

# European Concerted Action on Anticoagulation. Evaluation of a Method for International Sensitivity Index Calibration of Two Point-of-Care Prothrombin Time (PT) Monitoring Systems (CoaguChek Mini and TAS PT-NC) with Fresh Plasmas Based on Whole-Blood Equivalent PT

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**Background:** The International Sensitivity Index (ISI) calibration of whole-blood prothrombin time (PT) monitors for point-of-care testing (POCT) described by Tripodi et al. (*Thromb Haemost* 1993;70:921–4) has been shown to be dependable but is too complex and demanding. The use of plasma would simplify calibration of whole-blood POCT PT monitors, but important differences may exist between the ISI for whole blood and plasma calibrations.

**Methods:** In a 10-center calibration study of two POCT whole-blood monitoring systems (CoaguChek Mini and TAS PT-NC), we characterized the relationship between the log PT for whole blood and fresh plasma with use of single lots of test strips/cards. This relationship (linear) was used to correct the difference between the whole-blood and plasma ISI. The reliability of the correction with different lots of test strips/cards was assessed at

three centers. The linear relationship was used to correct the difference in the whole-blood and plasma ISI with four other lots of TAS PT-NC cards and with two additional lots of CoaguChek Mini test strips.

**Results:** The correction decreased the ISI difference from 13.3% to 0.9% for the TAS PT-NC and from 5.7% to 0.6% for the CoaguChek Mini. In assessments at three centers, which included different lots of test strips/cards, the mean ISI difference was markedly decreased with the TAS PT-NC but not with the CoaguChek Mini, for which the mean ISI difference increased slightly.

**Conclusions:** The proposed correction resolves the discrepancy between whole-blood and fresh plasma ISI calibrations with TAS PT-NC test cards. The CoaguChek Mini systems could be calibrated without this correction.

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To conform to the WHO prothrombin time (PT)<sup>6</sup> standardization scheme for safe and effective warfarin administration, whole-blood PT monitors for point-of-care testing (POCT) need to be calibrated in terms of their International Sensitivity Index (ISI) (1, 2). For conventional PT testing, the ISI is obtained from a comparison of the PT results on the test system with the results on the

<sup>6</sup> Nonstandard abbreviations: PT, prothrombin time; POCT, point-of-care testing; ISI, International Sensitivity Index; IRP, International Reference Preparation; ECAA, European Concerted Action on Anticoagulation; INR, International Normalized Ratio; and CI, confidence interval.

same blood specimens obtained with the manual method using the relevant International Reference Preparation (IRP) for thromboplastin. The results are plotted on the log scale, and the orthogonal regression line is determined. The slope of this line provides the ISI. Unfortunately, this procedure cannot be applied to whole-blood POCT monitors, and a method for such ISI calibration was developed by Tripodi et al. (3). The method depends on the comparison, by orthogonal regression analysis, of PT results for whole-blood samples from 60 coumarin-treated patients and 20 healthy individuals obtained with the whole-blood PT monitor and results from manual PT tests on plasmas. Plasmas are from the same samples and are tested using the species-specific thromboplastin IRP.

The ISI calibration of whole-blood POCT PT monitors would be simplified if the same plasma samples could also be tested on the whole-blood monitors without the need for whole-blood tests. A recent European Concerted Action on Anticoagulation (ECAA) multicenter study (4) demonstrated that the ISI of one of the two POCT systems investigated (CoaguChek Mini) could be determined with reasonable reliability with use of plasma samples. With the second POCT system (TAS PT-NC), there was, however, a considerable difference between the ISI derived from whole blood and fresh plasma samples.

In an attempt to resolve this problem, the present study investigates the relationship between PT results obtained with the two types of monitoring systems for plasma and whole-blood samples from the same patients on oral anticoagulant treatment and from healthy individuals.

In the present study, we examined the possibility of using a correction derived from the resulting relationship to devise a simpler ISI calibration method based on plasma samples on the same two types of whole-blood monitors. To test whether the same correction could be applied when different lots of test strips/cards containing thromboplastin were used with the monitors, an additional three-center calibration was undertaken with different production lots of test strips/cards on the two monitoring systems.

## Materials and Methods

### INSTRUMENTS

For this study, a monitoring system is defined as a brand of instrument with a single numbered lot of a specific type of test strips/cards. Two different test systems, incorporating CoaguChek and TAS POCT monitors with their respective test strips/cards, were provided to each of the 10 ECAA centers by Roche Diagnostics (Mannheim, Germany) and Bayer AG (Leverkusen, Germany), respectively.

Three different numbered lots of "Mini" test strips (lot nos. 079, 164, and 175) incorporating rabbit thromboplastin were used with the CoaguChek monitors. Lot 164 was used in the 10-center study, whereas lots 079 and 175 were included with lot 164 in the additional three-center study.

The TAS has recently been redesignated the Rapid

PointCoag, but in this study the established name of TAS has been retained. The "PT-NC" test cards used with the TAS monitors in this study contained human placental thromboplastin. Five different lots of PT-NC test cards (lot nos. 307060002, 307020001, 307030003, 3050099801, and 975019902) were used. For ease of reference, these will be referred to as lots 1–5, respectively, in this report. Lot 1 was used in the 10-center study, and the four other lots (lots 2–5) were used in additional calibrations. Lots 2 and 3 were tested at three centers, lot 4 at two centers, and lot 5 at one center.

Both the CoaguChek Mini and TAS PT-NC systems are fully described in previous ECAA reports (5, 6).

### DISPLAYED SECONDS

For many years until the 1990s, most thromboplastins in North America had high ISI (>2.0), and laboratories were accustomed to a short PT for both patients and healthy individuals. The PT results displayed on the CoaguChek Mini and the TAS PT-NC are derived by conversion of the actual PT result (in seconds) to the PT that would have been obtained with a typical traditional North American thromboplastin. However, to perform a reliable ISI calibration, according to WHO recommendations, actual PT values are required (1, 2). Therefore, a master code chip to replace the conventional code chip was provided by the manufacturer of the CoaguChek Mini system to display actual PT values. In the case of the TAS PT-NC, a specific correction formula was supplied to us by the manufacturer to convert the monitor-displayed PT to actual PT. These corrections were applied at the ECAA Central Facility before the ISI calculations.

### IRP FOR THROMBOPLASTIN

Calibrations of the CoaguChek Mini (lot 164) and TAS PT-NC (lot 1) were performed in a 10-center exercise using the WHO human recombinant IRP (rTF/95) (7) and the WHO rabbit plain IRP (RBT/90) (8). These were provided for the study by WHO for species-specific calibrations because the CoaguChek Mini and TAS PT-NC use rabbit- and human-based thromboplastin, respectively. RBT/90 was used in the present study with 25 mmol/L calcium chloride, prepared and provided by the ECAA Central Facility. The rTF/95 reagent contains the requisite concentration of calcium chloride.

### ECAA RABBIT REFERENCE THROMBOPLASTIN

Additional calibrations of the CoaguChek Mini with numbered lots (164, 079, and 175) of test strips and of TAS PT-NC with two different lots of test cards (lots 2 and 3) were performed at three centers (Leiden, Manchester, and Milan). Calibrations of lot 4 of the TAS PT-NC test cards were performed at two of these centers, and lot 5 was calibrated at one of these centers only.

These calibrations incorporated the ECAA rabbit reference thromboplastin with 25 mmol/L calcium chloride prepared individually at each center. The ISI of the ECAA

rabbit thromboplastin had been determined in a previous collaborative study against the WHO rabbit IRP (RBT/90) (9).

#### TRAINING WORKSHOP

Scientific assistants from the 10 ECAA national centers attended a training workshop for familiarization with the methodology of the two types of monitor and to attempt to standardize the manual tilt-tube technique.

#### QUALITY-CONTROL EXERCISE

A preliminary exercise consisted of the testing of five abnormal lyophilized quality-control plasmas issued from the Central Facility. The purpose was to detect any monitoring system or operator consistently providing outlying results. All centers returned satisfactory results.

#### ISI CALIBRATION PROCEDURE

The 10 centers undertook whole-blood ISI calibrations as designed for POCT monitors by Tripodi and coworkers (3, 10). Noncitratd whole blood from 20 healthy individuals and 60 patients stabilized on long-term oral anticoagulant therapy was tested with both the CoaguChek Mini (lot 164) and the TAS PT-NC (lot 1) systems. PT testing was then performed on the monitors with citrated plasma from the same blood samples. The citrated plasmas from these same blood samples were then tested with the conventional manual PT test with both WHO IRPs (human and rabbit) for thromboplastin in a given sequence provided to all centers (4).

Three centers (Leiden, Manchester, and Milan) performed additional calibrations, testing blood samples from individuals other than those studied in the 10-center calibration exercise. Citrated plasmas from these same blood samples were then tested in parallel with the conventional manual PT method and ECAA rabbit reference thromboplastin. Blood samples were collected from 20 healthy donors and 60 long-term patients stabilized on oral anticoagulants within the therapeutic International Normalized Ratio interval (INR, 1.5–4.5). At one of the three centers, it was possible to collect blood samples from only 40 patients and 15 healthy donors with two lots of CoaguChek Mini test strips and two lots of TAS PT-NC test cards.

#### COLLECTION AND TESTING OF NONCITRATED WHOLE BLOOD ON THE MONITORS

In both the 10-center and the additional calibration exercise, noncitratd whole blood was collected by venipuncture using a needle and plastic syringe without citrate and was applied with minimum delay (not >15 s from venipuncture) to the test strips/cards in the monitors. All volunteers gave informed consent.

#### BLOOD COLLECTION FOR CITRATED PLASMA SAMPLES

After a sample was taken for testing on the POCT systems, the remainder of the whole blood sample was

drawn from the syringe into a Vacutainer containing 105 mmol/L sodium citrate or with a Monovette system containing 106 mmol/L citrate (the difference in citrate concentration between these systems is negligible). Samples were then centrifuged to obtain citrated plasma for testing in the monitoring systems and the manual PT test with the relevant reference thromboplastin. All tests were performed within 6 h of blood collection. Further details are provided in previous ECAA reports (4, 6)

#### TESTING OF CITRATED PLASMA ON THE MONITORS

Citrated plasma (0.1 mL) was transferred to a plastic tube, and 0.1 mL of 17 mmol/L calcium chloride was then added to the tube and mixed. The recalcified plasma was applied within 15 s to the test strip/cards, and the PT was recorded from the monitor display screens. Single tests were performed on the monitoring systems with all plasma samples. The 17 mmol/L calcium chloride was prepared and provided to all centers by the ECAA Central Facility.

In the additional calibration exercise, 0.1 mL of citrated plasma was recalcified with 0.1 mL of 16.3 mmol/L calcium chloride for testing on these monitoring systems. The optimum range of 16.3–17 mmol/L for recalcification of citrated plasma on these POCT systems was established previously (6).

#### STATISTICAL ANALYSIS

Results were analyzed at the ECAA Central Facility.

#### RELATIONSHIP BETWEEN PT OF WHOLE-BLOOD AND PLASMA SAMPLES

The relationship between the PT of whole-blood and plasma samples tested on the two POCT systems (TAS PT-NC lot 1 and CoaguChek Mini lot 164) was examined by plotting the logarithms of PT (log PT) for whole-blood samples against those for plasma from the same donations at all 10 centers. To comply with the WHO guidelines for ISI calibration (2), log PT results were analyzed and patients' samples with an INR outside the range 1.5–4.5 with the species-specific IRP were excluded. A line (hereafter called a "line of equivalence") was derived from the plot of log PT for whole-blood samples against log PT for plasma by use of orthogonal regression analysis and is described by the following equation:

$$\log(\text{whole-blood PT}) = \text{intercept} + \text{slope} \times \log(\text{plasma PT})$$

The SD of log PT on the monitors was estimated [defined as "residual SD" in the revised WHO guidelines (2)]. Samples were then excluded if the perpendicular distance from the line of equivalence was greater than three times the residual SD. The final line of equivalence was then derived from the remaining data. The number of samples excluded is reported in the *Results*.

To investigate whether the linear relationship was constant at all the centers, straight lines were also derived using the results from the 10 individual centers.

**WHOLE-BLOOD CALIBRATIONS**

The ISI for whole blood with the two monitoring systems was derived as detailed previously (5), according to the conventional WHO orthogonal regression procedure (1, 2). The ISI derivation procedure is described in the Appendix.

**PLASMA CALIBRATIONS**

PT results for the plasma samples tested on both monitors at each center were corrected by use of the appropriate line of equivalence (derived from samples tested at all 10 centers), which describes the relationship between whole-blood and plasma PT. An example of this correction is given in the Appendix. The resulting "corrected PT" values were then used to calibrate the POCT systems according to the conventional WHO procedure (1, 2), as described in the Appendix.

The mean ISI for the whole-blood and citrated plasma calibrations were compared by use of paired *t*-tests and 95% confidence intervals (CIs).

To allow a comparison, calibrations were also performed with the "uncorrected PT" results for the plasma samples (i.e., plasma PT without the transformation using the line of equivalence).

A flowchart describing the ISI calibration procedure with whole blood and plasma is given in Fig. 1.

**DIFFERENT LOTS OF TEST STRIPS/CARDS**

To investigate the applicability of the line of equivalence to different lots of test strips/cards, plasma PT results from the additional calibration exercise were transformed to corrected PT by use of the line of equivalence derived from results from the 10-center calibration exercise. The resulting ISI from calibrations with uncorrected and corrected plasma PT were compared with those for whole blood.

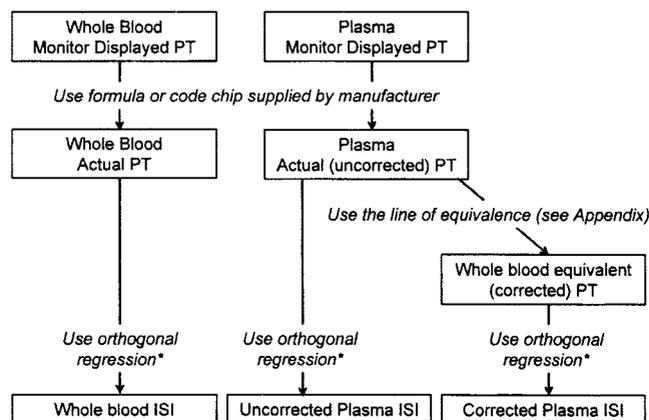


Fig. 1. Flowchart summarizing the whole-blood and plasma ISI calibrations for the CoaguChek Mini and TAS PT-NC systems.

\*, the orthogonal regression procedure uses the monitor-displayed PT and the plasma PT obtained with the manual method and the thromboplastin IRP (see Appendix).

**Results**

**LINE OF EQUIVALENCE**

Plots of log PT for whole blood and plasma on the two POCT systems are shown in Fig. 2. An overall straight-line relationship was observed with both systems for results from the 10 centers. The lines of equivalence for individual centers (Fig. 3) indicated that the relationship between whole-blood and plasma PT was relatively constant at the 10 centers for both systems.

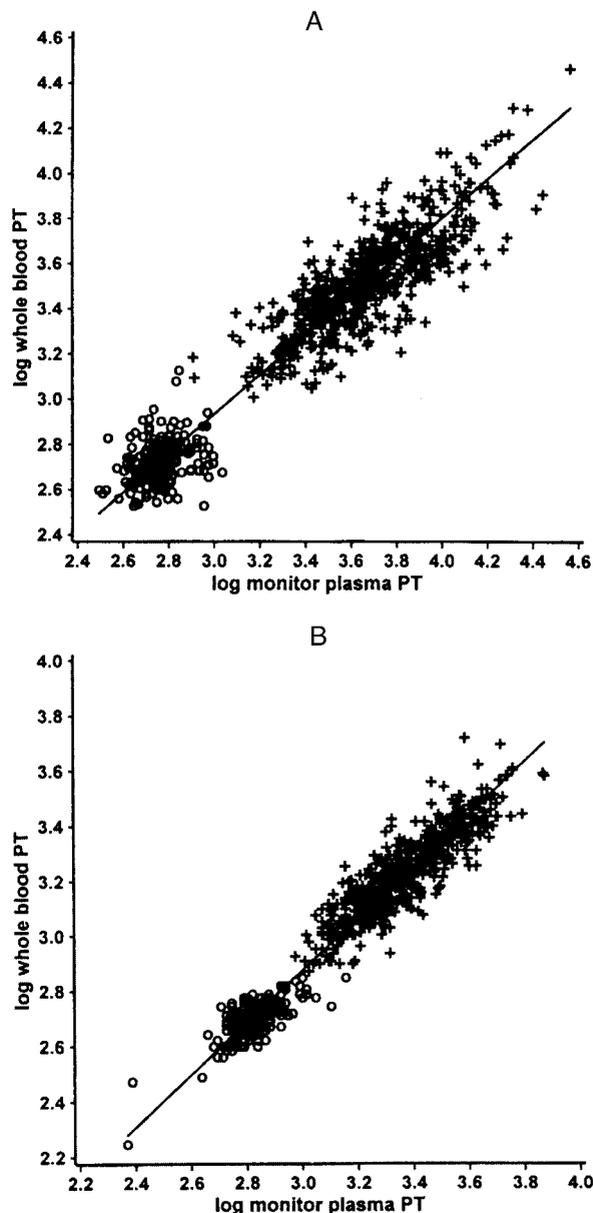


Fig. 2. Plots of log PT for whole-blood samples against log PT for plasma samples tested at the 10 centers with the TAS PT-NC system (A) and the CoaguChek Mini system (B).

+, patient sample; O, sample from healthy individual. The solid line, derived by orthogonal regression, is referred to as the line of equivalence.

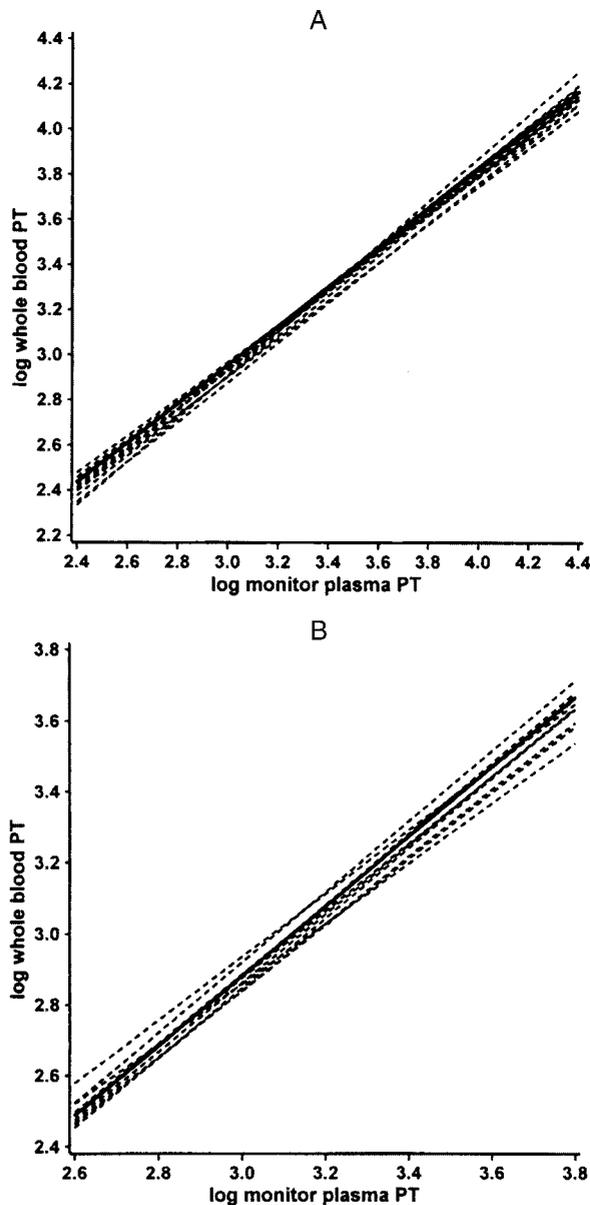


Fig. 3. Lines of equivalence relating log PT for whole-blood and plasma samples tested at individual centers with the TAS PT-NC system (A) and the CoaguChek Mini system (B).

The *dashed lines* are the lines derived from the results at individual centers, and the *solid line* is the line of equivalence derived from the results obtained at all 10 centers (as given in Fig. 2).

#### TAS PT-NC

Blood samples from 33 of the 600 patients tested at the 10 centers with INRs >4.5 and <1.5 with the same-species IRP were excluded according to WHO guidelines (2). In accordance with the WHO guidelines on eliminating outliers, samples from seven patients and one healthy individual were excluded because the perpendicular distance from the line of equivalence was greater than three times the residual SD. The line of equivalence derived from the remaining 560 results is shown in Fig. 2A and is described by the following equation:

$$\log(\text{whole-blood PT}) = 0.3383 + 0.8651 \times \log(\text{plasma PT})$$

where 0.3383 is the intercept and 0.8651 is the slope.

A plot of the lines derived using results from the individual centers (see Fig. 3A) showed that there was good agreement with the line of equivalence derived from the results obtained at all 10 centers.

*Ten-center calibration exercise.* The line of equivalence derived from the results at all 10 centers was then used to calculate corrected PT for the plasma samples tested on the monitors at each of the centers with lot 1 of the test cards.

Shown in Table 1 are the ISI calibration results with whole blood and with the uncorrected and corrected PT for the plasma samples on the TAS PT-NC system at each of the 10 centers. The results in Table 1 for calibrations with whole blood and uncorrected PT for plasma have been presented in an earlier report (4).

The mean whole-blood ISI of 1.13 (range, 1.05–1.29) was not significantly different from the mean ISI of 1.14 (range, 1.06–1.30; 0.9% difference) obtained with the corrected PT for plasma samples (CI for mean ISI difference, –0.02 to 0.03; paired *t*-test, *P* = 0.5). With the uncorrected plasma PT, the mean ISI of 0.98 (range, 0.92–1.12) was significantly lower than the mean ISI of 1.13 for whole blood (13.3% difference; CI for mean ISI difference, 0.12–0.18; paired *t*-test, *P* < 0.0001). The between-center CV of the ISI at the 10 centers was lower for plasmas than for whole blood.

**Table 1. TAS PT-NC (lot 1) and rTF/95: ISI and CV of calibration slope [CV(b)] for whole-blood and plasma calibrations.**

| Center                 | Whole blood |      | Plasma                |                              |                            |
|------------------------|-------------|------|-----------------------|------------------------------|----------------------------|
|                        | CV(b), %    | ISI  | CV(b), <sup>a</sup> % | Uncorrected ISI <sup>b</sup> | Corrected ISI <sup>c</sup> |
| 1                      | 4.4         | 1.07 | 3.2                   | 0.97                         | 1.13                       |
| 2                      | 2.6         | 1.29 | 1.6                   | 1.12                         | 1.30 <sup>d</sup>          |
| 3                      | 3.4         | 1.07 | 2.6                   | 0.96                         | 1.12                       |
| 4                      | 3.2         | 1.16 | 2.5                   | 0.97                         | 1.12                       |
| 5                      | 2.7         | 1.26 | 2.3                   | 1.06                         | 1.23 <sup>d</sup>          |
| 6                      | 3.6         | 1.17 | 5.4                   | 0.96                         | 1.12                       |
| 7                      | 3.1         | 1.05 | 2.8                   | 0.94                         | 1.09                       |
| 8                      | 3.8         | 1.06 | 2.5                   | 0.92                         | 1.06                       |
| 9                      | 3.8         | 1.11 | 3.8                   | 0.97                         | 1.13                       |
| 10                     | 4.7         | 1.08 | 4.0                   | 0.93                         | 1.08                       |
| Overall <sup>e</sup>   | 3.5         | 1.13 | 3.1                   | 0.98                         | 1.14                       |
| ISI CV, <sup>f</sup> % |             | 7.6  |                       | 6.6                          | 6.4                        |

<sup>a</sup> CV(b) was identical for both uncorrected and corrected plasma ISI calibrations.

<sup>b</sup> ISI derived from the uncorrected plasma PT.

<sup>c</sup> Derived by use of the line of equivalence.

<sup>d</sup> Outlying result.

<sup>e</sup> Mean CV(b) and ISI at 10 centers.

<sup>f</sup> Between-center CV of the ISI.

At 9 of the 10 centers, there was better precision (CVs of the plasma calibration slopes were lower) than for whole blood, with mean slope CVs of 3.1% and 3.5% for plasma and whole-blood calibrations, respectively.

At two centers (centers 2 and 5), plasma calibration ISI values were detected as outlying by use of the algorithm described by van den Besselaar (8). None of the whole-blood calibration ISI were outliers.

When calibration results for the two centers with outlying ISI values were excluded, the mean ISI for whole-blood calibrations (1.10) was not significantly different from the mean ISI obtained with corrected PT for the plasma samples (1.11; CI for mean ISI difference, -0.02 to 0.04; paired *t*-test, *P* = 0.4). The mean ISI with whole blood remained significantly higher than the mean ISI with uncorrected PT (0.95; CI for mean ISI difference, 0.11–0.18; paired *t*-test, *P* < 0.0001).

*Additional three-center calibrations.* Shown in Table 2 are the results for the additional calibration exercise with the four different lots of test cards (lots 2–5). In each instance, the difference between the whole-blood and plasma ISI was greatly reduced by correction of plasma PT from the line of equivalence.

The mean ISI values derived with uncorrected plasma PT (mean ISI, 1.01–1.04) were markedly lower than the mean whole-blood ISI values (mean ISI, 1.15–1.21) for lots 2–4. Plasma PT, corrected by use of the line of equivalence derived from TAS PT-NC lot 1, gave a mean ISI for lots 2–4 (mean ISI, 1.17–1.21) consistent with whole-blood results. With lot 5, the difference between the whole-

blood ISI and the plasma ISI was considerably reduced after correction with the line of equivalence.

#### CoaguChek MINI SYSTEM

Of the blood samples collected from the 600 patients at the 10 centers, 57 samples that had an INR outside the range 1.5–4.5 with the RBT/90 species-specific IRP were excluded according to WHO guidelines (1, 2). Of the 200 samples from healthy donors tested at the 10 centers, 194 samples gave PT results when tested as both whole blood and plasma on the monitor. Nine other samples from patients and three from healthy donors were excluded because the perpendicular distance from the line of equivalence was greater than three times the residual SD.

For the remaining samples, a plot of log PT for whole blood against log PT for plasma from the same donations is given in Fig. 2B. The resulting line of equivalence is described by the following equation:

$$\log(\text{whole-blood PT}) = 0.05223 + 0.9405 \times \log(\text{plasma PT})$$

There was, as in the case of the TAS PT-NC system, good agreement between the lines derived from the results from the 10 individual centers (see Fig. 3B), and again the relationship appeared relatively constant between centers.

*Ten-center calibration exercise.* Shown in Table 3 are the results of the ISI calibration of the CoaguChek Mini system with whole-blood samples and with both corrected and uncorrected PT for the plasma samples at each center with lot 164 test strips. The results in Table 3 for

**Table 2. TAS PT-NC (lots 2–5) and ECAA rabbit: ISI and CV of calibration slope [CV(b)] for whole-blood and plasma calibrations.**

| Lot no. | Center                 | Whole blood |      | Plasma                |                              |                            |
|---------|------------------------|-------------|------|-----------------------|------------------------------|----------------------------|
|         |                        | CV(b), %    | ISI  | CV(b), <sup>a</sup> % | Uncorrected ISI <sup>b</sup> | Corrected ISI <sup>c</sup> |
| 2       | A                      | 4.3         | 1.29 | 3.2                   | 1.02                         | 1.18                       |
|         | B <sup>d</sup>         | 5.8         | 1.16 | 3.6                   | 1.01                         | 1.17                       |
|         | C                      | 4.7         | 1.18 | 3.3                   | 0.99                         | 1.15                       |
|         | Overall <sup>e</sup>   | 4.9         | 1.21 | 3.4                   | 1.01                         | 1.17                       |
| 3       | A                      | 3.9         | 1.24 | 2.7                   | 1.11                         | 1.29                       |
|         | B <sup>d</sup>         | 5.3         | 1.18 | 4.0                   | 0.97                         | 1.13                       |
|         | C                      | 3.5         | 1.21 | 3.4                   | 1.04                         | 1.21                       |
|         | Overall <sup>e</sup>   | 4.2         | 1.21 | 3.4                   | 1.04                         | 1.21                       |
| 4       | A                      | 2.8         | 1.24 | 2.1                   | 1.06                         | 1.22                       |
|         | B                      | 3.9         | 1.06 | 3.2                   | 0.96                         | 1.12                       |
|         | Overall <sup>e,f</sup> | 3.4         | 1.15 | 2.7                   | 1.01                         | 1.17                       |
| 5       | C                      | 4.7         | 1.20 | 3.2                   | 1.01                         | 1.17                       |

<sup>a</sup> CV(b) was identical for both uncorrected and corrected plasma ISI calibrations.

<sup>b</sup> ISI derived from the uncorrected plasma PT.

<sup>c</sup> Derived by use of the line of equivalence.

<sup>d</sup> Calibrations based on results from 40 patients and 15 healthy individuals.

<sup>e</sup> Mean CV(b) and ISI at the centers.

<sup>f</sup> Mean of results from two centers (A and B) only.

**Table 3. CoaguChek Mini (lot 164) and RBT/90: ISI and CV of calibration slope [CV(b)] for whole-blood and plasma calibrations.**

| Center                 | Whole blood      |      | Plasma                |                              |                            |
|------------------------|------------------|------|-----------------------|------------------------------|----------------------------|
|                        | CV(b), %         | ISI  | CV(b), <sup>a</sup> % | Uncorrected ISI <sup>b</sup> | Corrected ISI <sup>c</sup> |
| 1                      | 4.6              | 1.63 | 4.0                   | 1.63                         | 1.74                       |
| 2                      | 3.4              | 2.10 | 2.5                   | 1.86                         | 1.98                       |
| 3                      | 3.2              | 1.70 | 3.1                   | 1.70                         | 1.81                       |
| 4                      | 3.0 <sup>d</sup> | 1.89 | 3.5                   | 1.79                         | 1.91                       |
| 5                      | 1.9              | 1.78 | 1.7                   | 1.66                         | 1.76                       |
| 6                      | 4.7              | 1.52 | 4.1                   | 1.44                         | 1.54                       |
| 7                      | 2.8              | 1.67 | 2.7                   | 1.55                         | 1.65                       |
| 8                      | 2.6              | 1.76 | 2.8                   | 1.72                         | 1.83                       |
| 9                      | 3.6              | 1.92 | 5.1                   | 1.67                         | 1.78                       |
| 10                     | 3.3              | 1.57 | 4.4                   | 1.50                         | 1.61                       |
| Overall <sup>e</sup>   | 3.3              | 1.75 | 3.4                   | 1.65                         | 1.76                       |
| ISI CV, <sup>f</sup> % |                  | 10   |                       | 7.8                          | 7.7                        |

<sup>a</sup> CV (b) was identical for both uncorrected and corrected plasma ISI calibrations.

<sup>b</sup> Derived from the uncorrected plasma PT.

<sup>c</sup> Derived by use of the line of equivalence.

<sup>d</sup> CV(b) is >3.0 but <3.05.

<sup>e</sup> Mean CV(b) and ISI for 10 centers.

<sup>f</sup> Between-center CV(%) of the ISI.

calibrations with whole blood and uncorrected PT for plasma have also been presented previously (4).

There was close agreement in ISI after correction of plasma PT from the line of equivalence.

The mean ISI of 1.75 (range, 1.52–2.10) for whole blood was not significantly different from the mean ISI of 1.76 (range, 1.54–1.98; 0.6% difference) obtained with the corrected PT for the plasma samples (CI for mean ISI difference, –0.05 to 0.07; paired *t*-test, *P* = 0.8). With the uncorrected plasma PT, the mean ISI of 1.65 (range, 1.44–1.86) was significantly lower than the mean ISI for whole blood (5.7% difference; CI for mean ISI difference, 0.04–0.16; paired *t*-test, *P* = 0.006). The between-center variation of ISI was lower with plasma calibrations than with the whole-blood calibrations.

With whole blood, the CV of the calibration slopes ranged from 1.9% to 4.7% (mean, 3.3%), and 3 of the 10 centers had a CV of the slope within the 3% recommended limit (2), although all were <5%. With plasma, the CV of the calibration slopes ranged from 1.7% to 5.1% (mean, 3.4%), and 4 of the 10 centers had a CV of the slope within the 3% limit; the CV for 1 center, however, was slightly >5%. None of the ISI were deemed to be outliers.

*Additional three-center calibrations.* The results for the additional calibration exercises are shown in Table 4. The original lot of test strips used in the 10-center exercise to derive the line of equivalence (lot 164) was included in the 3-center calibration with blood samples from different patients and healthy individuals. The mean ISI for whole

**Table 4. CoaguChek Mini (lots 164, 079, and 175) and ECAA rabbit: ISI and CV of calibration slope [CV(b)] for whole-blood and plasma calibrations.**

| Lot no. | Center               | Whole blood      |      | Plasma                |                              |                            |
|---------|----------------------|------------------|------|-----------------------|------------------------------|----------------------------|
|         |                      | CV(b), %         | ISI  | CV(b), <sup>a</sup> % | Uncorrected ISI <sup>b</sup> | Corrected ISI <sup>c</sup> |
| 164     | A                    | 2.7              | 1.85 | 2.3                   | 1.74                         | 1.85                       |
|         | B <sup>d</sup>       | 3.3              | 1.75 | 3.8                   | 1.71                         | 1.82                       |
|         | C                    | 3.2              | 1.61 | 3.5                   | 1.57                         | 1.68                       |
|         | Overall <sup>e</sup> | 3.0              | 1.74 | 3.2                   | 1.67                         | 1.78                       |
| 079     | A                    | 2.1              | 1.62 | 1.9                   | 1.56                         | 1.66                       |
|         | B                    | 3.2              | 1.50 | 3.2                   | 1.58                         | 1.69                       |
|         | C                    | 2.9              | 1.42 | 2.9                   | 1.32                         | 1.41                       |
|         | Overall <sup>e</sup> | 2.8              | 1.51 | 2.7                   | 1.49                         | 1.59                       |
| 175     | A                    | 2.5              | 1.76 | 2.1                   | 1.72                         | 1.83                       |
|         | B <sup>d</sup>       | 3.2              | 1.74 | 4.0                   | 1.71                         | 1.83                       |
|         | C                    | 3.0 <sup>f</sup> | 1.69 | 2.8                   | 1.63                         | 1.73                       |
|         | Overall <sup>e</sup> | 2.9              | 1.73 | 2.9                   | 1.69                         | 1.80                       |

<sup>a</sup> CV(b) was identical for both uncorrected and corrected plasma ISI calibrations.

<sup>b</sup> Derived from uncorrected plasma PT.

<sup>c</sup> Derived by use of line of equivalence.

<sup>d</sup> Calibrations based on results from 40 patients and 15 healthy individuals.

<sup>e</sup> Mean CV(b) and ISI at the centers.

<sup>f</sup> CV(b) is between 2.95 and 3.00.

blood was 1.74. Corrected plasma PT gave a mean ISI of 1.78, which was, as in the 10-center study, closer to the whole-blood mean ISI than the mean ISI (1.67) for uncorrected plasma PT.

With two other lots of test strips (lots 079 and 175), the correction from the line of equivalence derived from lot 164 in the 10-center calibration exercise gave slightly poorer agreement than the uncorrected plasma PT.

The absolute differences between the mean whole-blood and plasma ISI before correction were 1.3% and 2.3% for lots 079 and 175, respectively, and increased to 5.3% and 4.0% after correction.

## Discussion

The method for calibration of the POCT PT monitors derived from whole-blood PT (3), which was validated by the previous ECAA multicenter study (5), has provided the ISI reference values to assess the simplified calibration procedure in the present study. This study shows that both the CoaguChek Mini and TAS PT-NC test systems can be calibrated reliably with fresh plasmas provided that an appropriate procedure is used. In the previous ECAA report on the 10-center calibration of the two monitoring systems (TAS PT-NC and CoaguChek Mini), the overall differences in ISI between plasma and whole-blood calibrations were, however, considerable for the TAS PT-NC system (13.3%), but much lower for the CoaguChek Mini system (5.7%) (4).

The present study demonstrates that a linear relationship exists between log PT for whole blood and plasma in both monitoring systems and that this was similar at the 10 centers. The good agreement between the overall lines of equivalence and those for the individual centers suggests that this may be a constant relationship irrespective of test samples and between-center differences. After the plasma PT values obtained with both test systems were corrected by use of this line, the differences in ISI between plasma and whole blood were considerably lower (0.9% for the TAS PT-NC and 0.6% for the CoaguChek Mini).

We had to establish, however, that this correction was applicable to all lots of test strips/cards on the two monitoring systems. The reliability of the line of equivalence to compensate for differences between whole-blood and plasma PT was therefore tested with different lots of strips/cards on both test systems in additional three-center calibrations.

With the TAS PT-NC test cards, the line of equivalence again gave good agreement between the whole-blood- and plasma-derived ISI for all four additional lots of test cards. With the CoaguChek Mini system, however, use of the line of equivalence failed to produce an improvement with two additional lots of test strips. This evidence of interlot differences in the relationship of plasma and whole-blood PT with the CoaguChek Mini test strips therefore appears to invalidate the use of the established line of equivalence with this test system.

The line of equivalence can therefore be used with confidence with the TAS PT-NC system, which was the system in which the plasma calibration was in need of correction to achieve results comparable to the whole-blood ISI. Fortunately, this correction is not required with the CoaguChek Mini system, which, unlike the TAS PT-NC, gives a comparable ISI with plasma and whole blood. The line of equivalence therefore appears to provide a solution to the problems of plasma calibration with the TAS PT-NC.

It is anticipated that calibration of the monitors should be the responsibility of the manufacturers. The original method described by Tripodi et al. (3) should be performed with a master lot of test strips/cards when a new whole-blood POCT PT monitoring system is introduced to the market, but this needs to be performed on a multicenter basis (Poller et al., manuscript in preparation). Subsequently, when a new batch of test strips/cards is marketed, the simplified calibration may be performed with the full number of plasma samples (60 abnormal and 20 normal) used in a conventional ISI calibration (11). The line of equivalence described in this report needs to be used for such calibrations with the TAS PT-NC system but not with the CoaguChek Mini. These calibrations, using plasma samples, could also be performed on individual monitors for which quality-control tests performed by users indicate that the INR results are significantly different from reference values.

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## Appendix

### ISI CALIBRATIONS

The ISI value for whole blood and plasma with the two monitoring systems were derived according to the conventional WHO orthogonal regression methodology (1, 2). Individual patient results were excluded if the INR with the species-specific IRP was outside the range 1.5–4.5. Individual log PT values for healthy individuals and for coumarin-treated patients obtained with the IRP were plotted, as recommended, on the vertical axis against the log PT obtained from the monitoring system on the horizontal axis (1, 2). The slope of the calibration line was calculated by orthogonal regression analysis. The SD of log PT on the monitors and with the relevant IRP was estimated (defined as residual SD in the revised WHO guidelines) (2). Additional samples were excluded if the perpendicular distance from the orthogonal regression line exceeded three times the residual SD. The final orthogonal regression line was determined from the remaining data.

The precision of the slope of the calibration line ( $b$ ) was measured by its CV (%):

$$CV(b) = SE(b)/b \times 100\%$$

where  $SE(b)$  is the standard error of  $b$ .

$ISI = b \times ISI_{ref}$ , where  $ISI_{ref}$  is the ISI of the IRP. Between-center ISI variation was measured by use of the CV (%). Outlying ISI were detected by use of an algorithm described and used by van den Besselaar (8) in the calibration of the WHO rabbit plain IRP (RBT/90) and also by Tripodi et al. (7) in their calibration of the WHO human plain IRP (rTF/95).

## CORRECTION OF PLASMA PT

An example is given to show how to correct a plasma PT of 25 s on the TAS PT-NC system.

With the TAS PT-NC system, the line of equivalence is described by the equation:

$$\log(\text{whole-blood PT}) = 0.3383 + 0.8651 \times \log(\text{plasma PT})$$

The log of the whole-blood equivalent PT is first calculated using the line of equivalence and the uncorrected PT for plasma:

$$\log(\text{whole blood PT}) = 0.3383 + 0.8651 \times \log(25) = 3.12$$

Finally, the whole-blood equivalent PT (i.e., the corrected PT) is calculated by taking the exponential of 3.12:

$$\text{Whole-blood PT} = \exp(3.12) = 22.6 \text{ s}$$

For the CoaguChek Mini system, the correction procedure is the same, except that the different line of equivalence for this monitoring system would be used.

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