mean changes were <10%. The percentage of samples with negative changes in INR that exceeded the 10% limit was moderate (25% of samples) after 6 h and was large (>25% of samples) for tube 4 after 24 h. All changes were statistically significant except in tube 1a after 3 h and 24 h.

Table 2. Experiments, conditions, and participating centers.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Conditions</th>
<th>Center(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a. Time between blood collection and PT/INR test on specimens stored at room temperature</td>
<td>Tests after 0.5–1, 3, 6, and 24 h; centrifugation just before the tests</td>
<td>1 and 2</td>
</tr>
<tr>
<td>1b. Time between blood collection and PT/INR test on specimens stored at 4–6 °C</td>
<td>Tests after 0.5–1, 3, 6, and 24 h; centrifugation just before the tests; storage, in refrigerator</td>
<td>1 and 2</td>
</tr>
<tr>
<td>1c. Time between blood collection and PT/INR test on specimens stored at 37 °C</td>
<td>Tests after 0.5–1, 3, 6, and 24 h; centrifugation just before the tests; storage, in waterbath (Center 1) or incubator (Center 2)</td>
<td>1 and 2</td>
</tr>
<tr>
<td>2a. Time between blood collection and PT/INR test with mechanical agitation and storage at room temperature</td>
<td>Tests after 0.5–1, 3, 6, and 24 h; centrifugation just before the tests; agitation, tubes lying horizontal on roller mixer</td>
<td>1 and 2</td>
</tr>
<tr>
<td>2b. Time between blood collection and PT/INR test with mechanical agitation and storage at 4–6 °C</td>
<td>Tests after 0.5–1, 3, 6, and 25 h; centrifugation just before the tests; storage, in refrigerator; agitation, tubes vertical in orbital shaker</td>
<td>3</td>
</tr>
<tr>
<td>2c. Time between blood collection and PT/INR test with mechanical agitation and storage at 37 °C</td>
<td>Tests after 0.5–1, 3, 6, and 25 h; centrifugation just before the tests; storage, in incubator; agitation, tubes vertical in orbital shaker</td>
<td>3</td>
</tr>
<tr>
<td>3a. Time between centrifugation and PT/INR test</td>
<td>Tests after 0.5–1, 3, 6, and 24 h; centrifugation just after blood collection; plasma left on the spun-down blood cells</td>
<td>1 and 2</td>
</tr>
<tr>
<td>3b. Centrifugation with and without control of temperature</td>
<td>Control at 20 °C, 1892g, 3000 rpm, and 10 min</td>
<td>4</td>
</tr>
<tr>
<td>3c. Centrifugation at different times and platelet counts</td>
<td>Times, 5 and 10 min; 2500g, 3822 rpm; temperature, 4 °C</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3. Questionnaire: Questions and answers from 58 Thrombosis Services.

<table>
<thead>
<tr>
<th>Preanalytical variables</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum time between blood collection and PT test</td>
<td>4–6 h 56</td>
</tr>
<tr>
<td>Minimum time between blood collection and PT test in the central laboratory</td>
<td>&gt;0.5 h 50, &lt;0.5 h 8</td>
</tr>
<tr>
<td>Position of the tubes during transport</td>
<td>Vertical 49, Horizontal 5, Both 4</td>
</tr>
<tr>
<td>Temperature: special measures during transport</td>
<td>Yes 46, No 12</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>Just before determination 52, Not applicable (whole blood) 6</td>
</tr>
<tr>
<td>Time</td>
<td>2420–4700 rpm; 1000–6240g Differ widely</td>
</tr>
<tr>
<td>rpm and g</td>
<td>Yes 21, No 31</td>
</tr>
<tr>
<td>Cooled</td>
<td>10 min 35, &gt;10 min 8, &lt;10 min 6, Other 3</td>
</tr>
<tr>
<td>Duration</td>
<td>In plasma 52, In whole blood 6</td>
</tr>
</tbody>
</table>
Experiment 1c: Time between blood collection and PT/INR determinations in samples stored at 37 °C (Table 3 in the online Data Supplement).

For Center 1, the mean percentage changes were negative for all tubes except tube 4 (24-h incubation), for which the change in INR was positive; all mean changes were <10%. The samples with negative changes that exceeded the 10% limit were mainly from one patient. The percentage of samples with positive changes for tube 4 (24-h incubation) that exceeded the 10% limit was large. All changes were statistically significant, except in tube 3 (incubation for 6 h).

For Center 2, the mean percentage changes in INR for all samples were negative except for tubes 1a and 4 after 24 h, for which the changes in INR were positive; all mean changes were <10% except for tube 1 after 24 h. The percentage of samples with positive changes that exceeded the 10% limit was moderate (≤25% of samples) after 6 h and large (>25% of samples) after 24 h. All changes were statistically significant, except for tube 1 after 24 h. For Center 2 [n = 21 patients except for tube 1 after 3, 6, and 24 h (n = 20 patients)], the mean percentage changes in tube 1 were negative and were <10%; in the other tubes, they were positive after 3 h (tube 2), 6 h (tube 3), and 24 h (tube 4) of mechanical agitation and were >10% after 24 h. The percentage of

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### Table 4. Summary of the results of experiments 1a, 1b, 1c, 2a, 2b, 2c, and 3a.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Tube</th>
<th>Time, h</th>
<th>Center 1</th>
<th>Center 2</th>
<th>Center 3</th>
</tr>
</thead>
</table>
| 1a         | 1    | 0.5–1   | -1.4 (0.182) | -10.2 (<0.001)
|            | 2    | 3       | 0.0 (0.832)  | -4.6 (0.013)
|            | 3    | 6       | -2.2 (0.101) | -8.2 (<0.001)
|            | 4    | 24      | -1.4 (0.182) | -10.2 (<0.001)
| 1b         | 1    | 0.5–1   | 3.4 (0.037)  | -8.6 (<0.001)
|            | 2    | 3       | 0.2 (0.740)  | -3.8 (0.004)
|            | 3    | 6       | -1.1 (0.180) | -5.0 (0.001)
|            | 4    | 24      | 7.5 (0.002)  | -8.1 (0.004)
| 1c         | 1    | 0.5–1   | -3.1 (0.003) | -7.7 (<0.001)
|            | 2    | 3       | -1.5 (0.154) | -5.2 (0.012)
|            | 3    | 6       | 6.0 (<0.001) | 5.8 (0.032)
|            | 4    | 24      | 39.0 (<0.001) | 22.9 (<0.001)
| 2a         | 1    | 0.5–1   | -1.5 (0.003) | 6.0 1.4
|            | 2    | 3       | -2.6 (0.001) | 7.9 1.0
|            | 3    | 6       | -6.8 (<0.001) | 14.3 0.0
|            | 4    | 24      | 1.5 (0.048)  | 0.6 (0.816)
| 2b         | 1    | 0.5–1   | -2.5 (<0.001) | 4.9 0.0
|            | 2    | 3       | 0.1 (0.858)  | 0.1 (0.858)
|            | 3    | 6       | 31.5 (<0.001) | 46.5
| 2c         | 1    | 0.5–1   | -2.7 (0.001) | 1.0 (0.575)
|            | 2    | 3       | -1.8 (0.003) | -0.4 (0.750)
|            | 3    | 6       | -0.7 (0.281) | -1.8 (0.211)

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a See Table 2 for a description of the experiments.
b Time between blood collection and PT/INR determination.
c Reference value.
d Statistically significant difference (P ≤0.05).
samples with positive changes that exceeded the 10% limit was moderate after 6 h and large after 24 h. All changes were statistically significant except in tube 2 (3-h incubation).

Experiment 2b: Time between blood collection and PT/INR determinations with mechanical agitation and storage at 4–6 °C (Table 5 of the online Data Supplement). This experiment was performed only at Center 3 (n = 20 patients). The mean percentage changes were negative except for tube 1a after 0.5–1 h and 25 h, when the changes were near zero; all mean changes were <10%. The percentage of patients with negative changes that exceeded the 10% limit was moderate at 25 h of incubation (tube 4). All changes were statistically significant, except in tube 1 after 25 h and in tube 1a after 0.5–1, 6, and 25 h.

Experiment 2c: Time between blood collection and PT/INR determinations and mechanical agitation and storage at 37 °C (Table 6 of the online Data Supplement). This experiment was performed only at Center 3 (n = 20 patients). The mean percentage changes in INR were negative or near zero for all tubes until 6 h and were <10%; for tubes with mechanical agitation and storage at 37 °C, they became positive after 6 h and were >10% after 25 h. The percentage of samples with positive changes in INR that exceeded the 10% limit was large after 25 h. All changes were statistically significant except in tube 1 after 25 h and in tubes 1a and 3 after 6 h.

Experiment 3a: Time between centrifugation and PT/INR determinations (Table 7 of the online Data Supplement). For Center 1, the mean percentage changes were negative; all mean changes were <10%. All maximum changes were also within the 10% limit. All changes were statistically significant, except in tube 4 after 24 h. For Center 2, the mean percentage changes were positive or negative, and all were <10%. The percentage of negative or positive changes that exceeded the 10% limit was moderate. There were no statistically significant differences.

Experiment 3b: Centrifugation with and without control of temperature (Table 8 of the online Data Supplement). This experiment was performed only at Center 4. The mean
percentage difference for tube 2 compared with tube 1 was negative. The difference was within the 10% limit and was not statistically significant.

Experiment 3c: Centrifugation at 10 and 5 min, and number of platelets (Table 9 of the online Data Supplement). This experiment was performed only at Center 1. The mean percentage difference for tube 2 compared with tube 1 was negative. The difference was within the 10% limit and was not statistically significant. The mean numbers of platelets were 68 × 10^9/L after 5 min of centrifugation and 18 × 10^9/L after 10 min.

**Discussion**

Our survey showed that off-site blood collection for the control of OAT produces various preanalytical conditions.

A NCCLS guideline (8) recommends determination of the PT/INR within 24 h of blood collection and maintenance of sample tubes at 2–4 °C or at room temperature (18–24 °C). Several studies (9–17) have generally confirmed that such storage for 24 h does not lead to clinically important changes in the mean PT/INR results. These studies, however, considered only the mean values and possibly overlooked changes in individual samples that could lead to a change in the dose of coumarin derivatives. Other studies have taken into consideration that INR values of individual patients can fall outside the chosen limits (9,12,14). Leeming et al. (12) concluded that for some individual patients, storage of samples for 24 h changes the PT/INR values, leading to a dosage adjustment; these authors do not support that practice. Studies of mechanical agitation that simulate the conditions in a car during transport are few. In one study, the tubes were subjected twice to a gentle agitation of only 30 min (14). We found no studies on storage at 37 °C in the literature. Centrifugation at high speed compared with routine centrifugation showed comparable results (18).

The statistical significance of the differences in INR cannot be used as a criterion to draw conclusions about the daily practice of calculating the dose of coumarin derivatives. The mean PT/INRs and mean percentage changes are important to give an overall picture of the course of the results during the experiments. We were particularly interested in clinically relevant changes. For the preanalytical variables, there are no guidelines for percentage changes compared with reference value that are acceptable. For calibration procedures and for testing of the local PT systems, a deviation of the INR of >10% of the reference value is defined as clinically important (19,20). We decided to consider an INR change outside the positive or negative limits of 10% of the reference value to be clinically relevant.

Centers 1 and 2 did the same experiments. In Center 1 (Monovette, Hepato Quick, and STA Rack), for most of the experiments the results of the PT/INR test changed less than results in Center 2 (Venoject, Recombiplastin, and Electra). We have no explanation for this difference between centers.

During storage time at room temperature, 4–6 °C, and 37 °C (experiments 1a, 1b, and 1c), changes in PT/INR were mostly negative until 6 h, but were positive after 24 h at 37 °C. Center 1 considered it acceptable to store at room temperature and in the refrigerator for 24 h, but at Center 2 only storage for up to 6 h is acceptable. In both centers, storage at 37 °C longer than 6 h is not acceptable.

Mechanical agitation with the roller mixer (horizontal position) at room temperature (experiment 2a) showed in Centers 1 and 2 an increase in changes after 6 h; after 24 h, all changes were positive and exceeded the 10% limit. The mechanism of this phenomenon is unknown. In Center 3 (Vacutainer, Simplastin HTF, and Thrombolyzer), mechanical agitation with the orbital shaker (vertical position) at 4–6 °C and 37 °C (experiments 2b and 2c) showed no relevant changes until 6 h. At 4–6 °C, the changes remained negative after 24 h, and only a small number exceeded the limit of 10%. However, with mechanical agitation at 37 °C, all changes were positive and exceeded the limit of 10%. Comparing the two experiments at 37 °C (experiments 1c and 2c), we see that this temperature always changes the results from negative to positive, and that mechanical agitation will enhance this phenomenon. We assume that mechanical agitation in a horizontal position stimulates coagulation more than when the tube is in a vertical position. We cannot explain this phenomenon. We recommend transporting the tubes vertically, and after transporting the tubes in a car to determine the PT/INR within 6 h after blood collection. The temperatures inside cars vary widely, but a high temperature must be avoided, not exceeding 37 °C for 6 h.

When the samples were centrifuged immediately after blood collection (experiment 3a), the results were stable at room temperature at both Centers 1 and 2. Thus, it is possible to carry out centrifugation immediately after blood collection and store the samples at room temperature. However, off-site blood collection does not always offer an opportunity to centrifuge immediately before the tubes are transported.

When we looked at the results obtained for tubes 1 and 1a in all experiments in Centers 1, 2, and 3 (experiments 1a through 3a), we saw that a 24-h delay at room temperature or at 4–6 °C caused a negative change in INR in all centers, with larger changes in center 2 than in Centers 1 and 3. These tubes were also centrifuged immediately after blood collection, between 0.5–1 h, but the PT/INR was determined several times in the same tube.

For determination of PT/INR, a temperature-controlled centrifuge is not required. The duration of centrifugation (5 or 10 min) and the platelet count did not influence the results.

Many different combinations of blood collection systems, thromboplastin reagents, and coagulation meters are used. The influence of preanalytical variables that change the INR values by >10% must be minimized. In
laboratories that have not investigated the influences of the preanalytical variables mentioned above on PT/INR results, we recommend a maximum of 6 h of storage at room temperature, 4–6 °C, or 37 °C or after mechanical agitation. This recommendation does not agree with the NCCLS guideline (8). Storage at a temperature of 37 °C or mechanical agitation for 24 h appears to be unacceptable. Storage at room temperature or at 4–6 °C for 24 h before determination of PT/INR may be acceptable, but this must be determined by each laboratory for its combinations of assays and blood collection methods.

We thank the personnel at the laboratories who accurately performed the experiments. We would particularly like to thank Masja de Punder and Afzal Kariman (‘s-Hertogenbosch), Annelies van der Smissen-van Meel (Etten-Leur), and Ellen van Eekelen (Veldhoven). We also thank Roche Diagnostics for providing the thromboplastin reagent Hepato Quick.

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