

Influence of Three Types of Automated Coagulometers on the International Sensitivity Index (ISI) of Rabbit, Human, and Recombinant Human Tissue Factor Preparations

A Multicenter Study

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Summary

Five tissue factor reagents and three types of automated instruments for prothrombin time (PT) determination were studied in an international multicenter collaborative exercise. The purpose of this work was to determine the international sensitivity index (ISI) for each combination of reagent and instrument against the international reference preparation RBT/90. Each type of instrument was used by 3 or 4 centers to assess the interlaboratory variation of the ISI. The interlaboratory variation of the ISI for each combination of reagent and instrument ranged between 0.4% and 7.8% coefficient of variation. For three reagents, the mean ISI values for ACL (nephelometric) and STA (mechanical) were practically identical, but the mean ISI values for MLA (photo-optical) were at least 10% higher. For two other reagents prepared from rabbit tissue, the mean ISI values increased in the order ACL, STA, MLA. The widest range of mean ISI values was noted with one rabbit tissue factor reagent: 1.68 for ACL and 2.00 for MLA. Exclusion of patient specimens with INR <1.5 and INR >4.5 determined by the international reference preparation resulted in a slight decrease of the mean ISI.

The interlaboratory variation of the International Normalized Ratio (INR) was assessed from the results obtained with common lyophilized and deep-frozen plasmas. The use of instrument-specific ISI values resulted in reduced interlaboratory variation of the INR. It is recommended that thromboplastin manufacturers provide instrument-specific ISI values.

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Introduction

The prothrombin time (scientific name: tissue factor – induced coagulation time) is widely used for monitoring oral anticoagulant treatment. The result of the test is influenced by the tissue factor (thromboplastin) reagent and the instrument used. It is recommended to report prothrombin times in terms of the international normalized ratio (INR). The INR is calculated with the formula $INR = (PT/MNPT)^{ISI}$ in which PT is the patient's prothrombin time, MNPT the mean normal prothrombin time, and ISI the international sensitivity index of the prothrombin time system used. The ISI is obtained by calibration of the system against an international reference preparation using fresh plasma samples from healthy persons and patients on long-term oral anticoagulant treatment (1). Traditionally, prothrombin times were determined with manual techniques and ISI values for thromboplastins related to the manual techniques. Today, many laboratories use automated coagulometers. Several studies have demonstrated that the ISI value is influenced by the type of instrument (2, 3). Some of these studies were performed by a single laboratory and their value is therefore limited. Several multicentric studies of coagulometer effects were carried out with lyophilized plasmas (4, 5).

The present study is the first multicentric study in which fresh plasma samples were used to assess the influence of different types of instrument on the ISI. The purpose of this study is to determine ISI values for five tissue factor reagents (two from rabbit tissue, two from human tissue, and one recombinant human) and three automated instruments. The tissue factor reagents represent materials currently used by many clinical laboratories in various countries. Two reagents were provided with instrument-specific ISI values by their respective manufacturers but the others were not.

In the present study the international reference preparation (IRP) for thromboplastin rabbit, plain (RBT/90) was used as yardstick. When this

Table 1 Thromboplastin preparations used in this study

Thromboplastin	Stated ISI
T1: RBT/90	1.00 (manual technique)
T2: USP Reference Standard	0.96 (manual technique)
T3: Thromborel-S	1.02 (ACL) 1.07 (bead coagulometers) 1.19 (MLA)
T4: Hemolab ISIMAT	0.95 (Hemolab, ACL, STA, ST4, KC10) 1.02 (MLA 1000C, Coag-a-mate X2)
T5: Néoplastine CI	1.82 (instrument not specified)
T6: Néoplastine CI Plus	1.22 (instrument not specified)

study was carried out, stocks of the international reference preparation for thromboplastin human, plain (BCT/253) were practically exhausted and could not be included.

Several lyophilized and deep-frozen plasma samples were provided to the participants. It could be shown that the use of instrument-specific ISI values improved the interlaboratory variation of the INR for lyophilized and frozen plasma samples.

Materials and Methods

Thromboplastin Reagents

RBT/90, the international reference preparation (IRP) for thromboplastin, rabbit, plain, was obtained from the World Health Organization (WHO) (6). The USP Thromboplastin Reference Standard, recombinant human type, was obtained from the United States Pharmacopeial Convention, Inc., Rockville, Maryland (7). Thromborel-S (lot number 505705), prepared from human placenta, was provided by the manufacturer Behringwerke AG, Marburg, Germany. Hemolab Isimat (lot number 091115 623595), prepared from human placenta, was provided by the manufacturer BioMérieux SA, Marcy-l'Etoile, France. Néoplastine CI (lot number 951193), and Néoplastine CI Plus (lot number 952186), both prepared from rabbit brain, were given by the manufacturer Diagnostica Stago, Asnières-sur-Seine, France. These six reagents are coded as T1, T2, T3, T4, T5, and T6, respectively, in the present study (Table 1). Reconstitution fluids for T2, T4, T5, and T6 were provided by the respective manufacturers.

Other Reagents and Equipment

Each participating laboratory was also provided with the following: evacuated blood collection tubes (Vacutainer, Becton Dickinson, Meylan, France), siliconized, containing 0.105 mol/l buffered sodium citrate solution, for blood collection in the proportion of 1 volume of anticoagulant to 9 volumes of blood; sterile distilled water for reconstitution of T1, T3, and lyophilized test plasmas; sterile calcium chloride, 0.025 mol/l, for recalcification of plasma/T1 mixtures. Lyophilized pooled coumarin plasma (coded L3) was provided by Immuno AG, Vienna, Austria.

Methods

All thromboplastins were used according to the instructions of the manufacturers. The clotting times with T1 were determined by the manual technique (tilt tube) by all participants but one which used a Coagtester Epsilon 104 (Labover, Montpellier, France).

The clotting times with the other five thromboplastins were determined with an automated coagulometer, either STA (Diagnostica Stago), or ACL (Instrumentation Laboratory), or Electra of Medical Laboratory Automation (MLA, Pleasantville, New York). The STA is a bead coagulometer detecting a change of viscosity, the ACL is a nephelometric instrument, and the Electra is measuring light absorbance.

Design of the Study

All participants received detailed instructions on how to collect blood specimens, to handle thromboplastins and to determine prothrombin times. Calibration was carried out independently by each participant using T1 as reference system. All thromboplastins were to be tested over 10 working days. On each day a different set of fresh plasmas from 2 healthy individuals and 6 patients on oral anticoagulant treatment were used. All levels of intensity of anticoagulation were acceptable provided the patients were on oral anticoagulants for at least 6 weeks in the range of 1.5 to 4.5 INR. To minimize the effect of instability of plasma samples and reconstituted thromboplastins the testing order of thromboplastins was changed from day to day according to a form provided. Single PT determinations were performed. On each day, the same set of 13 lyophilized plasmas and 5 deep-frozen pooled plasmas were analyzed with T2, T3, T4, T5, and T6. Due to limited availability of T1, it was not possible to analyze all lyophilized and frozen plasmas with this reagent.

Statistical Analysis

ISI values were estimated for each laboratory according to the calibration protocol recommended by the WHO (1). Briefly, PTs for fresh plasmas from healthy individuals and patients were plotted on a double-logarithmic scale with T1 on the vertical axis and T2, T3, T4, T5, or T6 on the horizontal axis. Orthogonal regression lines were calculated for the 5 combinations of T1 and the other thromboplastins (8). In one analysis, all patient specimens were included irrespective of the INR determined with T1. In another analysis, specimens with INR outside the 1.5-4.5 INR interval (determined with T1) were excluded. Data points lying at a perpendicular distance from the orthogonal regression line greater than 3 standard deviations around the line were identified as outliers and were discarded for calculation of the final line. The ISI values for T2 to T6 were the product of the respective slope of the orthogonal regression line and the manual ISI for T1. Since the manual ISI for T1 is equal to 1.0, the ISI values for the thromboplastins are equal to the values for the slope. The within-laboratory precision of the calibration line was estimated by the standard deviation (SD) of the slope of the orthogonal regression line, and was expressed as coefficient of variation (CV): CV = 100 SD/slope. It should be noted that the within-laboratory SD of the slope is not the result of repeated slope assessments by one center but was derived from the scatter of individual

Table 2 Number of normals' and patients' specimens, exclusions, and outliers. INRs of specimens were determined with RBT/90. After excluding specimens with INR <1.5 and those with INR >4.5, outliers were identified for calibration lines of T2, T3, T4, T5, and T6

Lab nr.	normals	patients	INR < 1.5	INR > 4.5	outliers T2	outliers T3	outliers T4	outliers T5	outliers T67
1	20	60	-	2	1	-	-	-	1
2	20	57	2	1	1	1	-	-	-
3	20	60	-	7	1	-	-	1	1
4	20	60	1	5	1	-	-	-	1
5	20	60	-	5	-	-	-	-	-
6	20	60	-	9	1	1	1	1	1
7	20	60	2	2	-	1	1	-	-
8	20	60	-	4	-	-	1	2	1
9	20	60	-	2	-	-	-	1	1
10	20	60	-	1	1	1	1	1	1
12	20	60	-	1	-	1	1	-	-

Table 3 Slopes and within-laboratory CV (in %) of slope for orthogonal regression lines. Specimens with INR <1.5 or >4.5 were excluded for calculation of regression lines

Laboratory number	Instrument	Slope/CV for T2	Slope/CV for T3	Slope/CV for T4	Slope/CV for T5	Slope/CV for T6
3	ACL	0.862/ 2.9	1.088/ 2.1	0.990/ 2.7	1.715/ 1.8	1.310/ 1.9
4	ACL	0.896/ 2.5	1.037/ 2.3	0.985/ 2.4	1.682/ 2.2	1.312/ 2.4
8	ACL	0.886/ 2.1	1.070/ 2.0	0.993/ 2.3	1.650/ 1.5	1.276/ 1.7
2	STA	0.855/ 2.7	1.071/ 2.1	0.976/ 2.8	1.698/ 1.4	1.346/ 2.3
5	STA	0.895/ 2.6	1.087/ 2.2	1.002/ 2.6	1.788/ 2.0	1.370/ 2.2
9	STA	0.863/ 2.8	1.058/ 2.3	0.979/ 2.7	1.861/ 2.0	1.464/ 2.2
1	MLA	1.019/ 2.6	1.132/ 2.5	1.145/ 2.4	1.970/ 3.0	1.373/ 2.8
7	MLA	1.008/ 2.4	1.296/ 2.8	1.097/ 2.5	2.075/ 2.4	1.516/ 2.1
10	MLA	0.863/ 3.2	1.183/ 3.3	0.973/ 3.3	1.832/ 3.2	1.397/ 2.8
12	MLA	1.009/ 1.8	1.338/ 1.7	1.134/ 1.6	2.105/ 1.5	1.579/ 1.5

Table 4 Mean ISI and between-laboratory CV (in %) for each instrument group, excluding plasma specimens with INR <1.5 or >4.5

Reagent		ISI for ACL (n = 3)	ISI for STA (n = 3)	ISI for MLA (n = 4)
T2	Mean:	0.88	0.87	0.97
	Range:	0.86 - 0.90	0.86 - 0.90	0.86 - 1.02
	CV:	2.0	2.4	7.7
T3	Mean:	1.07	1.07	1.24
	Range:	1.04 - 1.09	1.06 - 1.09	1.13 - 1.34
	CV:	2.4	1.4	7.8
T4	Mean:	0.99	0.99	1.09
	Range:	0.98 - 0.99	0.98 - 1.00	0.97 - 1.14
	CV:	0.4	1.4	7.3
T5	Mean:	1.68	1.78	2.00
	Range:	1.65 - 1.71	1.70 - 1.86	1.83 - 2.10
	CV:	1.9	4.6	6.2
T6	Mean:	1.30	1.39	1.47
	Range:	1.28 - 1.31	1.35 - 1.46	1.37 - 1.58
	CV:	1.6	4.5	6.7

Table 5 Slopes and within-laboratory CV (in %) of slope for orthogonal regression lines including all plasma specimens

Laboratory number	Instrument	Slope/CV for T2	Slope/CV for T3	Slope/CV for T4	Slope/CV for T5	Slope/CV for T6
3	ACL	0.923/ 2.8	1.167/ 2.3	1.069/ 2.7	1.831/ 2.0	1.388/ 2.0
4	ACL	0.937/ 2.6	1.087/ 2.5	1.028/ 2.6	1.741/ 2.3	1.374/ 2.6
8	ACL	0.893/ 2.0	1.079/ 1.9	1.005/ 2.2	1.675/ 1.6	1.290/ 1.7
2	STA	0.859/ 2.6	1.075/ 2.0	0.988/ 2.7	1.697/ 1.4	1.364/ 2.3
5	STA	0.917/ 2.6	1.089/ 2.0	1.033/ 2.5	1.820/ 1.9	1.409/ 2.2
9	STA	0.880/ 2.8	1.076/ 2.3	0.997/ 2.7	1.891/ 2.1	1.478/ 2.2
1	MLA	1.014/ 2.5	1.141/ 2.4	1.160/ 2.4	1.973/ 2.9	1.375/ 2.7
7	MLA	1.013/ 2.3	1.302/ 2.5	1.097/ 2.4	2.045/ 2.3	1.521/ 2.0
10	MLA	0.870/ 3.2	1.169/ 3.2	0.982/ 3.2	1.855/ 3.2	1.399/ 2.7
12	MLA	1.011/ 1.8	1.347/ 1.8	1.148/ 1.7	2.113/ 1.4	1.586/ 1.5

Table 6 Interlaboratory variation (CV, in %) of clotting times and INR for lyophilized coumarin plasma L3. INRs were calculated using local ISI based on fresh plasma testing

	CV of clotting times				CV of INR			
	ACL (n = 3)	STA (n = 3)	MLA (n = 4)	All (n= 10)	ACL (n = 3)	STA (n = 3)	MLA (n = 4)	All (n= 10)
T2	0.8	5.5	1.9	3.8	2.4	8.4	8.7	8.6
T3	3.0	1.5	4.6	3.9	2.1	4.6	7.5	7.6
T4	1.1	4.3	1.4	3.2	3.7	9.3	7.8	8.1
T5	1.5	2.0	2.0	2.9	2.3	4.3	8.3	7.7
T6	2.2	6.7	3.9	4.6	2.2	6.7	8.4	7.9

Table 7 Mean INR and interlaboratory variation (n = 10) for lyophilized coumarin plasma L3. INRs were calculated with the manufacturer's stated ISI and with each laboratory's own ISI determined in this study. Interlaboratory variation is expressed as coefficient of variation (CV, in %)

	Manufacturer's calibration		Local calibration	
Thromboplastin	Mean INR	CV	Mean INR	CV
T2	3.34	8.5	3.15	8.6
T3	2.99	4.8	3.09	7.6
T4	3.13	6.2	3.32	8.1
T5	3.25	10.9	3.25	7.7
T6	3.01	11.2	3.49	7.9

points about the orthogonal regression line. For each laboratory and for each reagent, INR values for the lyophilized and frozen plasmas were calculated from the mean PTs (i.e., the mean PT of 10 days' determinations), the local mean of 20 fresh normal plasma PTs, and the manufacturer's stated ISI or the local ISI determined in the present study. All calculations were performed centrally in the first author's laboratory.

Results

Eleven laboratories completed the exercise. Table 2 shows the numbers of normals and patients. Some patient specimens had INR values smaller than 1.5 or greater than 4.5 as calculated with the international reference preparation RBT/90 (T1). When these specimens were excluded for further calculations, a few outlier points were rejected because their distance from the orthogonal regression line was greater than 3 standard deviations. Table 3 shows the slopes of the orthogonal regression lines and associated standard errors for the 5 reagents obtained by each laboratory. The within-laboratory CV of the slopes ranged from 1.5% (laboratory nr. 12) to 3.3% (laboratory nr. 10). Since laboratory nr. 6 determined PTs with T1 using an instrument rather than the manual tilt-tube technique as required by the protocol, the results of this center were excluded.

When the laboratories were grouped according to the instrument used, the mean ISI increased in the order ACL, STA, MLA for T5 and T6 (Table 4). For T2, T3, and T4, the mean ISI values with ACL and STA were practically the same, but the mean ISI values for MLA were at least 10% greater. The between-laboratory variation for the centers using MLA was greater than the CV for the centers using ACL or STA.

Table 5 shows ISI values calculated for all data, i.e., including patient specimens with INR <1.5 and INR >4.5. The mean ISI values of Table 5 were greater than the corresponding mean values of Table 3.

The interlaboratory variation of clotting times and INRs could be assessed from the results of lyophilized and deep-frozen plasma samples provided to the participants. The results for one lyophilized coumarin plasma are shown in Tables 6 and 7. The interlaboratory variation of the clotting times was smaller than the variation of the INRs calculated with each laboratory's own ISI as determined in this study (Table 6). INRs for this lyophilized plasma were also calculated using the manufacturers' stated ISI values (Table 7). If the manufacturer provided instrument-specific ISI values (i.e., for T3 and T4, see Table 1), the appropriate ISI was used. The stated ISI for T2 was for the manual technique only (Table 1) and this value was used for calculation of INRs in Table 7. The inter-laboratory variation of the INRs calculated with the manufacturer's instrument-specific ISI values (i.e., the values for T3 and T4) was smaller than the variation calculated with the stated non-specific ISI values (i.e., for T2, T5, and T6). Similar results were obtained with frozen coumarin plasma (not shown).

Discussion

The purpose of the present study was to determine ISI values for 5 thromboplastin reagents used with 3 automated coagulometers and to compare these with the manufacturers' stated values. It is recommended that human thromboplastins should be calibrated with the international reference preparation (IRP) for human thromboplastin and rabbit thromboplastins with the IRP for rabbit thromboplastin (1). This is because the precision of calibration is improved when similar thromboplastins are being compared. In the present study both human and rabbit thromboplastins were calibrated with the IRP for rabbit thromboplastin. Even though this may have resulted in suboptimal precision for the human thromboplastins, the mean within-laboratory CVs of the calibration slopes were not greater than the 3% limit imposed by the WHO requirements (Table 3). The *relative* effect of different instruments on the ISI of the human and rabbit thromboplastins is not influenced by the IRP, because all participants used the same IRP and the same (manual) technique for the IRP. We felt that the use of a single IRP was justified for the purpose of the present study.

The coagulometers were based on different end point detection principles: nephelometric (ACL), viscometric (STA), and measurement of light absorbance (MLA). There was good agreement between the three laboratories using ACL instruments (Tables 3 and 4). Among the four laboratories using MLA instruments, there was one (nr. 10) which had >10% smaller ISI for T2 and T4 than the other three laboratories. The cause of this difference is not known.

The between-laboratory CV of the ISI for the MLA laboratories was considerably greater than the within-laboratory CV, suggesting that there were systematic differences between the laboratories. Part of the systematic differences may be caused by small local modifications of the manual technique for the international reference preparation RBT/90 (T1), and/or technical differences between the instruments. The magnitude of the ISI differences between the individual laboratories was not the same for all reagents (Table 3), suggesting that local modification of the method used for T1 was not the only cause.

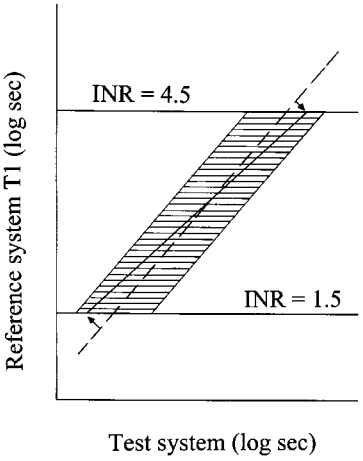

Fig. 1 Effect of selection of patient specimens on calculation of calibration line. Log prothrombin times determined with the reference system (in this case T1) are plotted on the vertical axis; log prothrombin times with the test system (either T2, T3, T4, T5, or T6) on the horizontal axis. The dashed line represents the true line of relationship. The shaded area represents the scatter of individual patient data obtained after data points were selected by using cut-off lines parallel to the horizontal axis corresponding to INR values of 1.5 and 4.5 respectively

There was a trend of greater ISI values for the laboratories using MLA instruments than those obtained by the three laboratories using ACL instruments. This trend was observed for all five reagents. Our results support the notion that it is not possible to assign a single ISI value to a reagent which is valid for all coagulometer systems. Two reagents (T3 and T4) were supplied with instrument-specific ISI values by their respective manufacturers. The ISI values provided by the manufacturer for T3 and various instruments, were 0-5% smaller than the mean values obtained in the present study (cf. Tables 1 and 4). Likewise, the ISI values provided by the manufacturer for T4 were 4-7% smaller than the corresponding mean values of the present study. The manufacturer of T6 supplied a single ISI value (1.22) which was considerably smaller than all values determined in the present study. The manufacturer informed us that the stated ISI had been determined using samples collected with 0.129 mol/l sodium citrate. The participants of the present study used 0.105 mol/l sodium citrate. It is possible that the lower ISI value stated by the manufacturer is the result of using a higher sodium citrate concentration (9, 10).

The ISI values for T2 determined in the present study are slightly different from the mean value reported for the manual technique, i. e. 0.96 (7). Apart from a different technique of clotting time determination, the use of different reference preparations may account for minor ISI differences (11). RBT/90 was used in the present study, but BCT/441 was used by the manufacturers of T2, T4, and T6. According to WHO guidelines, rabbit thromboplastins should be calibrated with RBT/90, and human thromboplastins with BCT/253. BCT/253 stocks were practically exhausted when the present study was carried out (11). However, recent multicenter studies have shown that mean ISI values obtained with RBT/90 were very similar to those obtained with BCT/253 (12).

Selection of patient samples within the range 1.5-4.5 INR is required because the calibration relation should be valid for this range. Patient samples with INR <1.5 or >4.5 are likely obtained from patients in the induction phase of treatment or from other non-stabilized patients, i. e. samples with discrepant levels of coagulation factors VII, X, and II. Non-stabilized samples are associated with greater scatter of points in a calibration plot and greater imprecision of the calibration line (13).

ISI values were slightly influenced by selection of the patient specimens for the calibration. When all specimens were used, ISI values were on average 2.4% greater than the values calculated by excluding INRs smaller than 1.5 and greater than 4.5 (cf. Tables 3 and 5). The excluded INRs were calculated with T1, that is the thromboplastin which was used as reference system for calculation of the slope and ISI. A simple geometrical consideration can account for the systematic decrease of the slope induced by excluding values in the vertical direction only, as shown in Fig. 1 (14). As a result of this method of patient specimen selection, the data points are not distributed symmetrically about the true calibration line. The slope of the orthogonal regression line calculated for the selected specimens is smaller than the slope of the true line. The magnitude of the slope bias is related to the scatter of points around the line: the greater the scatter, the greater the bias. The bias may be avoided by using a third system for selection of samples. We recommend that patient samples for calibration should be selected by using an independent prothrombin time system. Since the specimens are usually provided by an anticoagulant clinic, the system used by the clinic for routine monitoring of the patients could be used to select the specimens that fit the 1.5-4.5 INR interval suggested by the WHO guidelines. Once selected, the specimens should be used for the calibration even if the INRs determined with the reference system appear to be outside the 1.5-4.5 INR range.

The lyophilized plasmas included in this study could be used to assess the interlaboratory variation of the clotting times (Table 6). The differences between the three laboratories using ACL instruments were smaller than the differences between the participants using STA or MLA. The interlaboratory variation of the corresponding INRs was greater (Table 6), but the differences between the three ACLs were again smaller than those between the three STAs or between the four MLAs. It is no surprise that the interlaboratory variation of the INRs is greater than that of the clotting times, because the error in the INR is a result of error in both the clotting times and the ISI values. The interlaboratory variation of the INRs in this study compared well to that in previously published reports (15-17).

The interlaboratory variation of the INR of lyophilized coumarin plasma was lower with instrument-specific ISI values than with a single ISI for all instruments (Table 7). This finding is to be expected when instruments have a significant influence on the ISI. In fact, the interlaboratory variation of the INRs calculated with the instrument-specific ISI values stated by the manufacturers of T3 and T4 was even lower than the CVs with the local ISI values (Table 7). These results suggest that the stated ISI values for T3 and T4 were accurate, and that the instruments used in the present study were very similar to those used by the manufacturers. It is recommended that thromboplastin manufacturers provide instrument-specific ISI values for their products.

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