Multicentre ISI assignment and calibration of the INR measuring range of a new point-of-care system designed for home monitoring of oral anticoagulation therapy

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Summary
The new CoaguChek XS system is designed for use in patient self-testing. It is the successor of the current CoaguChek S system (1) which is used by more than 150,000 patients worldwide in self-monitoring of oral anticoagulation therapy (OAT).

To conform to the World Health Organisation (WHO) prothrombin time (PT) standardization scheme for safe and effective warfarin administration, whole-blood PT monitors for point-of-care (POC) testing need to be calibrated in terms of their International Sensitivity Index (ISI) (2). The WHO procedure for plasma-based methods was adapted to whole-blood monitors by Tripodi et al. (3), and depends on the comparison of ln(PT) results for whole-blood samples from 60 patients on OAT and 20 healthy normal donors obtained with the whole-blood PT monitor with results from manual PT tests on corresponding plasmas using international reference preparations for thromboplastin (IRP). This procedure was used in multiple studies to either assign the ISI or check the validity of the International Normalized Ratio (INR) results of the current CoaguChek S system (4–8).

The CoaguChek XS system contains recombinant human thromboplastin, and for calibration of this system the IRP for human thromboplastin (rTF/95) is required. It was decided to include the European Reference Material for rabbit thromboplastin against the human recombinant reference thromboplastin rTF/95 at each site using the samples from stabilized patients in the International Normalized Ratio (INR) range between 1.5 and 4.5 only. The new point-of-care system’s measuring range between 0.8 and 8 INR was calibrated against the mean INR of rTF/95 and AD149 using polynomial regression. ISIs were (CV of the slope): Site 1: ISI 0.99 (1.1%); Site 2: ISI 1.02 (2.0%); Site 3: ISI 1.03 (1.1%); Site 4: ISI 1.00 (1.4%). All regression lines calculated from patient-only data pass through the normal donor data points. All CVs of the slopes of the orthogonal regression lines are well below 3%, thus fulfilling the requirements of the WHO guidelines. The mean ISI for the new CoaguChek XS PT Test is 1.01.

Keywords
INR calibration, ISI assignment, point-of-care testing, patient self-testing, CoaguChek

Introduction
The new CoaguChek XS system is designed for use in patient self-testing. It is the successor of the current CoaguChek S system (1) which is used by more than 150,000 patients worldwide in self-monitoring of oral anticoagulation therapy (OAT).

This study was sponsored by Roche Diagnostics which is the manufacturer of the system. Financial support was given to the institutions participating in the studies.

Footnote:
Ingrid Leichsenring, Winfried Plesch, and Volker Unkrig are employees of Roche Diagnostics GmbH, Mannheim, Germany. COAGUCHEK is a trademark of Roche.

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tin (AD149) as well, because previous studies indicate that there are differences in INR between human and rabbit reference preparations (6, 9), and the new CoaguChek XS system will be applied in clinical centres using either human or rabbit thromboplastins for laboratory control of oral anticoagulation. Including AD149 in the present study provides an opportunity to reduce INR differences between the new CoaguChek XS system and both human and rabbit thromboplastins used in clinical practice.

The study we report here was performed to assign the ISI to the new test according to the WHO guidelines for thromboplastins and plasmas used to control anticoagulant therapy, and to calibrate the measuring range of the new system from 0.8 to 8 INR against the mean INR of rTF/95 and AD149. A master lot of test strips was calibrated to be used as the Working Reference Preparation for the manufacturer.

Materials and methods

Point-of-care system

The CoaguChek XS system consists of the CoaguChek XS monitor and the CoaguChek XS PT test strips. It quantitatively determines the PT in INR, %Quick or seconds using capillary blood from a fingertip or untreated venous whole blood. The test strip contains a human recombinant thromboplastin and a peptide substrate. The enzyme thrombin (factor IIa) cleaves the peptide substrate, generating an electrochemical signal. This signal is corrected for haemocrit, converted into INR by means of an algorithm and the result is displayed. The required lot specific information is stored on a code chip which comes with each vial of test strips. The code chip must be inserted into the CoaguChek XS monitor.

Displayed seconds

To perform a reliable ISI calibration according to WHO recommendations, actual PT values are required (2) which are referred to as instrument PT (IPT). Therefore, a master code chip to replace the conventional code chip was provided by the manufacturer of the CoaguChek XS system to display the actual PT values of the master lot test strips.

IRP for thromboplastin

Calibrations of the CoaguChek XS PT test were performed using the WHO human recombinant IRP (rTF/95) and the rabbit plain IRP (CRM149S, actually encoded ERM AD149). The latter is currently used in calibration exercises for commercial rabbit thromboplastins. Both IRPs were tested using the manual tilt tube technique as requested in the WHO guidelines (2).

Study design

The study was conducted at four study sites. Non-citrated venous whole blood was applied to the test strips of the new system. Each site performed testing on two monitors in parallel (monitor A and B). From the corresponding plasma samples PT results were obtained using the international reference preparations rTF/95 and AD149 by the manual tilt tube method.

Each center included a minimum of 22 normal donors and 62 patients on OAT, resulting in a total of 90 samples of normal donors and 291 samples of warfarin-, phenprocoumon- or acenocoumarol-treated patients. Fourteen samples of donors under oral anticoagulation had to be omitted from the analysis due to missing data, with 277 data sets of patients on OAT remaining for further analysis.

Sample collection for whole blood testing on the monitors and for plasma testing using IRPs

From a venipuncture approximately 10 ml of blood was drawn into a syringe either directly or by means of a butterfly. Blood was transferred into two 4.5 ml Vacutainer tubes (Becton-Dickinson lot 5006351; 105 mM Citrate) for reference testing. The same batch of tubes was used at all four study centers. With the remaining blood in the syringe the test strips were dosed.

The citrated blood in the Vacutainer tubes was processed to plasma in agreement with the WHO guidelines (2). The manual tilt tube method was performed with the international reference thromboplastins rTF/95 and AD149 from fresh plasmas.

Calibration of the INR scale of the CoaguChek XS system

The calibration was carried out through the INR measuring range of the system from 0.8 to 8.0 INR. Therefore the 12 data sets with INRs of the IRPs greater than 4.5 were also included in the standardization procedure. In total samples of 90 normal donors and 277 patients on OAT were evaluated. A polynomial regression was calculated to calibrate the new test versus the mean INR of the two IRPs used in this study. The INR values for the IRPs were regressed against the raw IPT seconds of the master lot PT test strips, and an equation was generated which described the relationship. This equation was used to convert the CoaguChek XS system IPT times to IRP equivalent INR values. The master strip lot, for purposes of traceability to the IRPs, is considered a Working Reference Preparation (WRP).

Statistical analysis

The ISI value of the new test was assigned species-specific against the human recombinant reference thromboplastin rTF/95 at each site according to the procedure given in the WHO guidelines. The PT in seconds of the rTF/95 was plotted against the IPT of the master lot in a logarithmic scale. Only the first determination on monitor A was used for the ISI assignment. Orthogonal regression was applied to the lnPTs of normal donors and stable patients on OAT, and the coefficient of variation (CV) of the slope was calculated. Additionally the orthogonal regression lines for patient data only were calculated, and checked if passing through the data points of the normal donors (see appendix for calculations concerning lin-
Leichsenring et al. INR calibration of a new point-of-care system

## Results

### ISI assignment

The basis of the ISI calibration model is necessarily an empirical one. While there is good evidence that the calibration relationship defined in a double-logarithmic plot of prothrombin times is usually linear, and that the same line represents data points for both patients and healthy donors, the possibility of departure from these assumptions cannot be ruled out. In Table 1 it is shown that the lines for all data (i.e. patients and healthy donors) are not identical to the lines for patient data only. In the case of marked deviation, the assignment of an ISI would not be meaningful. For practical purposes, the assignment of an ISI is acceptable if INRs calculated with the ISI derived from the overall regression line (i.e. for patients plus healthy donors) do not differ by more than 10% from INRs calculated with the equation describing the regression line for patients only. In Table 1 it is shown that the differences in INR are less than 2%. This finding is equivalent to the requirement that all regression lines calculated from patient data pass through the normal donor data points. Furthermore all CVs of the slopes of the orthogonal regression lines are below 3%, thus fulfilling the requirements of the WHO guidelines. The mean ISI for the new CoaguChek XS PT Test is 1.01.

### Table 1: ISI assignment of the CoaguChek XS PT master lot versus rTF/95.

<table>
<thead>
<tr>
<th>Site</th>
<th>ISI</th>
<th>Orthogonal regression line (all data /patient data only)</th>
<th>slope CV</th>
<th>n (normals / patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indianapolis</td>
<td>0.99</td>
<td>$y = 1.0493x + 0.1412 / y = 1.0492x + 0.1414$</td>
<td>1.1%</td>
<td>91 (22 / 69; 1 outlier removed)</td>
</tr>
<tr>
<td>Mannheim</td>
<td>1.02</td>
<td>$y = 1.0857x + 0.0452 / y = 1.1538x – 0.1819$</td>
<td>2.0%</td>
<td>85 (23 / 62)</td>
</tr>
<tr>
<td>Zwolle</td>
<td>1.03</td>
<td>$y = 1.1009x – 0.0589 / y = 1.0656x + 0.0627$</td>
<td>1.1%</td>
<td>88 (22 / 66)</td>
</tr>
<tr>
<td>Sheffield</td>
<td>1.00</td>
<td>$y = 1.0610x + 0.0918 / y = 1.0330x + 0.1874$</td>
<td>1.4%</td>
<td>89 (23 / 66; 1 outlier removed)</td>
</tr>
</tbody>
</table>

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**Out of range in CoaguChek XS data set**

**Figure 1:** Method comparison of AD149 versus rTF/95 (Bland-Altman plot). Mean bias (represented by the broken line) = 0.21 INR; mean relative bias = 6.5%; $n = 368$ (data including normal donors); Lower limit (solid line) = –0.83 INR; Upper limit (solid line) = 1.26 INR; y: AD149 [INR]; x: rTF/95 [INR].
Calibration of the INR measuring range

The measuring range of the new test had to be calibrated in the range between 0.8 and 8.0 INR. The two IRPs showed slight deviation in the upper INR range (> INR 4), and non-linearity in comparison versus each other (Fig. 1, the indicated sample at mean INR 8.5 was out of range for the CoaguChek XS system and not used for calibration). The mean relative bias between IRPs in the most relevant therapeutic range from 2.0 to 3.0 INR was 5.7% (n = 128), and in the range from 3.0 to 4.5 INR it was 8.0% (n = 85).

Therefore it was decided to use a polynomial regression with the mean INR of the IRPs for each sample in fixing the INR scale of the CoaguChek XS system. The relative bias between INRs calculated with the assigned ISI for the CoaguChek XS and INRs derived from the polynomial regression was −0.5% at INR 2.0 and increased to −7% at INR 4.5 (Fig. 2).

For the CoaguChek XS master lot the calibration procedure was double-checked by converting the raw PT clotting times by means of the polynomial curve to INR, and performing a method comparison versus the mean INR values of the IRPs which were used for calibration.

The regression line after Bablok-Passing was $y = 0.996x - 0.001$ (y: INR of CoaguChek XS system / x: mean INR of IRPs; slope CI: 0.976 – 1.019), the mean bias for patient data only was −0.01 INR or −0.04% (Fig. 3).

The duplicate determinations on CoaguChek XS monitor B versus monitor A are shown in Figure 4. The data correspond to a CV of 1.5% for the CoaguChek XS master lot INR determinations from venous blood.

Discussion

For a new POC system in PT monitoring it is crucial in the first step that the calibration is performed in agreement with the WHO recommendations and guidelines. This is the prerequisite to receive reliable INR results on which warfarin dosing decisions can be made. The CoaguChek XS PT test was successfully calibrated in terms of ISI assignment in this study. At all four sites the requirements of the WHO guidelines were met. All CVs of the slopes were below 3%, and all regression lines using patient data only passed through the data points of the normal donors. The between-center variation of the assigned ISIs was low reflected by a narrow range of ISIs between 0.99 and 1.03. The mean ISI of the new test was calculated to be 1.01.

For ISI assignment according to the WHO guidelines (2), samples with INRs outside the 1.5 – 4.5 range shall be excluded. Consequently, the ISI/INR system can be accurate only in the INR range explored by the calibration procedure, i.e. 1.5 –4.5. The manufacturer of the CoaguChek XS decided that this new

![Figure 2: Relative bias between ISI and polynomial curve-derived INRs of the master lot.](image)
Leichsenring et al. INR calibration of a new point-of-care system

The system must have a measuring range up to an INR of 8. Furthermore, it was demonstrated that there is a considerable bias in the high INR range (INR > 4) between rTF/95 and AD149 (Fig. 1). A similar finding was reported previously when comparing the rabbit IRP RBT/90 and rTF/95 (9). Therefore, the ISI obtained according to the WHO Guidelines (2) would not be the optimal parameter to define the INR for the CoaguChek XS. It was decided to use the mean INR of rTF/95 and AD149 as the reference for the INR scale, and use the polynomial regression for calculation of INRs from IPTs measured by the CoaguChek XS. This procedure will give most reliable INR values when comparing the test results versus different thromboplastins (IRPs and routine laboratory methods), and will compensate for potentially non-linear relationship between the new test and the reference.

The relative bias between INRs calculated with the assigned ISI for the CoaguChek XS and INRs derived from the polynomial regression was only –0.5% at INR 2.0 and increased to –7% at INR 4.5. For comparison it should be considered that in this investigation the relative bias between INR results of the reference preparations AD149 and rTF/95 was 8.0% in the INR range between 3.0 and 4.5, increasing to >10% at higher INRs. Overall the relative bias in the therapeutic range is less than ±10% and is not of clinical importance.

The imprecision of the CoaguChek XS master lot was found to be very low as demonstrated in Figure 4, reflected by a CV of 1.5%.

In summary, the CoaguChek XS PT test was calibrated in agreement with WHO guidelines according to the method of Trippodi et al. (3) to give an ISI of 1.01, and the measuring range was calibrated against the mean INRs of rTF/95 and AD149 between 0.8 and 8.0 INR.

**Acknowledgements**

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**Appendix**

Checking the linearity of the relationship between the PTs of the master lot (EBC03) and rTF/95 in the ISI assignment:

In Table 2 the mean normal PTs (MNPT) for rTF/95 and for the master lot (EBC03) at the four study centers are given. According to the instructions in Appendix 1 of the WHO guidelines (2) the PTs of rTF/95 (ISI = 0.94) corresponding to an INR of 2 and 4 were calculated (Table 2). Therewith, using the regression equation from patient data only (see Table 1), the equivalent PT of the master lot (EBC03) was calculated. The corresponding INR of the master lot was determined using the specific ISI and MNPT of the study center (see Table 1). The relative bias to the INR of rTF/95 must not exceed 10%. No bias was detected greater than ±2% (Table 2), therefore it can be concluded that the ISI assignment for the CoaguChek XS master lot at the four study centers is valid.

![Figure 4: Method comparison of duplicate determinations with the CoaguChek XS master lot.](https://example.com/figure4)

Table 2: Checking the linearity of the relationship between the PTs of the master lot (EBC03) and rTF/95 in the ISI assignment according to the instructions of the WHO guidelines (2).

<table>
<thead>
<tr>
<th>Site</th>
<th>MNPT</th>
<th>rTF/95 INR = 2</th>
<th>rTF/95 INR = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rTF/95</td>
<td>EBC03</td>
<td>PT (rTF)</td>
</tr>
<tr>
<td>Indianapolis</td>
<td>13.8 s</td>
<td>10.7 s</td>
<td>28.8 s</td>
</tr>
<tr>
<td>Mannheim</td>
<td>13.8 s</td>
<td>10.7 s</td>
<td>28.8 s</td>
</tr>
<tr>
<td>Zwolle</td>
<td>13.0 s</td>
<td>10.9</td>
<td>27.2 s</td>
</tr>
<tr>
<td>Sheffield</td>
<td>13.5 s</td>
<td>10.7 s</td>
<td>28.2 s</td>
</tr>
</tbody>
</table>

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References