European Concerted Action on Anticoagulation (ECAA). An assessment of lyophilised plasmas for ISI calibration of CoaguChek and TAS whole blood prothrombin time monitors

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P oint of care testing (POCT) whole blood monitors are being used on an increasing scale for prothrombin time (PT) monitoring of warfarin treatment. In some countries, they are also being used by patients for self testing and self dosage. The recommended method for the international sensitivity index (ISI) calibration of whole blood PT monitors was developed by Tripodi and has rarely been performed because it is too demanding in patient samples and requires parallel manual PT tests with a thromboplastin international reference preparation (IRP).

Nevertheless, ISI calibration is essential if POCT monitors are to be used with safety and confidence. The European Concerted Action on Anticoagulation (ECAA) aims to provide a simpler ISI calibration for whole blood PT monitors. Two widely used, European Union manufactured, whole blood PT monitors (CoaguChek Mini and TAS PT-NC, recently redesignated RapidPointCoag) have been studied.

Progress was made in previous ECAA studies, by showing that fresh plasma samples can provide reliable ISI with the CoaguChek Mini monitor, although not with the TAS PT-NC.

"International sensitivity index calibration is essential if point of care test monitors are to be used with safety and confidence"

Aims: The recommended method for the international sensitivity index (ISI) calibration of whole blood point of care testing (POCT) prothrombin time (PT) systems was originally described by Tripodi et al in 1993 but is too complex and demanding. The present European Concerted Action on Anticoagulation (ECAA) study aimed to assess the reliability of simpler ISI calibration using lyophilised plasma samples.

Methods: ISI calibrations using three different types of ECAA lyophilised plasma samples (artificially depleted, individual, and pooled) were compared with whole blood calibrations on CoaguChek Mini and TAS PT-NC POCT monitors at 10 centres.

Results: With CoaguChek Mini systems, lyophilised coumarin plasma samples (both single donation and pooled) gave ISI and international normalised ratio (INR) values comparable to whole blood. With artificially depleted plasma, ISI and INR values were too high. With TAS PT-NC systems, all three types of lyophilised plasma samples gave inaccurate ISI and unreliable INR results, similar to previous ECAA findings with fresh plasma calibrations.

Conclusions: With CoaguChek Mini systems, ISI calibration can be simplified by the use of ECAA lyophilised plasma samples from coumarin treated patients. Further study is needed to devise a simpler calibration method for the TAS PT-NC system.

MATERIALS AND METHODS

Instruments
A monitor system is defined as a combination of a brand of monitor with a single lot of test strips/cards. Two different test systems (CoaguChek Mini and TAS PT-NC) were loaned to each of the 10 ECAA centres by Roche Diagnostics (Mannheim, Germany) and Bayer AG (Leverkusen, Germany), respectively.

The CoaguChek system used lot 164 “Mini” test strips, incorporating rabbit thromboplastin.

The TAS system, recently redesignated the RapidPoint-Coag, was used with “PT-NC” test cards (lot 307060002), containing human placental thromboplastin.

Both test systems have been described in detail in previous ECAA reports.

Abbreviations: ECAA, European Concerted Action on Anticoagulation; CV, coefficient of variation; INR, international normalised ratio; IRP, international reference preparation; ISI, international sensitivity index; MNPT, mean normal prothrombin time; POCT, point of care testing; PT, prothrombin time; RBT/90, rabbit plain international reference preparation; rTF/95, human recombinant international reference preparation; WHO, World Health Organisation
Displayed PT
To perform a reliable ISI calibration, real PTs (in seconds) are required. Therefore, a master code chip to replace the conventional code chip was provided by the manufacturer of the CoaguChek Mini system to display real PT values. In the case of the TAS PT-NC, a specific correction formula was supplied to us by the manufacturer to restore the monitor displayed PT to the real PT value.

IRPs for thromboplastin
The World Health Organisation (WHO) human recombinant (rTF/95) and the rabbit plain (RBT/90) IRPs were provided by the WHO for same species ISI calibrations because the CoaguChek Mini and TAS PT-NC test systems use rabbit and human based thromboplastin, respectively.

Training workshop
Scientific assistants performing the exercise at the 10 ECAA centres attended a training workshop to familiarise them with the two types of monitor and to standardise the manual tilt technique.

Quality control exercise
A preliminary quality control exercise consisted of the testing of five abnormal lyophilised plasma samples prepared at the central facility. The purpose was to detect any monitor system or operator consistently providing outlying results. Results from all centres were satisfactory.

Whole blood and fresh plasma samples
The collection and testing of non-citrated whole blood on the monitors and the collection of fresh plasma samples for manual PT testing with the IRP were described in detail in a previous ECAA report. All donors gave informed consent.

Lyophilised plasma samples
Abnormal lyophilised plasma samples (artificially depleted, individual coumarin, pooled coumarin) and lyophilised normal plasma samples were prepared at the ECAA central facility.

Artificially depleted plasma samples
Sets of 60 artificially depleted lyophilised plasma samples were prepared using ECAA methodology from plasmapheresis donations. They were selected to span the INR range 1.5–4.5 with ECAA rabbit reference thromboplastin, a secondary reference thromboplastin. All the lyophilised, artificially depleted plasma samples had a fibrinogen content greater than 1.5 g/litre and factor V values greater than 50%, with inter-vial coefficient of variation (CV) values of less than 3%.

Individual coumarin plasma samples
Sets of 60 lyophilised coumarin plasma samples were prepared. Venous blood samples (20 ml) were collected from 60 adult patients stabilised on long term warfarin treatment in the anticoagulant clinic, Manchester Royal Infirmary, UK. For ethical reasons, the volume of blood collected from each patient had to be limited and therefore was insufficient for inter-vial CV measurements.

Pooled coumarin plasma samples
These were collected at Leiden University Medical Centre, The Netherlands, into 10 separate pools from 75–80 patients stabilised on long term oral anticoagulant treatment. The samples spanned the INR range 1.5–4.5, with an inter-vial CV of less than 3%.

Normal plasma samples
Plasma samples, collected from 20 healthy adult volunteers, were lyophilised by the ECAA central facility. The donations had to be limited to 20 ml of whole blood, so the volume was insufficient for inter-vial studies.

Reconstitution and testing of lyophilised plasmas on POCT PT monitors
Each lyophilised plasma sample was restored to its original volume of 0.5 ml with distilled water and left at room temperature for at least 10 minutes before testing. Plasma samples were tested within 30 minutes of reconstitution.

Reconstituted plasma (0.1 ml) was transferred to a plastic tube, 0.1 ml of 17mM calcium chloride was added, and the sample was mixed. The recalcified plasma was applied within five seconds to the test strip/cards and the displayed monitor PT recorded. The calcium chloride was provided to all centres by the ECAA central facility.

The 10 ECAA laboratories tested all lyophilised plasma samples on the monitors using single tests because of the restricted number of test strips/cards of the same individual lots. The 60 artificially depleted plasma samples, 60 individual coumarin plasma samples, 10 pooled coumarin plasma samples, and 20 normal plasma samples were tested on both POCT monitor systems using the same lots of test strips/cards at all centres.

Certification of lyophilised plasma samples
Three centres (Leiden, Manchester, and Milan) provided the certified PT values for the lyophilised plasmas by duplicate tests with both WHO rabbit (RBT/90) and human (rTF/95) IRPs using the manual technique. Certified PTs were the geometric means of the duplicate PTs obtained with the relevant IRPs at the three centres. The certified INR values were obtained using the certified PT, the mean normal prothrombin time (MNPT) derived from the lyophilised normal samples, and the stated ISI of the IRP.

Statistical analysis
The ISI for whole blood and lyophilised plasma calibrations with the two monitor systems were derived by the recommended WHO orthogonal regression procedure. Results with coumarin or artificially depleted plasma samples were excluded if their INR values, calculated with the relevant IRP, were outside the INR range 1.5–4.5. For the calibrations with whole blood, individual normal and abnormal log PTs obtained with the IRP were plotted, in the customary way, on the vertical axis against the log PT obtained from the monitor system on the horizontal axis, and the slope of the calibration line was calculated using orthogonal regression analysis. For the calibrations using lyophilised plasma samples, certified log PTs were plotted on the vertical axis in place of the log PT results obtained with the manual technique and the thromboplastin IRP in conventional ISI calibrations.

The SD of the log PT on the monitor and with the IRP was estimated (defined as “residual SD” in the WHO guidelines), and samples were excluded if the perpendicular distance from the orthogonal regression line was greater than three times the residual SD. The final orthogonal regression line was determined from the remaining data. The precision of the calibration slope (b) was measured by its CV, CV(b) = 100 × SE(b)/b, where SE(b) is the standard error of b.

The ISI was derived as ISI = ref × ISIref, where ISIref is the ISI of the IRP. Between centre ISI variation was measured using the CV (%). Paired t-tests were performed to compare the mean ISI derived from whole blood calibrations with the mean ISI derived from the three types of lyophilised plasma calibrations. Outlying ISI values were detected using an algorithm described and used by van der Besselaar in the calibration of the WHO rabbit plain IRP (RBT/90) and also used by Trippoli and colleagues in their calibration of the WHO human plain IRP (rTF/95).

Calibrations with the 60 lyophilised artificially depleted and 60 individual coumarin plasma samples incorporated the 20
normal plasma samples. Because only 10 pooled coumarin plasma samples were available, the number of normal plasma samples was reduced to three to preserve the proportion of normals to abnormals recommended by the WHO guidelines. The first three normal plasma samples were selected arbitrarily at each centre for inclusion.

**INR comparison for target patient samples: reliability of INR**

The relative reliability of lyophilised plasma ISI calibration of whole blood POCT monitors was assessed from the 600 fresh coumarin samples collected at the 10 centres. Five hundred and thirty five of these (target samples) had INR values within the 1.5–4.5 INR range with both the IRPs. For each target sample, monitor INR (INRm) were determined using the whole blood sample PT (PTwb) and the individual centre MNPT of whole blood (MNPTwb), with the respective individual centre ISI derived from the lyophilised plasma calibration (ISIwb); that is:

\[
\text{INR}_m = \left( \frac{\text{PT}_{wb}}{\text{MNPT}_{wb}} \right)^{\text{ISI}_{wb}}
\]

Monitor displayed INR values were compared with the reference (“true”) INR (INR) obtained on the same target blood (MNPTwb), with the respective individual centre MNPT of whole blood POCT monitors was assessed from the 600 fresh plasma samples. Between centre precision, expressed as CV of the ISI, was significantly greater than with whole blood calibrations with all three types of lyophilised plasma samples, but was significantly less than the mean ISI of 1.92 with artificially depleted plasma samples. Between centre precision, expressed as CV of the ISI, was greater than with whole blood calibrations with all three types of lyophilised plasma calibrations, but the whole blood samples were from different patients and normals at each centre, whereas the same lyophilised plasma samples were tested at all 10 centres.

**RESULTS**

Table 1 shows the range of the certified INR values, derived from the manual PT results at the three centres for the three types of abnormal plasma samples with both IRPs. The INR results cover a wide range of values with higher limits observed with the WHO rabbit IRP (RBT/90) than with the human IRP (rTF/95) with all three types of lyophilised plasma.

**ISI calibration of CoaguChek Mini system**

Table 2 compares the ISI calibration results for the CoaguChek Mini system for whole blood with those from the three types of certified lyophilised plasma samples. The WHO guidelines recommend that abnormal plasma samples with certified INR values outside the therapeutic INR range of 1.5 to 4.5 should be excluded from calibrations. With the rabbit IRP (RBT/90), used for the calibration of this system, nine artificially depleted plasma samples and two individual coumarin plasma samples were therefore excluded.

The mean ISI of 1.75 for whole blood calibrations was not significantly different from the mean ISI for the two types of lyophilised coumarin plasma samples, but was significantly less than the mean ISI of 1.92 with artificially depleted plasma samples. Between centre precision, expressed as CV of the ISI, was greater than with whole blood calibrations with all three types of lyophilised plasma calibrations, but the whole blood samples were from different patients and normals at each centre, whereas the same lyophilised plasma samples were tested at all 10 centres.

Artificially depleted plasma samples

ISI ranged from 1.80 to 2.05 at the 10 centres. Precision, as measured by calibration slope CV, was greatest with artificially depleted plasmas.

Individual coumarin plasma samples

Across the 10 centres, ISI ranged from 1.66 to 1.91. There was little difference between the mean calibration slope CV with

Table 2

<table>
<thead>
<tr>
<th>Centre</th>
<th>Whole blood</th>
<th>Artificially depleted plasma</th>
<th>Individual coumarin plasma</th>
<th>Pooled coumarin plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>ISI</td>
<td>CV(b)</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>73</td>
<td>1.63</td>
<td>4.6</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>72</td>
<td>2.10</td>
<td>3.4</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>1.70</td>
<td>3.2</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>1.89</td>
<td>3.0*</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>73</td>
<td>1.78</td>
<td>1.9</td>
<td>70</td>
</tr>
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<td>6</td>
<td>77</td>
<td>1.52</td>
<td>4.7</td>
<td>70</td>
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<td>7</td>
<td>75</td>
<td>1.67</td>
<td>2.8</td>
<td>71</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>1.76</td>
<td>2.6</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>71</td>
<td>1.92</td>
<td>3.6</td>
<td>71</td>
</tr>
<tr>
<td>10</td>
<td>71</td>
<td>1.57</td>
<td>3.3</td>
<td>71</td>
</tr>
<tr>
<td>Overall</td>
<td>1.75</td>
<td>3.3</td>
<td>1.92</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean ISI difference</td>
<td>0.17</td>
<td>0.02</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>(0.04 to 0.30)</td>
<td>(−0.14 to 0.17)</td>
<td>(−0.08 to 0.22)</td>
<td></td>
</tr>
<tr>
<td>p Value</td>
<td>0.02</td>
<td>0.8</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>CV (ISI %)</td>
<td>10.0</td>
<td>3.8</td>
<td>5.2</td>
<td></td>
</tr>
</tbody>
</table>

The values shown are the ISI derived from the calibration slope b, the CV (%) of the calibration slope, and the number of samples used in the calibration (N). The “Overall” row gives the mean CV(b) and ISI at the 10 centres. The ISI CV; row gives the between centre CV (%) of the ISI. Mean ISI difference [lyophilised plasma ISI – whole blood ISI] and corresponding confidence interval (CI) and p value are provided. *The absolute % INR deviations from theoretical INR of 2.0 and 4.5, respectively, are given in parentheses. See World Health Organisation guidelines for further details. CV, coefficient of variation; ISI, international sensitivity index; RBT/90, rabbit plain international reference preparation.
individual coumarin plasma samples and with whole blood samples. Five centres gave slope CV greater than 3% but all were less than 5%.

**Pooled coumarin plasma samples**

Calibrations at the 10 centres with the 10 pooled coumarin plasma samples and three normal plasma samples gave ISI values ranging from 1.66 to 1.98. Eight centres gave calibration slope CVs greater than 3%; four were greater than 5%, with two being 14.8% and 15.2%.

**ISI calibration of the TAS PT-NC system**

Table 3 gives the ISI of the TAS PT-NC system calibrated against WHO recombinant human plain IRP (rTF/95). The mean whole blood ISI of 1.13 was significantly higher than the mean ISI with lyophilised plasma calibrations, all of which were of a similar order (0.96 to 0.98).

The between centre CV of the ISI was lower for the calibrations using 60 artificially depleted and 60 individual coumarin lyophilised plasma samples than for whole blood calibrations. The between centre CV of the ISI with the 10 lyophilised coumarin pools was slightly higher than with the 60 whole blood coumarin samples.

**INR comparison for target patient samples: reliability of INR**

Figure 1 relates the difference between monitor and reference INRs to the intensity of anticoagulation (INR level) for the 535 target samples. The dotted line shows the difference between monitor and reference mean INR and the solid line represents zero difference. (A) Artificially depleted samples; (B) individual coumarin samples; (C) pooled coumarin samples.

![Figure 1](http://jcp.bmj.com/content/56/2/114)
Table 4  Monitor and reference INR for 535 target samples at 10 centres

<table>
<thead>
<tr>
<th>POC T system</th>
<th>Species specific IRP</th>
<th>Mean reference INR</th>
<th>Lyophilised plasma calibration</th>
<th>Mean monitor INR</th>
<th>Mean % difference</th>
<th>CI for mean % difference</th>
<th>p Value (paired t test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoaguChek Mini</td>
<td>RBT/90</td>
<td>2.50</td>
<td>Artificially depleted</td>
<td>2.77</td>
<td>10.8</td>
<td>8.9 to 12.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Individual coumarin</td>
<td>2.56</td>
<td>2.2</td>
<td>0.5 to 3.9</td>
<td>0.0099</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pooled coumarin</td>
<td>2.63</td>
<td>5.0</td>
<td>3.3 to 6.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TAS PT-NC</td>
<td>rTF/95</td>
<td>2.46</td>
<td>Artificially depleted</td>
<td>2.16</td>
<td>−12.0</td>
<td>−13.2 to −10.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Individual coumarin</td>
<td>2.13</td>
<td>−13.4</td>
<td>−14.5 to −12.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pooled coumarin</td>
<td>2.14</td>
<td>−12.9</td>
<td>−14.1 to −11.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Geometric means of reference and monitor INR values, percentage difference in geometric mean INR, corresponding 95% confidence intervals (CI), and p value are given.

INR, international normalised ratio; IRP, international reference preparation; POC T, point of care testing; RBT/90, rabbit plain international reference preparation; rTF/95, human recombinant international reference preparation.

Target samples with the two POC T systems and the three types of certified lyophilised plasma calibrations. With both POC T systems, a similar pattern was seen with the three types of lyophilised plasma: that is, the differences increased as the INR increased but only differed greatly when INR values were higher than 3.0.

For the target samples, table 4 gives the mean reference and monitor INRs, confidence intervals for the percentage difference between mean INR, and the corresponding p values for the two POC T systems.

CoaguChek Mini system
Table 4 shows that with the CoaguChek Mini system, the mean monitor INR was significantly (p < 0.01) greater than the mean reference INR with all three types of lyophilised plasma. The difference in mean INR was the greatest with artificially depleted plasma samples (10.8%). However, the difference in mean INR was only 2.2% for the ISI derived with the individual lyophilised coumarins.

Figure 1 shows that for a small number of the 535 target samples (1.1% and 1.3% with the ISI derived using the individual and pooled coumarin plasmas, respectively) the difference between the monitor and reference values was more than 2.0 INR.

TAS PT-NC system
With the TAS PT-NC system, the mean monitor INR was significantly (p < 0.0001) less than the mean reference INR with all types of lyophilised plasma (table 4). The difference in mean INR ranged from 12.0% to 13.4%.

DISCUSSION
The attempt to simplify ISI calibration by using certified lyophilised plasma samples in place of fresh whole blood samples has proved satisfactory with the CoaguChek Mini but not with the TAS PT-NC POC T monitor system.

With the CoaguChek Mini, lyophilised samples from individual patients on oral anticoagulant treatment gave reliable ISI results. The small number (10) of pooled plasma samples from coumarin treated patients were promising because they gave reasonably reliable ISI results, although they had a lower precision, presumably as a result of the smaller number of samples. The possibility of further simplification of ISI calibration of the CoaguChek Mini system by reducing numbers of plasma samples from coumarin patients by pooling appears worthy of further investigation.

In contrast, with the TAS PT-NC system, all three types of lyophilised plasma samples gave considerably lower mean ISI values than whole blood and unreliable INR results. In previous ECAA studies,7,8 we found that the TAS PT-NC system also gave unsatisfactory ISI values in fresh plasma calibrations. Our present report is therefore consistent with these findings, indicating the failure of plasma samples, whether fresh or lyophilised, to provide reliable INR results with this POC T monitor system.

Therefore, the conclusions of our study are encouraging with the CoaguChek Mini system, in that reliable ISI results were obtained with ECAA certified lyophilised plasma samples from individual coumarin treated patients and reasonably satisfactory ISI values were obtained from a small number of pooled coumarin samples.

“…The possibility of further simplification of international sensitivity index calibration of the CoaguChek Mini system by reducing numbers of plasma samples from coumarin patients by pooling appears worthy of further investigation”

In the case of the TAS PT-NC system, the problem of simplification of the calibration procedure has not yet been resolved by the use of lyophilised plasma samples and further work is in progress to develop a simple calibration method for this POC T monitor based on lyophilised plasma samples. We have demonstrated in a separate study13 that the use of a “line of equivalence” of whole blood and plasma PT on the TAS PT-NC system permits the use of fresh plasma samples for reliable ISI calibration of this system. It remains to be established whether the same approach will prove to be successful using lyophilised plasma samples with this whole blood PT monitor system.

ACKNOWLEDGEMENTS
Additional multicentre study participants were J Conard (Laboratoire Central D’Hématologie, Paris, France), D Dias (Service Immunotherapie, Hospital de S Joao, Vila Nova de Famalicão, Portugal), N Egberg (Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden), J A Iriarte (Instituto de Epidemiologia y Prevencion de Enfermedades Cardiovasculares, Hospital Civil de Basurto, Bilbao, Spain), I Kontopoulou-Griva (Anticoagulant Unit, Hippocratie General Hospital, Athens, Greece), and B Otridge (Haematology Department, Mater Misericordiae Hospital, Dublin, Ireland). We are grateful...
to the following additional scientific staff at the various centres: G Anthi (Athens), M Clerici (Milan), H Fitzgerald (Dublin), MH Horel-lou (Stockholm), K Overgaard (Esbjerg), M Vacas Rius (Bilbao). This study was supported by EC grant number SMT4-CT98–2269 and an additional grant from the Manchester Thrombosis Research Foundation. Gratitude is expressed to Roche Diagnostics (Germany) and Bayer AG (Germany) for the loan of the CoaguChek and TAS instruments and donation of test strips/cards, respectively. WHO Biologicals supplied the RBT/90 and rTF/95 IRPs.

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