Evaluation of European Concerted Action on Anticoagulation Lyophilized Plasmas for INR Derivation Using the PT/INR Line

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Key Words: PT/INR Line; Prothrombin time; ECAA plasmas; International normalized ratio; INR correction; Coagulometers; Thromboplastin

Abstract

The prothrombin time/international normalized ratio (PT/INR) Line method based on 5 certified European Concerted Action on Anticoagulation (ECAA) plasmas provides reliable local INR values without conventional World Health Organization international sensitivity index calibrations. The present study investigated the use of different numbers and types of ECAA calibrant plasmas to derive accurate PT/INR Lines and reliable INR values. The numbers ranged from 3 to 10 plasmas in a set with normal or abnormal samples. Sets were selected, and sampling was repeated 1,000 times for each center to derive PT/INR Lines. The lines were selected randomly or from clusters. The INR values of 5 independent “validation” plasmas were compared before and after correction.

In 56 calibrations, 5 ECAA plasmas gave better results than did fewer plasmas. Plasmas with wide-ranging INR values gave better results than randomly selected sets, and including a normal plasma was not essential. The INR deviations of validation plasmas from certified values were reduced with sets of human, bovine/combined, and rabbit reagents. Deviations of more than 10% from certified INR values were significantly reduced (P < .001).

The prothrombin time/international normalized ratio (PT/INR) Line is a recently described simple method that provides a dependable local INR. There is no need for local manual PT testing using the World Health Organization (WHO) thromboplastin international reference preparations (IRPs) required in the conventional WHO method for international sensitivity index (ISI) calibration to obtain INRs. The PT/INR Line is a development of the “direct INR” method of Houbouyan and Goguel, later modified in the Scientific and Standardization Committee Guidelines and in the Clinical and Laboratory Standards Institute document, that gave discouraging results in a previous European Concerted Action on Anticoagulation (ECAA) study.

The WHO ISI calibration scheme has been limited in application because of its heavy demands such as the requirement for multicenter testing with the now-discarded manual (tilt-tube) PT method of samples from 60 patients and 20 healthy subjects. There is also a requirement for local availability of the relevant species WHO thromboplastin and the need to perform orthogonal regression analysis to derive the local ISI.

The PT/INR Line is derived by using a set of 5 ECAA-certified plasmas with stated INR values derived by manual PT testing using the thromboplastin IRP of human, rabbit, or bovine origin by manual testing at experienced certifying centers. The PT results of plasmas with the local technique and reagents are then plotted against their certified values on a natural logarithm scale. The PT/INR Line is then determined by using linear regression, and a local INR is derived directly from the estimated PT/INR Line. In the WHO ISI calibrations, the more complex orthogonal regression analysis is required. We have previously shown that using simple linear regression to determine the PT/INR Line gave similar INR results.
In 2 recent studies, we showed that the PT/INR Line based on the testing of only 5 ECAA lyophilized plasmas certified with INR using the manual PT technique provides reliable INR values.1,2 In our previous studies on the PT/INR Line, calibrant plasmas were specifically selected to give a spread of INR values across the therapeutic interval. Normal plasmas were not included in the set, and the possible additional value of including a normal plasma was not studied. The main aims of the present study were therefore to investigate the following: (1) the use of calibrant plasma sets varying in number (3-10) to derive the PT/INR Line; (2) the use of different calibrant sets with varying INR values; (3) whether sets of calibrant plasmas specifically selected from clusters to provide a spread of INR values across and outside the therapeutic 2.0 to 4.5 interval gave better results than randomly selected plasmas regardless of their INR values; and (4) whether there was additional value from the inclusion of a normal plasma in the set.

In the present study, PT/INR Lines were determined from the different sets of calibrant plasmas that were used to obtain corrected INR values of a further independent set of 5 plasmas (termed “validation” plasmas). Results were compared with the certified INR value on the same validation plasmas from ECAA centers experienced in manual PT testing using the relevant IRP thromboplastins (human, rabbit, or bovine).

Materials and Methods

The reliability of the PT/INR Line in INR derivation, based on sets of calibrant plasmas with varying INR values and numbers, has been assessed in a multicenter study at selected centers with an established interest in oral anticoagulant control. The INR values obtained from the PT/INR Line have been compared with manual INR values provided by a group of experienced ECAA certifying centers using the relevant species thromboplastin IRP (human, rabbit, or bovine).

During a 5-year period, 28 participant laboratories collaborated in the program of repeated ISI calibrations in the ECAA randomized multicenter prospective study of computer-assisted anticoagulant dosage.8-10 All participants used an automated method for PT determination with a range of locally used commercial thromboplastins. The ISIs of the local PT systems were determined by ISI calibrations according to the US Food and Drug Administration–approved method using 20 ECAA artificially depleted lyophilized abnormal plasmas and 7 lyophilized normal plasmas.11 These 27 plasmas were then used as the calibrant plasmas to derive the PT/INR Line.

An additional independent set of 5 validation plasmas was tested in parallel by the participant centers as part of the ongoing external quality assessment for the ECAA computer-assisted dosage study. The INR values of the validation plasmas were determined by using the PT/INR Lines and were compared with certified values, described later in the article, and were also compared with INR values obtained without using the PT/INR Line. The present report describes the reliability of the PT/INR Line based on different combinations and numbers of calibrant plasmas selected from the set of 20 abnormal and 7 normal plasmas. The 20 abnormal calibrant plasmas included some samples with certified INRs outside the 2 to 4.5 INR range that had been excluded in previous reports on the PT/INR Line.1,2

Certified PT and INR Values

Certified PT and INR values for the individual plasmas from the set of 20 artificially depleted abnormal and 7 normal plasmas were provided by the 3 ECAA certifying centers (Leiden, the Netherlands; Manchester, England; and Milan, Italy). These values were based on their manual PT testing with the 3 species of thromboplastin IRP: WHO human plain rTF/95, ECAA rabbit plain reference EUTHR-1, and WHO bovine/combined OBT-79. In the absence of WHO rabbit IRP at the time of the study, ECAA rabbit plain EUTHR-1 was substituted. This reference reagent had been used in previous ECAA multicenter studies and certified in terms of the WHO rabbit IRP RBT/90.12-14 Calibrant plasmas were tested by the certifying centers once on 3 different days.

Certified INR values were provided similarly for the 5 independent validation plasmas at the same certifying centers. The validation plasmas gave a spread of INR over the therapeutic interval with the 3 species of IRPs. Individual plasmas were tested by the certifying centers once on 3 different days. The INR values were averaged and taken as certified values.

Certified INR Derivation

Certified INRs were derived by using the local mean normal PT (MNPT) as follows:

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

\[ \text{MNPT}_{\text{ref}} \] is the geometric mean PT of the 7 normal plasmas. \( \text{ISI}_{\text{ref}} \) is the ISI for WHO human IRP rTF/95 (ISI = 0.94), ECAA rabbit reagent EUTHR-1 (ISI = 1.67), or bovine/combined reagent OBT/79 (ISI = 1.0).

The observed INRs for each of the plasmas from the 3 certifying centers were averaged and taken as their certified values.

Local PT and INR Values

Local PTs for the set of 20 artificially depleted plasmas were provided for the calibration exercises by the participant centers. Plasmas were tested in duplicate on 1 occasion. Information on the type of local reagent and coagulometer system was also provided, together with the local system manufacturers’ thromboplastin ISI and participants’ local MNPT.
Effects of Random Sampling vs Cluster Sampling for the PT/INR Line

Randomly Selected Calibrant Plasmas

In one of our previous studies on the PT/INR Line, we reported that sets of 3 to 10 plasmas were used to derive the PT/INR Lines and then to correct the INRs of the 5 validation plasmas. The calibrant plasmas were purposely selected to provide a range of INRs that spanned the 1.7 to 4.0 interval (mean INR, 2.76) with the 3 IRPs for thromboplastin.

In contrast, in the present study, 3 calibrant plasmas were first randomly selected from the full set of 20 abnormal samples without reference to their INR. The PT/INR Line was derived from the results with each set of 3 plasmas obtained by the individual participants. This type of random sampling was repeated 1,000 times with each of the 56 sets of exercises from the 28 centers, and the PT/INR Lines were derived for each set. The procedure was repeated for sample sets of increasing numbers of 4 to 10 calibrant plasmas to derive their respective PT/INR Lines.

Calibrant Plasmas Randomly Selected From Clusters

For this analysis, 3 calibrant plasmas were first randomly selected from each of 3 different clusters. These clusters represented different INR ranges: 2.0 or less, between 2.0 and 3.0, and more than 3.0. A single calibrant plasma was then randomly selected from each of the 3 clusters, and the PT/INR Line was derived from these 3 plasmas. This was repeated 1,000 times, giving a total of 56,000 PT/INR Lines. This procedure was then performed with 3 and 4 calibrant plasmas in place of 3, each of these selected from 4 clusters, representing INRs of 2.0 or less, 2.01 to 2.5, 2.51 to 3.0, and more than 3.0. Finally, 5 calibrant plasmas were randomly selected from each of 5 different clusters representing INRs of 2.0 or less, 2.01 to 2.33, 2.34 to 2.66, 2.67 to 3.0, and more than 3.0.

The PT/INR Lines were determined with each random set and then used to derive the local INRs of the validation plasmas and compared with their certified values. In addition, INR deviation (as a percentage) from the certified values was examined before correction (the INR obtained at the participating center without using the PT/INR Line) and after correction (the INR derived at the participating center using the PT/INR Line).

Using Normal Plasmas to Derive the PT/INR Line

Additional analysis was performed in which a normal plasma randomly selected from the 7 lyophilized normal samples was also added to the calibrant plasma sets from the different clusters studied earlier, and the resultant PT/INR Lines were derived. Three calibrant plasmas and a normal plasma were first selected randomly from the results of each of the 56 exercises. This procedure was repeated 1,000 times, giving a combined total of 56,000 sets of plasmas and PT/INR Lines derived with each set. This was further repeated in turn with 4 and 5 calibrant plasmas including a normal plasma in each set with the same INR cluster groups mentioned earlier.

INR Derivation Before and After PT/INR Line Correction

The PT/INR Lines with each sample set were used to derive corrected INRs for the 5 validation plasmas, and their results were compared with their independently certified values before and after PT/INR Line correction.

Statistical Analysis

Certified INR

To assess the reliability of results from the ECAA certifying centers, a 1-way analysis of variance (ANOVA) was performed to test for significant differences in INRs between the centers.

INR Derivation With the PT/INR Line

For the PT/INR Line, the certified INRs were plotted on the horizontal axis \( x_i \) against the observed PT of the randomly selected set of artificially depleted calibrant plasmas on the vertical axis \( y_i \) on a natural log (Ln) scale. The PT/INR Line was fitted using linear regression analysis. Figure 1 shows an example of certified international normalized ratio (INR) plotted against the local prothrombin time (PT) results of 5 calibrant plasmas on a natural logarithm (Ln) scale. The PT/INR Line fitted using linear regression (solid line) is used to determine the INR after correction. The INR of a test plasma sample with a PT of 30 seconds (broken line, Ln (30 s) = 3.40) is derived using the PT/INR Line (INR, \( e^{1.16} = 3.19\)).

\[ y = 2.43 + 0.84x. \]
example of the derivation of a PT/INR Line that can be used to
directly determine the local INR of test plasma samples.

The INR values for the 5 validation plasmas were
calculated by using the intercept (a) and slope (b) estimates of the
regression lines as follows:
\[
y_i = a + bx_i
\]
When the local PT of a test plasma sample \([\exp(y_i)]\) is known,
this formula can be rearranged to derive the INR of the valida-
tion plasma \([\exp(x_i)]\) by:
\[
x_i = \frac{(-a/b)}{1/b} + (1/b)y_i
\]
Therefore, the INR of a test plasma = \(\exp(x_i)\).

**INR Derivation Before and After Correction**

The mean INRs for the 5 independent validation plas-
mas were calculated using the intercepts (a) and the slopes
(b) of the PT/INR Lines with the different sets of calibrant
plasmas (selected from 3 to 10). The dispersion of results was
examined by coefficient of variation (% CV). In addition,
INR deviation (as a percentage) from the certified values was
examined before correction and after correction with the PT/
INR Line. The results were outlined in difference plots and
tested for significance with the McNemar test. The analysis
was performed using the STATA statistical package. Random
samples were selected using the “sample” command in
STATA (StataCorp, College Station, TX).

**Results**

Results were obtained in 56 independent calibration
exercises at 28 participant centers during the 5-year period
of the ECAA computer-assisted dosage study. One of the
centers performed 4 exercises, 10 other centers performed 3
exercises, 6 centers performed 2, and 10 centers performed
only 1 exercise. All used an automated PT system, and none
reported manual PT values. In 42 results, a human thrombo-
plastin was used; in 8, a bovine/combined was used; and in 6,
a rabbit reagent was used.

**Certified Values**

**Calibrant Plasmas**

Certified INRs with the 20 calibrant plasmas ranged from
1.57 to 3.99 (overall mean INR, 2.55) with human thrombo-
plastin, 1.41 to 3.99 (mean INR, 2.30) with bovine/combined,
and higher values of 1.57 to 5.67 (mean INR, 3.09) with rabbit
thromboplastin. These calibrant plasmas not only provided a
range of INR over the therapeutic 2.0 to 4.5 interval but also
included extremes less than 2.0 and, with the rabbit IRP, more
than 5.0. Plasmas chosen for determining the PT/INR Lines
were randomly selected irrespective of whether the INRs were
in the therapeutic range. Certified INRs with the 7 lyophilized
normal plasmas ranged from 0.96 to 1.03 with human throm-
oblastin, 0.98 to 1.01 with bovine/combined, and 0.92 to
1.06 with rabbit.

**Validation Plasmas**

Mean INRs of the 5 independent validation plasmas
at the certifying centers ranged from 1.71 to 3.01 (overall
mean INR, 2.41) with human reference thromboplastin. With
bovine/combined, INRs on the same plasmas ranged from
1.48 to 2.59 (overall mean INR, 2.04), and with rabbit, INRs
were higher, ranging from 1.81 to 3.81 (overall mean INR,
2.80).

A 1-way ANOVA to test for differences in INRs between
the 3 certifying centers was not significant \((P = .88)\). A 1-way
ANOVA to test for differences between reagents (human,
bovine/combined, and rabbit) was significant \((P = .002)\).

**Results of Sample Selection for PT/INR Line Derivation**

The overall mean INR results of the 5 validation plasmas
before correction (not using the PT/INR Line) and after cor-
rection (using the PT/INR Line) are shown in Table 1 and
compared with certified values from the experienced certify-
ing centers. Table 2 shows INRs of the individual validation
plasmas obtained with the selected set of plasmas compared
with random plasmas with the 3 types of IRP.

When only 3 randomly selected calibrant plasmas were
used to derive the PT/INR Line, 104 sets of samples had to
be excluded from the 56,000 because INR results were too
close to each other to produce a reliable PT/INR Line. With 4
calibrant plasmas, 29 sets were similarly excluded, and with 5
plasmas, only 6 sample sets were excluded. No further sample
sets were excluded with other selected groups.

**Randomly Selected Calibrant Plasmas for the PT/INR Line**

Results with the 3 reference thromboplastins were as
follows:

**Human Reagents**

By using the manufacturers’ stated ISIs and locally
obtained MNPT, the absolute mean INR deviation from
mean certified INR of the 5 validation plasmas with human
reagents was 11.2% (mean INR, 2.68; 95% confidence inter-
val, 2.59-2.77). With the PT/INR Line, the absolute mean INR
deviation from the certified INR was reduced to 2.1% with 3
and 4 randomly selected calibrant plasmas and to less than
2.0% with 5 or more calibrant plasmas. Mean INR dispersion
assessed using % CV was reduced from 11.8% to 7.0% with 3
calibrant plasmas and to 5.0% or less with 4 or more calibrant
plasmas. Figure 2 shows the total proportion of validation
Table 1
Absolute Mean INR (% CV) and the Proportion of INRs Within ±10% Deviation From Mean Certified INR of the 5 Independent Validation Plasmas Before and After Correction With Increasing Numbers and Different Sets of European Concerted Action on Anticoagulation Calibrant Plasmas

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Bovine/Combined</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean INR</td>
<td>INRs With &lt;10% Deviation</td>
<td>Mean INR</td>
</tr>
<tr>
<td></td>
<td>(% CV)</td>
<td>From Certified INR, % (SE)</td>
<td>(% CV)</td>
</tr>
<tr>
<td>Certified values</td>
<td>2.41</td>
<td>—</td>
<td>2.04</td>
</tr>
<tr>
<td>Before correction</td>
<td>2.68 (11.8)</td>
<td>34.3 (0.10)</td>
<td>1.93 (4.0)</td>
</tr>
<tr>
<td>PT/INR Line</td>
<td>2.80</td>
<td>—</td>
<td>2.51 (8.1)</td>
</tr>
<tr>
<td>No. of randomly selected calibrant plasmas</td>
<td></td>
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<tr>
<td>3</td>
<td>2.46 (7.0)</td>
<td>83.9 (0.09)</td>
<td>2.11 (7.2)</td>
</tr>
<tr>
<td></td>
<td>2.46 (5.0)</td>
<td>88.6 (0.07)</td>
<td>2.11 (4.3)</td>
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<tr>
<td></td>
<td>2.45 (4.3)</td>
<td>90.6 (0.06)</td>
<td>2.10 (3.0)</td>
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<tr>
<td></td>
<td>2.45 (4.1)</td>
<td>92.1 (0.05)</td>
<td>2.10 (2.6)</td>
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<tr>
<td></td>
<td>2.45 (2.9)</td>
<td>92.8 (0.05)</td>
<td>2.10 (3.3)</td>
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<td></td>
<td>2.44 (3.9)</td>
<td>93.3 (0.05)</td>
<td>2.10 (2.2)</td>
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<td></td>
<td>2.44 (3.8)</td>
<td>93.8 (0.05)</td>
<td>2.10 (2.1)</td>
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<tr>
<td></td>
<td>2.44 (3.7)</td>
<td>94.1 (0.05)</td>
<td>2.10 (2.0)</td>
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<tr>
<td>Selected set of plasmas from clusters</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>2.42 (4.5)</td>
<td>93.8 (0.09)</td>
<td>2.11 (3.2)</td>
</tr>
<tr>
<td></td>
<td>2.43 (4.1)</td>
<td>93.7 (0.05)</td>
<td>2.11 (2.8)</td>
</tr>
<tr>
<td></td>
<td>2.43 (3.7)</td>
<td>94.2 (0.05)</td>
<td>2.10 (2.6)</td>
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<tr>
<td>4</td>
<td>2.43 (4.5)</td>
<td>94.3 (0.05)</td>
<td>2.09 (4.0)</td>
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<td></td>
<td>2.43 (4.1)</td>
<td>94.7 (0.05)</td>
<td>2.09 (3.4)</td>
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<td></td>
<td>2.43 (4.4)</td>
<td>95.2 (0.05)</td>
<td>2.09 (3.1)</td>
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<tr>
<td>Selected plasmas + 1 normal plasma</td>
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<td></td>
<td></td>
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<tr>
<td>3 + 1N</td>
<td>2.42 (4.5)</td>
<td>94.3 (0.05)</td>
<td>2.09 (4.0)</td>
</tr>
<tr>
<td></td>
<td>2.43 (4.5)</td>
<td>94.7 (0.05)</td>
<td>2.09 (3.4)</td>
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<td></td>
<td>2.43 (4.4)</td>
<td>95.2 (0.05)</td>
<td>2.09 (3.1)</td>
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</table>
| CV, coefficient of variation; INR, international normalized ratio; PT, prothrombin time.

* Statistically significant (P < .001) compared with INR before correction using the McNemar test.

Figure 2
Proportion of plasmas deviating less than 10% from certified values before correction (BC; without the prothrombin time [PT]/international normalized ratio [INR] Line) and after correction with the PT/INR Line with 3 to 5 calibrant plasmas in a set. The sets of plasmas were selected randomly (R), from clusters with varying INR ranges (C), or from clusters and including a normal plasma (C + 1N).
### Table 2
Comparison of Calibrant Plasma Sets Selected From Clusters and Randomly Selected to Derive the INR of Individual Validation Plasmas With the PT/INR Line for Human, Bovine/Combined, and Rabbit Thromboplastins

<table>
<thead>
<tr>
<th></th>
<th>Mean INR (SD) of 5 Validation Plasmas</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td><strong>Human reagents</strong></td>
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<tr>
<td>Certified values</td>
<td></td>
<td>1.71</td>
<td>2.02</td>
<td>2.51</td>
<td>2.81</td>
<td>3.01</td>
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<tr>
<td>Before correction</td>
<td></td>
<td>1.88 (0.19)</td>
<td>2.25 (0.24)</td>
<td>2.74 (0.33)</td>
<td>3.17 (0.43)</td>
<td>3.37 (0.44)</td>
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<td>PT/INR Line derived using</td>
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<tr>
<td>Set of calibrant plasmas randomly selected</td>
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<tr>
<td>No. of plasmas</td>
<td></td>
<td>1.78 (0.31)</td>
<td>2.09 (0.25)</td>
<td>2.51 (0.19)</td>
<td>2.86 (0.29)</td>
<td>3.03 (0.34)</td>
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<td></td>
<td></td>
<td>1.78 (0.18)</td>
<td>2.09 (0.16)</td>
<td>2.51 (0.14)</td>
<td>2.85 (0.19)</td>
<td>3.02 (0.24)</td>
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<td>1.78 (0.13)</td>
<td>2.09 (0.13)</td>
<td>2.50 (0.11)</td>
<td>2.85 (0.16)</td>
<td>3.01 (0.20)</td>
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<td>Selected set of plasmas from clusters</td>
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<td>No. of plasmas</td>
<td></td>
<td>1.74 (0.08)</td>
<td>2.06 (0.10)</td>
<td>2.48 (0.11)</td>
<td>2.84 (0.16)</td>
<td>3.01 (0.20)</td>
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<td>1.75 (0.08)</td>
<td>2.07 (0.10)</td>
<td>2.49 (0.10)</td>
<td>2.84 (0.15)</td>
<td>3.02 (0.18)</td>
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<td>2.07 (0.10)</td>
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<td>3.01 (0.16)</td>
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<td>No. of plasmas</td>
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<td>2.04 (0.10)</td>
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<td>2.84 (0.16)</td>
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<td>1.73 (0.07)</td>
<td>2.05 (0.10)</td>
<td>2.48 (0.11)</td>
<td>2.84 (0.16)</td>
<td>3.02 (0.19)</td>
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<td>1.73 (0.06)</td>
<td>2.05 (0.10)</td>
<td>2.48 (0.11)</td>
<td>2.85 (0.16)</td>
<td>3.02 (0.18)</td>
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<td><strong>Bovine/combined reagents</strong></td>
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<tr>
<td>Certified values</td>
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<td>1.48</td>
<td>1.87</td>
<td>2.04</td>
<td>2.40</td>
<td>2.59</td>
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<td>Before correction</td>
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<td>1.69 (0.24)</td>
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<td>4.10 (0.52)</td>
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</table>

INR, international normalized ratio; PT, prothrombin time.
plasmas with INRs with less than 10% deviation from the certified INR, which was 34.3% before correction and significantly improved to between 83.9% and 94.1% with the PT/INR Line using 3 or more calibrant plasmas ($P < .001$).

**Bovine/Combined Reagents**

At centers using bovine/combined reagents, the mean deviation was 5.4% from the certified INR, and after correction using the PT/INR Line with 3 or more calibrant plasmas, it became 3% to 3.4%. The mean % CV increased from 4.0% to 7.2% with 3 calibrant plasmas but decreased to 3.0% or less with 5 or more calibrant plasmas. Figure 2 shows the total proportion of validation plasmas within ±10% of certified values before correction of 87.5% was significantly reduced to 81.1% after correction with 3 calibrant plasmas but improved significantly to 90.2% to 94.5% with 5 or more randomly selected calibrant plasmas ($P < .001$).

**Rabbit Reagents**

In centers using rabbit-type reagents, the deviation was 10.4% from the certified INR, but after correction using the PT/INR Line, this was reduced to 2.9% with 3 calibrant plasmas and to 2.1% to 2.5% with 4 or more calibrant plasmas. The mean % CV was 8.1% before correction and was reduced to 7.1% to 7.6% with the PT/INR Line using 6 or more calibrant plasmas. The total proportion of validation plasmas within ±10% of certified values before correction was 40.0%, which significantly increased to between 61.9% and 75.7% with the PT/INR Line ($P < .001$).

**Calibrant Plasmas Selected From Clusters for the PT/INR Line**

**Human Reagents**

With human reagents, the absolute mean INR deviation from the certified INR was reduced from 11.2% to less than 1% with 3 to 5 calibrant plasmas selected from a group of clusters. The mean INR dispersion was reduced from 11.8% CV to 3.7% to 4.5%. Figure 2 shows the total proportion of validation plasmas with INRs with less than 10% deviation from the certified INR before correction was 34.3%, which significantly improved to 93.7% to 94.2% with the PT/INR Line ($P < .001$) with only 3 to 5 calibrant plasmas. The total proportion of 94.2% with 5 calibrant plasmas from clusters gave similar results to sets of 10 plasmas randomly selected (94.1%).

**Bovine/Combined Reagents**

With bovine/combined reagents with inclusion of a normal plasma, the absolute mean INR deviation was reduced across all sets (Table 1). The proportion of validation plasmas within ±10% of the certified INR significantly increased from 87.5% before correction to between 89.2% and 93.1% when including a normal sample with 3 to 5 calibrant plasmas ($P < .001$). Using 3 calibrant plasmas with a single normal sample gave no difference in the proportion of results with less than ±10% deviation from certified values compared with 4 calibrant plasmas and no normal plasma ($P = .26$). The use of 4 calibrant plasmas with 1 normal plasma gave results 0.7% higher compared with 5 calibrant abnormal plasmas ($P < .001$).

**Rabbit Reagents**

With rabbit-type reagents, INR deviation from the certified INR was reduced across all plasma sets, similar to results without a normal plasma. There was no improvement
in mean % CV after correction. The proportion of validation plasmas within ±10% of certified values increased significantly to results between 71.5% and 74.1% with the PT/INR Line (P < .001).

Discussion

With the manual tilt-tube method of PT measurement now largely abandoned for oral anticoagulant control, it is now no longer easy or practical for nonspecialist laboratories to conform to the WHO-recommended procedure for ISI calibration. The less demanding method of ISI calibration using the US Food and Drug Administration–approved method based on sets of 20 certified ECAA lyophilized plasmas and 7 normal plasmas has proved to be of only limited value because of the lack of availability of the requisite control plasmas. These problems have led to the development of a simpler approach, the PT/INR Line, based on a relatively small number of manually certified reference plasmas in terms of the relevant WHO thromboplastin IRP by a group of experienced centers. The simple relationship of certified INRs obtained at the expert centers and the local PT results is then used to derive the local INRs of patient test plasmas.

In the present study, differing numbers of plasmas in calibrant sets were shown to result in varying INR correction using the PT/INR Line. As few as 3 plasmas used to derive the PT/INR Line gave improved results with human and rabbit thromboplastin reagents, although not with bovine/combined reagents. The greatest benefit was evident with the human reagents, the largest group. It was shown that 5 calibrant abnormal plasmas carefully selected from a group of clusters with INRs ranging across and outside the therapeutic limits gave optimal results. The results were similar to sets of 10 randomly selected ECAA calibrant plasmas irrespective of their INR values.

There was, however, only a small improvement with bovine/combined reagents, but results before correction were considerably better than with human and rabbit reagents. At the small group of centers using rabbit reagents, there was improvement toward certified values with the PT/INR, although not as great as with the human reagents, and INR dispersion was not improved.

A small benefit resulted from the inclusion of a normal plasma in a set to derive the PT/INR Line, which was shown to be statistically significant with bovine and rabbit reagents. With the human reagents, the additional benefit of including a normal plasma, although statistically significant owing to the large sample (56,000 plasma sets), was small in clinical terms (approximately 0.5%).

The availability of suitable normal plasmas is an essential requirement in conventional ISI calibrations as the geometric MNPT is required to derive the INR. The present study confirmed that with the PT/INR Line, the inclusion of normal plasma in a set of calibrant plasmas for INR derivation is not an essential prerequisite.

Overall, the PT/INR Line improved INR reliability between laboratories using a range of different commercial thromboplastins and automated PT systems and reduced INR dispersion. The proportion of test (independent validation) plasmas with less than 10% deviation from the certified values increased significantly after correction with the PT/INR Line.

The PT/INR Line can also be used to derive reliable INRs using different selected ECAA plasma sets. The present study showed that 3 to 10 calibrant plasmas can be used successfully for derivation of the PT/INR Line with the range of thromboplastin reagents and automated test systems included in this study. However, at least 5 ECAA lyophilized calibrated calibrant plasmas in a set is recommended, and these should provide a wide spread of INRs across the therapeutic interval.

From the 1EAA (ECAA) Central Facility, Faculty of Life Sciences, University of Manchester, Manchester, England; 2Department of Clinical Biochemistry, Hospital of South West Denmark, Esbjerg, and Department for Thrombosis Research, Institute of Public Health, University of Southern Denmark, Esbjerg; and 3Hart Biologicals, Hartlepool, England.

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ECAA plasma sets are available from Hart Biologicals, Hartlepool, England. Any income from the sales of these plasmas will be devoted to the registered charity, Manchester Thrombosis Research Foundation, to further research in anticoagulation.

Certifying center directors are as follows: A.M.H.P. van den Besselaar, The Haemostasis and Thrombosis Research Center, Leiden University Medical Center, Leiden, the Netherlands; A. Tripodi, The A. Bianchi Bonomi, Hemophilia & Thrombosis Centre, University and IRCCS Maggiore Hospital, Milan, Italy; and the EAA Central Facility.

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