The European Concerted Action on Anticoagulation (ECAA): Field Studies of Coagulometer Effects on the ISI of ECAA Thromboplastins

L. Poller¹, A. M. H. P. van den Besselaar², J. Jespersen³, A. Tripodi⁴, D. Houghton¹
Prepared on behalf of the ECAA by the above steering committee

Summary

Local calibration studies have been performed with lyophilized plasmas at 155 European laboratories to assess coagulometer effects on manual ISI of ECAA thromboplastins (low ISI human recombinant and high ISI rabbit brain). Common sets of 7 normal and 20 artificially depleted lyophilized plasmas were tested with the thromboplastins in the routine local coagulometers. With the human reagent, marked lowering of the manual ISI resulted with most coagulometers, which was associated with disproportionate shortening of the normals. Where this shortening did not occur, there was little coagulometer effect on the ISI. With the rabbit reagent, proportionate shortening with both normal and abnormal plasmas occurred in most instruments with little effect on ISI. INR correction by local ISI assignment appeared successful with the ECAA human reagent. There was negligible INR correction from local calibration of the ECAA rabbit reagent in coagulometers where thromboplastin ISI were unchanged from the ECAA established manual values.

Introduction

Prothrombin time (PT) methodology has changed since the introduction of the WHO scheme as most laboratories have now replaced the manual technique by automation. The ISI of thromboplastins may be considerably modified by coagulometers and subsequent INR derived from the ISI, i.e. INR = (prothrombin ratio)\^ISI, may consequently be inaccurate (1-5).

Some thromboplastin manufacturers have attempted to resolve the problem by providing a range of ISI for their reagents combined with different coagulometers (i.e. system ISI) but this method of correction is limited by the varied effects of individual instruments of the same brand (6).

Lyophilized plasmas with certified manual PT values with the relevant international reference preparation (IRP) for thromboplastin have been shown to correct for local effects of coagulometers on thromboplastin ISI (5-7). A previous European Concerted Action on Anticoagulation (ECAA) multicentre report showed that artificially depleted and coumarin lyophilized plasmas when certified with manual ISI values with a thromboplastin IRP and used as reference values in the WHO orthogonal regression procedure gave a close approximation to the ISI value based on fresh plasma from coumarin-treated patients (8).

The present multicentre study was designed to evaluate coagulometer effects on thromboplastin ISI by provision of the same lyophilized artificially depleted test plasmas and ECAA reference thromboplastins to 155 participant laboratories. This was to evaluate the effects of a range of coagulometers on the manual ISI of the two ECAA reference thromboplastins, i.e. the low ISI human recombinant and high ISI rabbit brain established by the ECAA multicentre calibration (9). Sets of the same lot of 20 artificially depleted lyophilized plasmas and 5 lyophilized coumarin test plasmas together with 7 lyophilized normals were tested on the local routine coagulometer with either the ECAA rabbit or human thromboplastin in two consecutive field studies. The type of ECAA reagent supplied to participants was dependent on whether the local routine thromboplastin was of human or rabbit type.

Because variations in the local fresh plasma mean normal prothrombin time (MNPT) have an important effect on prothrombin ratios as well as ISI and to reduce the workload of participants, a set of 7 lyophilized normal plasmas was provided for the study participants. The performance of these 7 lyophilized normal plasmas has been described separately in a recent report as the first part of the results of this study (10). The MNPT of fresh plasmas and the means of the 7 lyophilized plasmas were shown to be closely comparable at the participant centres using most routine rabbit thromboplastins, but were marginally longer with the human type thromboplastins employed locally.

Reproducibility of performance was assessed by a repeat exercise at an interval of 3 months with a set of 20 certified lyophilized artificially depleted plasmas from a different lot.

Materials and Methods

Lyophilized Artificially Depleted Plasmas

Because of the established discrepancies in INR arising from testing of individual plasmas by the human and rabbit IRP routes of thromboplastin calibration (11-13) all plasmas were certified in terms of both human and rabbit ECAA reference thromboplastins. Two different lots of twenty plasmas in sequential order of manufacture, certified with manual PT values (sec) in terms of the respective IRP, were selected from 60 artificially depleted plasmas prepared for the ECAA exercise. Each set of twenty provided a spread of INR over the therapeutic range of 1.5 to 4.5 when tested with the International Council for Standardisation in Haematology (ICSH) human plain thromboplastin IRP (BCT/441) (14). Two field studies were performed at an interval of three months.

The artificially depleted plasmas were prepared from blood plasma obtained from individual blood donations from healthy subjects which was snap frozen as soon as possible at -40°C prior to lyophilization. After thawing, the plasmas
were adsorbed with barium sulphate for variable periods to provide INR between 2.0 and 4.5. Separate donations were obtained for each of the depleted plasma samples.

The certified manual PT of the artificially depleted lyophilized plasmas were the averages of four individual replicate tests on each plasma at all 6 certifying ECAA national centres using both ECAA reference thromboplastins. The overall mean PT of the four replicate PT was calculated for each of the plasmas at each centre. The assigned INR was from the mean PT of the result from the 6 centres. The PT technique had been standardised at a preliminary “wet workshop” (9). The ISI values of the ECAA thromboplastins had been established by the previous multicentre manual PT exercise at 14 ECAA national centres employing the conventional WHO ISI calibration procedure based on 60 fresh plasmas from long term stabilised coumarin treated patients and 20 healthy adults (15).

Because of possible complicating effects of depletion of the non-coumarin-dependent clotting factors, factor V and fibrinogen on the PT results, factor V assays (16) and fibrinogen determinations (17) were performed on all the artificially depleted plasmas to ensure adequate levels. Samples containing less than a minimum level of 50% factor V or 1.5 g/l fibrinogen were excluded. These minimum levels are regarded as adequate for prothrombin time measurement and only when the individual clotting factors V or fibrinogen fall below these levels do they prolong the PT test. The factor V of all the artificially depleted plasmas included in the study ranged from 50% to 100% and fibrinogen content from 1.5 g/l to 4.0 g/l. It has been shown that when factor V levels are above 40% they do not influence international normalised ratio (INR) results (18). Inter-vial variation, accelerated degradation and long-term stability studies were also performed on each of the depleted lyophilized plasmas. All plasmas gave a CV of less than 3% in inter-vial studies and minimum heat degradation stability of seven days at 40° C. Long term stability studies also proved satisfactory up to a minimum of twelve months.

Lyophilized Normal Plasmas

These were prepared at the Central Facility in response to requests to reduce the workload for participants by eliminating the need for local MNPT on a minimum of 20 fresh plasmas from healthy adults. The use of a common set of lyophilized normals also controlled the effect of possible variability of the MNPT in the different populations of the EU member states. The aim was to obtain a mean value from the 7 plasmas which approximated to the fresh plasma MNPT of healthy adults (i.e. 14.3 s) when tested with the ICSH human plain international reference preparation (IRP) for thrombolastin, BCT/441 (14).

Lyophilized Coumarin Test Plasmas

Five coumarin test plasmas were obtained from donations of 50 ml whole blood from individual patients stabilised on oral anticoagulant therapy. The plasmas were lyophilized at the ECAA Central Facility in Manchester in the same way as the artificially depleted plasmas. These plasmas were certified with manual PT values by 3 ECAA national laboratories (“certifying centres”). Four replicate tests were performed on each plasma, using both human and rabbit ECAA thromboplastins. The overall mean PT of the four replicate PT was calculated for each of the plasmas at each centre. The assigned INR was the mean of the results from the 3 centres. INR were calculated using the ECAA established ISI of the reference thromboplastins (ISI ECAA human = 0.95; ISI ECAA rabbit = 1.67) and the manual geometric mean of the 7 lyophilized normal plasmas as follows:

\[
\text{INR}_t = \left( \frac{\text{PT (manual)}}{\text{mean normal}} \right)^{\text{ISI}_t}
\]

The lyophilized normals were also used to obtain local PT ratios for INR derivation with the coumarin test plasmas. The differences between the fresh plasma MNPT and mean PT of the lyophilized normals with ECAA reagents in the two ECAA field studies have been described in detail in a previous report (10).

Study Thromboplastins

A preliminary survey had elicited the brand and species of the routine thromboplastin at each centre. The ECAA human or ECAA rabbit thromboplastin was provided to individual centres according to whether they used a human or rabbit tissue thromboplastin for routine work. As supplies of the bovine IRP were not available for the study, users of the bovine commercial reagent Thrombotest also were provided with the ECAA rabbit reagent.

Participants

The ECAA national directors in each member state were asked to nominate at least ten centres in their country with an interest in laboratory control of anticoagulation and known to employ a coagulometer for routine PT tests. The invited participants listed in the appendix were instructed to test each of the artificially depleted plasmas in duplicate with the relevant ECAA thromboplastin reagent (human or rabbit). The coagulometer employed for routine prothrombin time testing was to be used. Thrombotest-users were to perform a standard prothrombin time by the recommended method with the ECAA rabbit reagent in parallel according to instructions provided.

In both field studies, participants received a package consisting of sets of 20 lyophilized depleted plasmas, the set of 7 lyophilized normals, the 5 lyophilized coumarin plasmas and the relevant ECAA reference thromboplastin (rabbit or human).

Statistical Analysis

Orthogonal regression analysis was used according to the established WHO procedure for prothrombin time standardisation (19, 20). The certified manual log PT values with the relevant ECAA reference thromboplastin obtained at the certifying ECAA laboratories were placed on the vertical (y-axis) and the local coagulometer PT system on the horizontal (x-axis). Two calibration equations were determined for each centre. The first used the conventional combined normal/abnormal ISI calibration with lyophilized abnormal and lyophilized normal plasmas, and the second was based on the lyophilized abnormal plasmas only to derive a calibration slope. The coincidence of the slopes of the abnormal/normal and abnormal-only line have been examined for significant deviation by testing the hypothesis that the mean log PT of the normals lie on the line of the abnormal plasmas (21).

Instruments used by five or more centres were tested for significant differences between the ISI of the various coagulometers (one way ANOVA, for unbalanced design). Paired t-tests were used to test for significant differences in the overall mean slopes and ISI of all centres between the two field studies.

Accuracy of Local Calibration Schemes

The accuracy or reliability of the individual field laboratory’s local calibration schemes has been determined by comparing local INR with the assigned INR (INR_L). The local system ISI (ISI_L) had been calculated using orthogonal regression and local INR (INR_L) determined in the conventional manner.

\[
\text{INR}_L = \left( \frac{\text{PT coumarin}}{\text{mean normal}} \right)^{\text{ISI}_L}
\]

where PT coumarin is the coagulometer result, the mean normal is the geometric mean on the coagulometer of the 7 normals and the ISI_L is the value from the local calibration using the 20 artificially depleted and the 7 lyophilized normals. The absolute percentage deviation of local system INR_L from the assigned INR_L has been calculated for each of the 5 coumarin test plasmas and the mean percentage deviation was calculated.

An attempt was made to examine further the mechanism of the coagulometer effect on ISI. The ratio of the mean local coagulometer PT to the certified mean manual PT has been determined for each coagulometer using both the 7 lyophilized normal plasmas and the 20 lyophilized artificially depleted plasmas. This was in order to determine whether the coagulometers altered the
relationship of either the normal or the abnormal plasmas i.e. changed the pro-thrombin ratio compared to the manual technique. Ratios were determined for each plasma by dividing local coagulometer PT results by certified manual PT results (i.e. ratio = PT<sub>local</sub>/PT<sub>certified</sub>). Mean ratios for the 20 abnormal and 7 normal plasmas have been calculated for each centre. The effect of coagulometers was studied further in plasmas at different INR levels to determine whether coagulometer effects varied with the severity of the coagulation defect.

**Results**

One hundred and forty three centres of the 155 invited laboratories returned complete sets of results. Four laboratories had to be excluded because they changed coagulometers between studies. Nineteen others were excluded because they either participated in only one field study or returned incomplete results. Of the remaining 120 centres, 45 laboratories tested the ECAA human thromboplastin reagent and 75 the ECAA rabbit thromboplastin reagent.

**ISI Calibrations**

After exclusions, six brands of coagulometer were found to be used by 5 or more laboratories. The overall effect of the coagulometers on the ISI of the two thromboplastins is seen in Figs. 1 and 2. Table 1 summarises the results of the two studies. The conventional fresh plasma ISI of the ECAA human reagent determined in a previous multicentre calibration was 0.95 and with the ECAA rabbit reagent was 1.67 (9). Table 1 shows that the overall coagulometer ISI with the human ECAA reagent was approximately 0.88 for the two field studies representing a marked overall lowering of 7.4% from the manual ISI of 0.95. In contrast the overall coagulometer mean ISI for the rabbit reagent was 1.65, which was a negligible (1.2%) overall mean reduction from the established manual ISI value of 1.67. The SD with the instruments was considerably higher in both studies with the high ISI rabbit than the low ISI human reagent. There was no significant difference between the mean slopes and subsequent ISI of the two field studies (p >0.05, paired t-test) in terms of either reference thromboplastin reagent even though two different lots of 20 abnormal lyophilized plasma calibrants were used. For simplicity, the presentation of the detailed results has therefore been limited to the first of the two serial studies.

Theoretically, the orthogonal regression calibration line should be representative of both normal and abnormal results. When the hypothesis was tested that the mean log PT of the normals lies on the same line as the log PT of the abnormal plasmas (21), there was a high proportion of discrepant results with lack of coincidence of the two lines. This provides additional evidence of the disproportionate effect of the coagulometers on the normal values compared to the abnormalities particularly with the human recombinant reagent, which is reported later. The abnormal-only slope did not coincide with the combined abnormal/normal slope at 62% of centres with the human recombinant reagent using the lyophilized abnormal and normal plasmas. The abnormal-only slopes underestimated the combined abnormal/normal slope in all but one case. With the rabbit thromboplastin, lack of coincidence of the two lines was observed in 41% of cases. For both routes of calibration the number of significant deviations of the two lines observed cannot be assumed to be due to chance alone.

![Fig. 1](image1.png)  Local ISI calibration distribution for ECAA human thromboplastin (ECH) (ISI<sub>ECH</sub> = 0.95) for first field study

![Fig. 2](image2.png)  Local ISI calibration distribution for ECAA rabbit thromboplastin (ECR) (ISI<sub>ECR</sub> = 1.67) for first field study

<table>
<thead>
<tr>
<th>Route of calibration</th>
<th>n</th>
<th>a&lt;sub&gt;0&lt;/sub&gt; (SD)</th>
<th>b&lt;sub&gt;0&lt;/sub&gt; (SD)</th>
<th>a&lt;sub&gt;1&lt;/sub&gt; (SD)</th>
<th>b&lt;sub&gt;1&lt;/sub&gt; (SD)</th>
<th>ISI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAA Human</td>
<td>45</td>
<td>0.2285 (0.1113)</td>
<td>0.8545 (0.0713)</td>
<td>0.1176 (0.0870)</td>
<td>0.9231 (0.0649)</td>
<td>0.8769 (0.0617)</td>
</tr>
<tr>
<td>ECAA Rabbit</td>
<td>75</td>
<td>-0.0644 (0.1615)</td>
<td>1.065 (0.0999)</td>
<td>0.0513 (0.1340)</td>
<td>0.9919 (0.0926)</td>
<td>1.6565 (0.1547)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Route of calibration</th>
<th>n</th>
<th>a&lt;sub&gt;0&lt;/sub&gt; (SD)</th>
<th>b&lt;sub&gt;0&lt;/sub&gt; (SD)</th>
<th>a&lt;sub&gt;1&lt;/sub&gt; (SD)</th>
<th>b&lt;sub&gt;1&lt;/sub&gt; (SD)</th>
<th>ISI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAA Human</td>
<td>45</td>
<td>0.2013 (0.0970)</td>
<td>0.8799 (0.0603)</td>
<td>0.1078 (0.0774)</td>
<td>0.9385 (0.0624)</td>
<td>0.8916 (0.0393)</td>
</tr>
<tr>
<td>ECAA Rabbit</td>
<td>75</td>
<td>0.0915 (0.1442)</td>
<td>0.9669 (0.1195)</td>
<td>0.0689 (0.1281)</td>
<td>0.9808 (0.0999)</td>
<td>1.6379 (0.1668)</td>
</tr>
</tbody>
</table>

Table 1  The mean abnormal only equations (y = a<sub>0</sub> + b<sub>0</sub>x) and the abnormal/normal (y = a<sub>1</sub> + b<sub>1</sub>x) equations intercepts (a), slopes (b) and estimated ISI together with between centre standard deviations for the two routes of calibration are presented. Calibration equations intercept and slope determined for each centre using orthogonal regression with certified log PT on the vertical (y) and local coagulometer log PT on the horizontal (x)
With the human reagent there was a disproportionate shortening of the normal values with the lyophilized plasmas in the coagulometers compared with the certified values for the same lyophilized normals with the manual technique. The ratio of the coagulometer normal PT to the manual normal PT was 0.93 (see Table 2) whereas for the abnormal samples the coagulometer/manual ratio was close to unity at 1.02.

In the case of the high ISI rabbit reagent both ratios of coagulometer/manual PT for normal and abnormal samples were reduced but to a similar degree (see Table 2). Thus the overall prothrombin ratio and hence INR was relatively undisturbed.

It will be seen from Table 3 that all coagulometers did not reduce the ISI of the human reagent. The MLA and STAGO (STA) which had the least effect on the established manual ISI of 0.95 showed the least disturbance of the relationship of the coagulometer PT to manual PT with the normal and abnormal lyophilized plasmas (see Table 4) and most disturbance (lowering) of the ISI of the coagulometers used in sufficient numbers with both reagents. In contrast Table 3 shows that at the 14 centres using the KC type coagulometers in the two studies with the rabbit reagent there was a measurable disproportionate disturbance of the normal ratio. The ISI was also most lowered with the ECAA rabbit reagent in the KC instruments and with the ACL instrument which also disturbed the prothrombin ratio disproportionately.

The mean ISI results in Table 3 differ significantly (p = <0.05) for the various instruments with both human and rabbit calibrations (one-way ANOVA). Two of the instruments, the ACL and KC, underestimated the ISI for both human and rabbit ECAA reagents although the ACL had relatively less effect on the ISI of the rabbit reagent. The Behring coagulometer only gave results in sufficient numbers for analysis with the human ECAA reagent but gave the lowest ISI of all the systems considered. Of the instruments used by five or more laboratories the MLA instruments most closely approximated to the manual ISI with the human reagent whereas the STAGO was marginally better than the MLA with the ECAA rabbit reagent.

INR Assessment of Local Calibration

Table 5 lists the mean INR of the five lyophilized coumarin test plasmas obtained at the three ECAA certifying centres using the geometric mean of the 7 lyophilized normals and the established ISI of the two thromboplastins. The ECAA human thromboplastin gives an overall mean INR of 4.04 whereas the ECAA rabbit thromboplastin gives 3.57. A single factor ANOVA reveals no statistically significant difference in results between the certifying centres’ INR for the two reagents although they are sufficiently different to be important clinically (mean difference = 13.7%) assuming a 10% difference is clinically relevant. Although variability exists between INR, the mean results obtained by the three centres have been regarded as the best estimates of the true INR with the respective thromboplastins using lyophilized coumarin plasmas. An important difference in INR according to the route of calibration (rabbit or human) is confirmed in this study. This has been reported previously (11–13, 22).

Laboratories which failed to test all five coumarin plasmas were excluded from the analysis. The overall mean INR by orthogonal regres-

Table 2

<table>
<thead>
<tr>
<th>Route of calibration</th>
<th>Mean Abnormal Ratio</th>
<th>Mean Normal Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECAA Human</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>ECAA Rabbit</td>
<td>0.92</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table 3a

<table>
<thead>
<tr>
<th>Instrument</th>
<th>n</th>
<th>Mean ECAA human PT field study 1</th>
<th>Mean ECAA human PT certified PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL</td>
<td>8</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>Behring</td>
<td>9</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>KC</td>
<td>6</td>
<td>0.86</td>
<td>0.86</td>
</tr>
<tr>
<td>MLA</td>
<td>7</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>STA</td>
<td>5</td>
<td>0.92</td>
<td>0.92</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
<td>0.88</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Table 3b

<table>
<thead>
<tr>
<th>Instrument</th>
<th>n</th>
<th>Mean ECAA rabbit PT field study 1</th>
<th>Mean ECAA rabbit PT certified PT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL</td>
<td>20</td>
<td>1.60</td>
<td>1.60</td>
</tr>
<tr>
<td>KC</td>
<td>14</td>
<td>1.55</td>
<td>1.55</td>
</tr>
<tr>
<td>MLA</td>
<td>8</td>
<td>1.76</td>
<td>1.76</td>
</tr>
<tr>
<td>STA</td>
<td>10</td>
<td>1.75</td>
<td>1.75</td>
</tr>
<tr>
<td>Thrombolyzer</td>
<td>8</td>
<td>1.77</td>
<td>1.77</td>
</tr>
<tr>
<td>Others</td>
<td>15</td>
<td>1.66</td>
<td>1.66</td>
</tr>
</tbody>
</table>
sion analysis for the laboratories who tested all five coumarin plasmas are presented in the form of histograms in Figs. 3 and 4. The vertical broken lines represent the assigned ("true") INR for the coumarin test plasmas obtained by the three certifying centres. Prior to correction there is a general trend towards overestimation of INR for the human thromboplastin in both field studies (Fig. 3). Once the ISI of the reference thromboplastin reagent is replaced with the local system ISI value the overestimation is corrected and local INR are distributed about the ‘true’ mean value. In contrast there appears to be no correction for the rabbit route of calibration (Fig. 4).

Table 6 summarises the results presented in Figs. 3 and 4. Mean INR prior to correction is 4.78 for the human thromboplastin for the two field studies, while after correction the overall mean is approximately 4.1 which is considerably closer to the assigned ‘true’ mean INR value of 4.04. This is reflected in the mean absolute percentage deviation (mean unsigned deviation %) which is reduced by approximately 10% in the two field studies. Although correction in this instance has re-dressed obvious bias observed when the stated (reference) ISI is used for INR calculation the dispersion about the assigned (‘true’) value does not appear to have been greatly reduced.

The uncorrected ECAA rabbit thromboplastin INR are much closer to the assigned value with the rabbit IRP than the human reagents were to the human IRP before correction and their deviation is only minimally reduced by the correction perhaps not unexpectedly in view of the

![Fig. 3 Field study 1, ECAA human thromboplastin calibrations. Mean INR distribution for 5 lyophilized coumarin test plasmas for human route. The vertical broken line represents the mean assigned INR value](image)

![Fig. 4 Field study 1, ECAA rabbit thromboplastin calibrations. Mean INR distribution for 5 lyophilized coumarin test plasmas for rabbit route. The vertical broken line represents the mean assigned INR value](image)

### Table 5

Assigned INR values of lyophilized coumarin test plasmas, obtained using MNPT of 7 lyophilized plasmas and ISI of ECAA thromboplastins ($\text{ISIEC Human} = 0.95; \text{ISIEC Rabbit} = 1.67$), and the manual technique

<table>
<thead>
<tr>
<th>Plasma</th>
<th>ECAA human</th>
<th>ECAA rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.74</td>
<td>3.82</td>
</tr>
<tr>
<td>2</td>
<td>6.13</td>
<td>5.82</td>
</tr>
<tr>
<td>3</td>
<td>2.57</td>
<td>1.95</td>
</tr>
<tr>
<td>4</td>
<td>1.69</td>
<td>1.40</td>
</tr>
<tr>
<td>5</td>
<td>5.05</td>
<td>4.87</td>
</tr>
</tbody>
</table>

| Mean | 4.04 | 3.57 |

### Table 6

Mean INR for the 5 lyophilized coumarin test plasmas for field study 1 for laboratories who tested all 5 plasmas. Before correction INR were determined using the stated ISI value of ECAA thromboplastins ($\text{ISIEC Human} = 0.95; \text{ISIEC Rabbit} = 1.67$) and after correction by locally determined ISI

<table>
<thead>
<tr>
<th>Route of calibration</th>
<th>n</th>
<th>Before Correction</th>
<th>After Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean INR (SD)</td>
<td>Mean INR (SD)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean %dev</td>
<td>Mean %dev</td>
</tr>
<tr>
<td>ECAA Human</td>
<td>31</td>
<td>4.78 (0.46)</td>
<td>4.12 (0.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17.04</td>
<td>6.70</td>
</tr>
<tr>
<td>ECAA Rabbit</td>
<td>66</td>
<td>3.59 (0.40)</td>
<td>3.55 (0.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.99</td>
<td>7.80</td>
</tr>
</tbody>
</table>
negligible coagulometer effect on ISI found with the rabbit thromboplastin. Table 1 shows a mean local coagulometer ISI of 1.65 was obtained against the manual stated ISI of the rabbit thromboplastin of 1.67.

Table 1 presents the mean INR and mean percentage deviation from assigned INR for the centres which tested the 5 lyophilized coumarin test plasmas on their local coagulometer with the test thromboplastin. Groups with as few as 3 coagulometers are displayed but less emphasis should be placed on these results because of the small numbers. Correction of INR is good across the range of coagulometers for the human thromboplastin, with the exception of Schnitzer & Gross instrument. For the rabbit calibration there was no overall correction across the range of coagulometers for the instruments used in sufficient numbers for analysis. The best correction was obtained with the KC coagulometers, the instruments with the most marked effect on ISI.

Discussion

There have been a number of previous reports showing marked effects of coagulometers on the ISI of thromboplastin reagents (1-6, 23, 24). In this study the effect of the coagulometers on ISI has been assessed in isolation from the other variables affecting ISI by providing to participants the same reference thromboplastins and sets of the same test plasmas (7 normals and of the 20 certified abnormal lyophilized plasmas). Opportunity has been taken in this study to compare coagulometer effects using a low ISI human recombinant thromboplastin and a high ISI rabbit thromboplastin on the PT of the same sets of 20 lyophilized abnormal plasmas certified with a standardised manual PT technique and of the lyophilized normals. As current recommendations are that thromboplastin reagents for routine anticoagulant control should have ISI less than 1.5 and preferably less than 1.2 (25) the relative performance in the coagulometers with the low ISI and high ISI reagent appears of considerable relevance.

Previous reports from the ECAA have demonstrated that lyophilized artificially depleted plasmas and lyophilized coumarin plasmas, although giving different ISI results, give a reasonable approximation to fresh plasma ISI value (8). They differed by approximately 5% from fresh plasma calibrations and approximately 11% from each other. In a further report it has been shown that a calibration based on a minimum of 20 of the lyophilized artificially depleted plasmas prepared at the ECAA Central Facility provided a reasonably reliable local system ISI assignment with the manual PT technique (26). Lyophilized plasmas however are necessary for external quality assessment and inter-laboratory exercises because fresh plasmas cannot be used owing to the instability of their clotting factor components. The use of lyophilized plasmas as substitutes for fresh or frozen plasma samples to assess INR correction in multicentre studies must therefore be regarded as a necessary procedure with some limitations.

This study, like previous multicentre studies (5, 6), has demonstrated coagulometer effects on ISI and the possibility of INR correction by local ISI calibration using certified lyophilized artificially depleted plasmas. The use of the same set of lyophilized normal plasmas in place of fresh plasma MNPT for local PT derivation and ISI calibration undoubtedly reduced the initial uncorrected error of the INR. It has been shown that variability of local MNPT of fresh plasmas at different laboratories has a considerable effect on the ISI and INR (27). In the majority of laboratories using the ECAA human reagent the orthogonal regression calibration slopes and the estimated ISI value were markedly lowered by some coagulometers with the exceptions of the MLA and STAGO instruments. The different types of coagulometer disturbed the relationship of the PT of the normal plasmas compared with the mean manual normal PT (MNPT) particularly with the low ISI human thromboplastin reagent. A previous report on the present two field studies has shown that shortening of the normal PT by the coagulometers compared to the manual technique disturbs the prothrombin ratio and the ISI (10). The overall ratio of the normal PT on the lyophilized normal samples in the coagulometers to the fresh plasma MNPT with the manual technique was 0.95 approximately. Thus the prothrombin ratios of the abnormal samples were increased by the coagulometers. In contrast the PT results with the abnormal samples in the coagulometers were of a similar order showing parallel shortening. Because of the increase of the prothrombin ratio, mainly detected with the KC coagulometers, the ISI are therefore lowered.

Results with the high ISI ECAA rabbit thromboplastin, although less precise than with the human recombinant, showed a parallel shortening with both abnormal and normal lyophilized plasmas compared with the manual technique. In the majority of cases the coagulometers under or overestimate the calibration slope of the rabbit reagent by only 5% or less. The exception was the KC and to a lesser extent the ACL which gave lower ISI in both field studies.

The level of INR correction obtained using local system ISI was inconsistent. The correction obtained for the human reagent (low ISI) was encouraging and effective for most coagulometers particularly where
the ISI was disturbed with the percentage deviation from the “true” INR substantially reduced by the local calibration. There was a less marked coagulometer effect on the ISI with the ECAA rabbit reagent and not unexpectedly comparatively little INR correction by local ISI determination. Generalisations on the performance of all commercial brands of rabbit tissue based thromboplastins should not be made from these results. The ECAA rabbit reagent studied and its manual ISI value (1.67) is no longer typical of many rabbit thromboplastins as newer more responsive (lower ISI) rabbit reagents are now being marketed on an increasing scale and may be more like the ECAA human reagent in performance with coagulometers.

With the relatively unresponsive high ISI reagent there was little or no local ISI correction by the local calibration. In some instances deviation from ‘true’ INR was in fact increased (ACL and KC) when the local system ISI was introduced.

Two different lots of 20 artificially depleted lyophilized plasma calibrants were used in the two consecutive exercises. They gave consistent ISI values between the two field studies performed at an interval of three months. The set of 7 lyophilized plasmas appeared to act as an anchor in the ISI calibrations and assisting agreement. This emphasises the importance of the inclusion of the normals in ISI calibration and their considerable influence on the slope of the calibration line.

The present study shows therefore that the effects of coagulometers on ISI may be considerable but are dependent on the type of thromboplastin used as well as the coagulometer. Some brands of coagulometer caused a marked lowering of the ISI, particularly with the low ISI human reagent, apparently due to the disturbance of the prothrombin ratios by disproportionate shortening of the normal controls. This is not a problem for all coagulometers as two of those studied in sufficient numbers in this study (MLA and STAGO) showed no disturbance of the prothrombin ratio and ISI even with the human recombinant reagent.

Acknowledgements

Gratitude is expressed to the field study participants listed in the appendix. This work was supported by grant number PL931349 of the EC Biomed programme.

Appendix

Field study participants
* = ECAA National Director

BELGIUM
Dr. J. Arnout, Leuven*
Dr. P. Capel, Brussels
Dr. M. de Weer, Leuven
Dr. B. Chatelain, Mont-Godinne
Dr. A. Criel, Brugge
Dr. L. Marcelis, Roeselare
Dr. A. Lust, Aalst
Dr. M. Vanderplanken, Edegem

GERMANY
Professor H. Beeser, Freiburg*
Dr. H. W. Tomesch, Werdau
Dr. S. Appel, München
Dr. med. Y. Schmitt, Darmstadt
Frau Dipl. med. B. Lüdtke, Stralsund
Herr Wilhelm, Bad Krozingen
Prof. Dr. med. G. Winckelmann, Wiesbaden
PD Dr. B. Ogemenöller, München
Prof. Dr. R. Zimmermann, Heidelberg

FRANCE
Professor M. Samama, Paris*
Dr. I. Houdebouyan, Boulogne Billancourt
Professor P. Sié, Toulouse
Dr. M. Gouault, Criteil
Dr. J. Roussi, Garches
Professor I. Juhan, Marseille
Professor J. Goudemand, Lille
Professor J. Sampol, Marseille
Professor M. C. Guillin, Clichy
Professor T. Lecompte, Vandoeuvre les Nancy

SPAIN
Professor Dr. J. A. Iriarte, Bilbao*
Dr. F. Martinez-Brotos, Barcelona
Dr. V. Vicente, Murcia
Dr. M. A. Fernández, Valencia
Dr. J. Fontcuberta, Barcelona
Dr. A. Ordinas, Barcelona
Dr. F. J. Batlle, La Coruña
Dr. J. Lasierra, Logroño
Dr. J. Montero, Madrid
Dr. J. L. Navarro, Madrid

DENMARK
Professor J. Jespersen, Esbjerg*
Dr. A. Bremmelgaard, Næstved
Dr. E. Magid, Copenhagen
Dr. K. Winther, Kolding
Dr. I. Brandslund, Vejle
Dr. S. Antonsen, Middelfart
Dr. K. Kynde, Roskilde
H. Jelert, Sønderborg

GREECE
Dr. I. Kontopoulou-Griva, Athens*
Professor T. Mandalaki, Athens
As. Professor S. Aroni, Athens
Dr. D. Zabouli, Thessaloniki
Dr. T. Tassopoulou, Athens
Dr. M. Antonopoulou, Piraeus
Dr. M. Parara, Athens
Dr. B. Tsoukanas, Piraeus
Professor A. Maniatis, Patras
R. Stathopoulou, Athens

ITALY
Professor P. M. Mannucci, Milan*
Dr. A. Tosetto, Vicenza
Dr. L. Del Buono, Milan
Dr. S. Testa, Cremona
Dr. N. Erba, Como
Dr. A. Gobbi, Milan
Dr. A. Galletti, Genoa
Dr. M. Pradella, Treviso
Dr. N. Ciavarella, Bari
Dr. G. Palareti, Bologna
Professor G. Mariani, Rome

THE NETHERLANDS
Dr. A. M. H. P. van den Besselaar, Leiden*
Drs. A. P. Anker, Dokkum
Dr. Ir A. A. M. Ermens, Eindhoven
J. van der Sloot, Hoorn
Dr. R. K. A. van Wermeskerken, The Hague
References


Received January 27, 1998 Accepted after revision June 5, 1998