Preparation of Control Blood for External Quality Assessment of Point-of-Care International Normalized Ratio Testing in the Netherlands

Anton M. H. P. van den Besselaar, PhD,1 Charmane F. Abdoel,1 Davina Ardanary,2 Gert van de Kamp,2 and Fanny A. C. Versluijs3

From the 1Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands; 2Department of Reagents Production, Sanquin Blood Supply, Amsterdam, the Netherlands; and 3Netherlands Federation of Thrombosis Services, Voorschoten, the Netherlands.

Key Words: External quality assessment; Point-of-care testing; International normalized ratio; Control blood

ABSTRACT

Objectives: The aim of this study was to prepare control blood for an external quality assessment scheme (EQAS) for international normalized ratio (INR) point-of-care testing (POCT) in the Netherlands and to assess the performance of the participants.

Methods: Control blood was prepared from dialyzed pooled patient plasma and washed human erythrocytes. Samples of control blood were mailed to participants of the Netherlands EQAS from October 2006 through December 2012.

Results: Most participants used CoaguChek XS (Roche Diagnostics, Mannheim, Germany) devices for POCT. The median between-center coefficient of variation (CV) of the reported INR decreased from 4.5% in 2006 to 2.6% in 2012. A few participants used the ProTime Microcoagulation System (ITC, Edison, NJ) for POCT. The median CV (per year) of the INR with the latter system was 7.0% to 10.6%.

Conclusions: The control blood samples were useful for external quality assessment in the Netherlands. The participants’ performance with the CoaguChek XS system improved with time, demonstrating the value of external quality assessment.

The point-of-care test (POCT) of the prothrombin time (PT) and the international normalized ratio (INR) is used in many countries for monitoring patients treated with vitamin K antagonists.1 Patients who have monitored their therapy at home with a point-of-care INR monitor have demonstrated an ability to achieve a high rate of time in a therapeutic range, as documented in clinical trials.2 Guidelines recommend that POCT users (eg, anticoagulation clinics) should participate in an external quality assessment scheme (EQAS).3 The purpose of an EQAS is to assess between-user variation. One study showed that patients were able to perform EQAS tests competently.4 Regular assessment through EQAS surveys also promotes a quality-conscious environment, so there may be indirect positive effects that derive from the use of POCT devices.5

The purpose of the present article is to describe the preparation of liquid control blood for a national EQAS for POCT of the INR. When the EQAS was set up, there were two brands of point-of-care devices for PT testing in the Netherlands: the CoaguChek (Roche Diagnostics, Mannheim, Germany) and the ProTime Microcoagulation System (ITC, Edison, NJ). The original CoaguChek was replaced by the CoaguChek XS device, which is based on electrochemical detection of thrombin formation.6,7 The ProTime Microcoagulation System is based on clot formation in a narrow capillary tube and optical detection of interrupted flow of the reaction mixture.8 The ProTime Microcoagulation System operates with blood specimens only. We tried to prepare control blood for an EQAS of the POCT systems, as described previously for an EQAS of conventional PT determinations.9 With some modifications of the methods described in that article, we managed to prepare control blood that could be used with both the CoaguChek XS and ProTime Microcoagulation System. The EQAS results...
obtained with control blood samples from October 2006 through December 2012 are described in the present article.

Materials and Methods

Collection and Photodynamic Treatment of Patients’ Plasma

Venous blood specimens of patients treated with vitamin K antagonists were drawn with plastic blood collection tubes (S-Monovette; Sarstedt, Nümbrecht, Germany), each containing 0.43 mL of 0.106 mol/L sodium citrate solution for 4.3 mL of blood. The citrated blood specimens were centrifuged for 10 minutes at 2,500 g. The INR was determined in each plasma specimen. Plasma was aspirated with plastic pipettes and transferred to plastic tubes. Samples with INRs in the 1.5 to 1.9 interval were pooled, as well as samples in the 2.0 to 2.4 INR interval. The pooled samples were centrifuged for 15 minutes at 2,500 g and then frozen and stored at −70°C. The time interval between phlebotomy and freezing of pooled plasma was 24 hours. After thawing, the pooled plasma was illuminated with incandescent light in the presence of methylene blue for inactivation of pathogenic viruses. After the photodynamic treatment, the pooled plasma was dialyzed against a solution containing sodium chloride (0.09 mol/L) and sodium citrate (0.0005 mol/L). After dialysis, 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES) buffer adjusted at pH 7.35, sodium azide, and thiomersal were added as preservatives to the pooled plasma, with final concentrations of 10 mmol/L HEPES, 0.1% sodium azide, and 0.01% thiomersal. The plasma was frozen and stored at −40°C.

Treatment of RBCs

Human packed cells of blood group ORhD+ had been in storage for 192 to 200 hours and were washed with phosphate-buffered saline. After centrifugation, the buffy coat and the supernatant were removed, and the cells were washed again with phosphate-buffered saline. Then the packed cells were mixed with an equal volume of HEPES buffer (formulation: 0.1 mol/L HEPES, 0.09 mol/L NaCl, and 0.0005 mol/L trisodium citrate, pH adjusted to 8.6 with sodium hydroxide) and incubated for 2 hours at room temperature. After centrifugation, the supernatant was removed from the packed cells.

Preparation of Control Blood

Frozen pooled plasma prepared as described above was thawed in a water bath at 37°C for 10 to 15 minutes. Samples of treated RBCs were weighed and mixed with pooled plasma so that the hematocrit of the control blood was approximately 30%. For example, when the weight of packed cells was 33 g, 72 g of plasma was added and carefully mixed with the cells. A mixture of penicillin and streptomycin was added to prevent microbial growth. While the bulk of the control blood was stirred mechanically with a Teflon magnetic bar of 3 cm (at approximately 60 revolutions per minute), 1.0-mL aliquots were pipetted into 2-mL plastic tubes (article number 72.693.100; Sarstedt). The tubes were closed with screw caps. Sets of two different control blood samples and a tube containing calcium chloride (0.05 mol/L) were distributed by mail to participants of the Dutch EQAS. Usually, the specimens were received by the participants within 1 day. The specimens were contained in a sealed plastic bag with absorbing paper. The plastic bag was contained in a cardboard box for mailing. There was no temperature monitoring for the specimens during transport.

External Quality Assessment

An EQAS for point-of-care users was organized by the Federation of Netherlands Thrombosis Services. There were five surveys per year, and two different control blood samples were provided in each survey. In each survey, control blood samples were prepared on Monday and mailed the next day. Participants of the EQAS received instructions for handling and testing the control blood samples. They were instructed to store the samples at room temperature and perform the tests on the fourth day after preparation. On the day of testing, the participants were instructed to homogenize the sample by gentle inversion of the tube, open the tube, add exactly 0.1 mL of calcium chloride solution, close the tube, mix the blood and the calcium chloride by gentle inversion, open the tube, and apply a drop of blood to the point-of-care test strip or cuvette. The inversions of the tube after calcium chloride addition were not standardized.

Statistical Evaluation of EQAS Results

INRs reported by the participants of the Netherlands EQAS were evaluated for each control sample and each group of participants using the same brand of point-of-care instrument. The statistical evaluation was done with a computer program. Outliers were detected by means of an algorithm described previously. Mean (M) and between-center standard deviation (SD) and coefficient of variation (CV) were calculated after exclusion of outlying results. Each center’s result (R) was compared with the mean value, and the percentage deviation D from the mean value was calculated with the following formula: $D = 100 \times \frac{(R - M)}{M}$. A participant’s result was judged as within consensus if the percentage deviation D was not greater than ±15%.

Results

When we started our study, it soon became clear that the citrate concentration in blood collected in 0.106 mol/L sodium citrate was too high for successful testing, after addition of
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calcium chloride, with the ProTime Microcoagulation System. Therefore, plasma was dialyzed against a sodium chloride solution containing a low citrate concentration (0.0005 mol/L) for preparation of control blood.

Control blood was prepared by mixing dialyzed pooled patient plasma with pretreated RBCs. The pooled plasma was derived from patients with various blood groups, and the RBCs were always blood group ORhD+.

Table 1

<table>
<thead>
<tr>
<th>Storage Time, d</th>
<th>INR Sample 1</th>
<th>INR Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.95</td>
<td>2.30</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>2.30</td>
</tr>
<tr>
<td>4</td>
<td>2.10</td>
<td>2.40</td>
</tr>
</tbody>
</table>

*International normalized ratio (INR) was determined in duplicate with a CoaguChek XS device (Roche Diagnostics, Mannheim, Germany). Mean values are shown.

Table 2

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of Participants (at End of Year)</th>
<th>Mean INR per Control Specimen</th>
<th>Percentage of Participants With D ≤ ±15%</th>
<th>Between-Center Coefficient of Variation per Control Specimen, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median Minimum-Maximum</td>
<td>Median Minimum-Maximum</td>
<td>Median Minimum-Maximum</td>
</tr>
<tr>
<td>2006</td>
<td>28</td>
<td>1.87 1.72-2.03</td>
<td>83.9 81.0-92.6</td>
<td>4.5 4.0-6.2</td>
</tr>
<tr>
<td>2007</td>
<td>37</td>
<td>2.29 1.60-3.17</td>
<td>93.9 91.1-100</td>
<td>4.1 2.9-7.5</td>
</tr>
<tr>
<td>2008</td>
<td>52</td>
<td>2.22 1.72-2.81</td>
<td>95.2 86.3-100</td>
<td>3.6 2.9-8.6</td>
</tr>
<tr>
<td>2009</td>
<td>59</td>
<td>2.49 1.82-2.82</td>
<td>95.7 85.7-100</td>
<td>3.1 2.2-5.1</td>
</tr>
<tr>
<td>2010</td>
<td>72</td>
<td>2.40 1.93-2.89</td>
<td>97.0 96.8-98.6</td>
<td>2.8 2.3-3.4</td>
</tr>
<tr>
<td>2011</td>
<td>75</td>
<td>2.55 2.00-2.84</td>
<td>98.0 94.7-100</td>
<td>2.8 2.5-4.1</td>
</tr>
<tr>
<td>2012</td>
<td>84</td>
<td>2.26 1.85-2.80</td>
<td>98.8 95.5-100</td>
<td>2.6 2.3-3.2</td>
</tr>
</tbody>
</table>

EQAS, external quality assessment scheme; INR, international normalized ratio.

* D is the deviation of a participant’s result relative to the mean INR of each control specimen. Minimum and maximum represent the range of values observed. The CoaguChek XS device is from Roche Diagnostics (Mannheim, Germany).
CoaguChek XS participants with D less than 15% (ie, within consensus) were calculated (Table 2). There was a trend to reduction of the between-center variation from 2007 through 2012. Furthermore, there was a trend to increasing percentage of participants within consensus. The between-center CV of the INR and the percentage of participants within consensus for the ProTime Microcoagulation System are presented in Table 3. The CV and percentage results within consensus were variable because of the low number of participants. The number of participants using the ProTime Microcoagulation System dropped below four in 2011, and evaluation of the results in terms of CV may be questionable.

Figure 2 shows the correlation between the mean INR determined with the CoaguChek XS and the mean INR determined with the ProTime Microcoagulation System from 2006 through 2011. Considerable scatter of the data points was observed. Despite the scatter, the correlation between the mean values was statistically significant ($P < .001$).

Discussion

The challenge of the present study was to prepare liquid control blood samples that could be used in two different types of POCT systems. The control blood samples must be stable for several days when they are mailed to the participants and stored until the requested date of testing. This could be achieved by removing citrate and calcium ions from the plasma to be used for the preparation of control samples. Preservatives such as sodium azide and thiomersal and HEPES buffer were added to improve the stability. The samples were not absolutely stable because a slight increase of the INR was observed in the course of 4 days (Table 1). We assumed that the change was the same in all samples and that the variation between participants’ results was not due to between-sample variation. It was necessary to add calcium chloride to the samples immediately before application to the POCT systems. The ProTime Microcoagulation System has two levels of onboard quality control integrated in each cuvette. The ProTime Microcoagulation System was very sensitive to the concentration of citrate in the samples, as measured by two quality control channels parallel to the sample channel. If the clotting times in the quality control channels were not within certain limits set by the manufacturer, the test failed. It should

<table>
<thead>
<tr>
<th>Year</th>
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<th>Mean INR per Control Specimen</th>
<th>Percentage of Participants With D &lt; ±15%</th>
<th>Between-Center Coefficient of Variation per Control Specimen, %</th>
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<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Minimum-Maximum</td>
<td>Median</td>
</tr>
<tr>
<td>2006</td>
<td>10</td>
<td>2.10</td>
<td>1.93-2.30</td>
<td>85.0</td>
</tr>
<tr>
<td>2007</td>
<td>9</td>
<td>2.54</td>
<td>1.73-2.98</td>
<td>82.9</td>
</tr>
<tr>
<td>2008</td>
<td>4</td>
<td>2.33</td>
<td>1.87-2.82</td>
<td>86.1</td>
</tr>
<tr>
<td>2009</td>
<td>4</td>
<td>2.26</td>
<td>1.68-2.87</td>
<td>100</td>
</tr>
<tr>
<td>2010</td>
<td>5</td>
<td>2.38</td>
<td>1.89-2.96</td>
<td>100</td>
</tr>
</tbody>
</table>

EQAS, external quality assessment scheme; INR, international normalized ratio.

D is the deviation of a participant’s result relative to the mean INR of each control specimen. Minimum and maximum represent the range of values observed. The ProTime Microcoagulation System is from ITC (Edison, NJ).
be realized that the ProTime Microcoagulation System was designed for blood samples without added citrate. By trial and error, we found that the citrate concentration in the control blood had to be reduced to be compatible with the control channels of the ProTime Microcoagulation System.

Patients’ plasma samples were collected within two INR ranges: 1.5 to 1.9 and 2.0 to 2.4. These INRs were determined in fresh samples (ie, within 4 hours after phlebotomy). We observed that the INRs of some control blood specimens prepared from the pooled plasma were higher than 2.4. This may be explained by the combined effects of the various steps in the preparation procedure. The photodynamic treatment of plasma caused partial deterioration of the clotting factors.10 The dialysis of the plasma may have caused a slight change of the INR, which could not be assessed accurately because of the different citrate concentrations before and after dialysis.

HEPES buffer, sodium azide, and thiomersal were added to the dialyzed plasma, resulting in a slight increase of the INR (not shown).15 Finally, storage of the specimens resulted in a slight increase of the INR (see Table 1).

For the evaluation of reported clotting times and INRs in the EQAS, outlying results should be detected and removed. Outlying results may be due to technical or clerical errors. The origin of an outlying result could not always be traced, but in some cases, it was obvious that a participant had reported the results of the two specimens in the wrong order. Clerical errors tend to exaggerate the variation between participants. We used an algorithm to detect and remove outliers.11,12

The relative number of outlying results was greater for the CoaguChek XS users compared with that of the ProTime Microcoagulation System, which may be related to the different numbers of participants using these systems.

The results obtained in the course of 6 years showed a gradual reduction of the between-center CV of the INR measured with the CoaguChek XS. There are several potential explanations for this observation. One may be an improved proficiency of the participants of the EQAS over the years (ie, a learning effect). At the same time, the number of participants increased from 37 to 84, which might have improved the average performance. An alternative explanation of the reduction of the between-center CV might be an improved performance of the test strips as a result of improved manufacturing procedures. It is interesting to note that the magnitude of the observed between-center CV is similar to the within-center reproducibility of the test reported previously.16 This observation suggests that the between-center variation hardly can be reduced any further. In comparison to the results obtained with the CoaguChek XS, the between-laboratory CV observed with the ProTime Microcoagulation System was higher and did not improve between 2006 and 2008. This observation suggests that the intrinsic imprecision of the ProTime Microcoagulation System is high and surpasses any between-laboratory variation. In an analytical evaluation of the ProTime Microcoagulation System by Van de Ven and coworkers,17 within-instrument precision of 14% was reported. In 2012, the ProTime Microcoagulation System was no longer used by EQAS participants in the Netherlands.

There are several limitations to the present study. The specimens were not absolutely stable, and there was no temperature monitoring for the specimens during transportation to the EQAS participants. The Netherlands climate is mild throughout the year. Average minimum and maximum temperatures fluctuate between 1°C and 6°C in December and between 12°C and 22°C in August. The INR results obtained with the two different POCT devices could not be combined because there were considerable differences between the mean INRs of individual specimens (see Figure 2). The scatter was probably due to the different measurement principles for the CoaguChek XS and the ProTime Microcoagulation System.

Our results may be compared with those of the CoaguChek XS and CoaguChek XS Plus EQA Programme in the UK National External Quality Assessment Scheme for Blood Coagulation.18 In the latter scheme period (2010-2011), CVs were 10.6% to 18.9% for CoaguChek XS Plus users, and a mean of 7.5% of CoaguChek XS users were more than 15% from the median of INRs determined from all user results.18 There are several differences between the Netherlands and the UK schemes. One difference is in the removal of any outliers. In our scheme, outliers were defined as results for which the chance of inadvertently marking the result as an outlier was 1% or less, but in the UK scheme, results more than five standard deviations from the median were considered outliers. This might influence the interlaboratory variation after the removal of outliers. Another difference is in the nature of the samples. In the UK scheme, lyophilized samples were provided with diluents for reconstitution, and no laboratory equipment was needed to complete the tests. One possible explanation for the greater proportion of “out with consensus” results in the UK scheme may be variable fluid transfer by some participants in the reconstitution and recalcification of the lyophilized samples.18 In the Netherlands scheme, participants were requested to use an accurate pipette for adding calcium chloride solution.

In conclusion, we have developed a procedure for the preparation and provision of control blood for external quality assessment of the INR with two types of POCT devices: the CoaguChek XS and ProTime Microcoagulation System. The results of external quality assessment with the control blood demonstrate improved interlaboratory precision for the CoaguChek XS and variable performance for the ProTime Microcoagulation System. Our system is useful only to professional EQAS participants because accurate pipetting of calcium chloride is required.
This study was supported by funds provided by the Netherlands Federation of Thrombosis Services.

References


