# A Comparison of INRs after Local Calibration of Thromboplastin International Sensitivity Indexes

DAVID L MCGLASSON

There are approximately 300 reagent/instrument combinations for performing prothrombin times/international normalized ratios (PT/INR) in the United States. Manufacturers and laboratories continually struggle to ensure that the International Sensitivity Index (ISI) of their thromboplastin is accurate for assaying PT/INR.

OBJECTIVE: This study reports the feasibility of a new method to locally calibrate ISI of thromboplastin using the mechanical STA automated coagulation analyzer (Diagnostica-Stago Inc.) and two photo-optic coagulation analyzers, the BCS (Dade-Behring) and CA-540 (Sysmex).

DESIGN: Neoplastine CI+ (CI+) (Diagnostica-Stago Inc); Thromboplastin C+ (TC+); Thromborel S (TRS); and Innovin (I) (Dade-Behring) were used in this study. A mean normal PT (MNPT) was determined for each reagent/instrument combination using samples from 25 normal individuals. Manufacturer instrument specific ISI values were not available for the STA with TC+, TRS and I. The CA540 had no ISI value for CI+ and the BCS system had no manufacturer assigned ISI values for TC+ and I; generic photo-optic and mechanical ISI manufacturer values were used for these two systems. Local on-site calibration was performed using frozen plasma calibrators to determine ISI values for each thromboplastin. Post-calibration, 95 patient samples were assayed for each reagent/instrument system combination using the manufacturer ISI and the local calibrated ISI to determine the INR result.

PATIENTS: Patients from whom samples were obtained included five with a lupus anticoagulant, 30 on heparin therapy, and 60 on coumadin therapy.

RESULTS: Differences between manufacturer versus local calibrated ISI ranged from 0.9% to 18.9% for normal sample INRs and from 0.8% to 16.4% for patient sample INRs. The number (or proportion) of patient specimens with clinically significantly different INR values (>10.0% difference) ranged from zero for several reagent combinations to more than half (or >50.0%) of those tested for several other combinations.

CONCLUSION: Our results indicated that by locally calibrating ISI values, each laboratory may eliminate variability and guesswork between different reagent/instrument systems for ISI values when performing PT/INR assays and potentially improve the clinical accuracy of their patients' PT/INR results.

ABBREVIATIONS: CI+ = Neoplastine CI+; INR = international normalized ratio; IRP = international reference preparation; ISI = international sensitivity index; MNPT = mean normal prothrombin time; OAT = oral anticoagulant therapy; PIVKAS = proteins induced by Vitamin K antagonists; PT = prothrombin time; TC+ = Thromboplastin C+; TRS = Thromborel S; WHO = World Health Organization.

INDEX TERMS: international normalized ratios; international sensitivity index; local calibration; prothrombin time; reagent/instrument combinations.

Clin Lab Sci 2002;15(2):91

*David L McGlasson is a Research Clinical Laboratory Scientist at the 59th Clinical Research Squadron, Lackland AFB TX.*

*Address for correspondence: David L McGlasson MS CLS/NCA, 59th Clinical Research Squadron/MSRL, 1255 Wilford Hall Loop, Lackland AFB TX 78236-5319. (210) 292-6555, (210) 292-6053 (fax). davemcglasson@hotmail.com*

Although the PT is the principal assay for monitoring patients undergoing oral anticoagulant therapy, there are many variables such as proper sampling and testing techniques that can have an effect on the accuracy of the assay. Probably the most important of these variables is the thromboplastin reagent selected for the assay. Commercial thromboplastins vary widely in their sensitivities to warfarin. Instrument choice can also play a significant role in the performance of the PT assay. With so many variables, clinicians can be easily confused when having to compare patient results from a variety of laboratories using different thromboplastin reagent/instrument combinations. High sensitivity thromboplastin reagents lead to greater prolongation of the PT time results than assays performed with a lower sensitivity. Thus, a patient may have a PT of 14 seconds with a low sensitive thromboplastin and a PT of 18 seconds with a more sensitive thromboplastin. Therefore, a patient monitored with insensitive thromboplastins would require a higher dosage of warfarin to result in an appropriate prothrombin time ratio.[1,2,3]

In 1977, the World Health Organization (WHO) recognized the difficulty of comparing PT times performed with different thromboplastins and introduced a thromboplastin that was to serve as an international reference preparation. Then in 1983, the WHO described a model for PT standardization based on a method in which the PT value is reported as an international normalized ratio (INR). Theoretically, the INR is the PT that one would obtain if the assay were performed using a WHO primary reference with

an ISI value of 1.0. The ISI compares the sensitivity of a given thromboplastin to an international reference plasma that has been calibrated using the WHO reference plasma. The INR is calculated using the formula:

$$INR = \frac{(\text{Patient PT})^{ISI}}{\text{Mean Normal PT}}$$

The advantage of the INR method of reporting is that the patient's result is compensated for between differing thromboplastins or instruments. While thromboplastins now have ISI values that may range from 0.9 to 3.0, as assigned by the manufacturer, the patient values can be compared using the calculated INR. The INR allows for a better regulation of the dosage of oral anticoagulation.[2]

Concerns regarding the INR system have concentrated primarily on the assignment of ISI values, method or instrument variations, and calculation errors. ISI values appear to be instrument-dependent. The ISI is used exponentially in calculating the INR and, therefore, any error in this number may result in significantly inaccurate values. INR variability, which may occur when there exists significant differences in ISI values from reagents with low ISI values versus reagents with high ISI values, has been reported.[4-10] These differences could result in inappropriate management of patients on oral anticoagulant therapy (OAT) with possible dire consequences of thrombotic or bleeding episodes.

The individual variability between reagent/instrument test systems suggests that each laboratory may need to calibrate its own PT/INR test systems. One method that has been somewhat reliable is the WHO protocol. The method uses an International Reference Preparation (IRP) of thromboplastin with the manual (tilt-tube) method with 20 fresh samples from normal subjects and 60 fresh samples from normal subjects on OAT, performed in quadruplicate. Obviously this is not a practical method for many clinical laboratories due to lack of practice with the manual procedure or the unavailability of so many patient samples.[11,12]

Local system calibration using well characterized plasma calibrants may be a practical alternative to the WHO protocol. However, until recently there have been no FDA cleared calibrator plasmas commercially available. Controversy exists regarding the use of artificially depleted vitamin-K plasmas or actual OAT plasmas. Results with these plasmas in various investigations have given disparate results, presumably due to the influence of proteins induced by Vitamin K antagonists (PIVKAS).[13-15] Lyophilization may also cause changes in plasma and affect results in coagulation assays.[15-17] This effect may vary for different reagent/instrument systems. In addition, there are many investigators who recommend using large plasma pools instead of individual patient plasmas.[12] One manufacturer uses 20 artificially depleted plasma calibrators prepared from normal donors that cover the OAT therapeutic range. This is a one-day protocol that uses the local reagent/instrument combination with the PT assays being performed in quadruplicate and analyzed with orthogonal regression analysis.[18] This protocol may not take into account the day to day variability seen in a laboratory setting.

Recently, the idea of frozen calibration plasmas for local calibration of ISI values has been introduced. Precision BioLogic (Dartmouth NS Canada) has produced an ISI calibration set consisting of five frozen OAT frozen plasmas and one frozen normal plasma that have been assayed against a standard WHO IRP of human recombinant thromboplastin (RTF/95).[11,19] In a study with 122 participating laboratories the normal frozen reference plasma gave an INR of 1.06. The frozen OAT calibrators had a range of 1.72 to 4.62. The INR results of the normal plasma and the OAT calibrator plasmas encompassed the four therapeutic categories used in OAT (<2.0; 2.0 to 3.0; 3.1 to 4.5; and >4.5).

In a study sponsored in part by Dade-Behring Inc (Deerfield IL), we evaluated the frozen plasma calibrants to determine if local calibration of ISIs for a variety of thromboplastins could be simplified to improve correlation of INR results between different reagent/instrument combinations.

## MATERIALS AND METHODS

**Instrumentation:** electro-mechanical STA automated coagulation analyzer (Diagnostica-Stago Inc, Parsippany NJ) and two photo-optic coagulation analyzers, the BCS (Dade-Behring, Deerfield IL) and CA-540 (Sysmex, Kobe Japan).

**Thromboplastins:** Neoplastine CI+ (CI+) (Diagnostica-Stago Inc), Thromboplastin C+ (TC+) (Deerfield IL), Thromborel S (TRS) (Deerfield IL), and Innovin (I) (Dade-Behring, Deerfield IL). Thromboplastin CI+ is a rabbit-brain source thromboplastin as is TC+. TRS is a human placenta source. I is human recombinant thromboplastin.

All of the specimens for this study were obtained after informed consent was obtained from each subject. All of the patient samples for this study were obtained previously and stored in the following manner. An atraumatic venipuncture was performed using a Vacutainer collection system with 3.2% sodium citrate in a 9:1 blood to anticoagulant ratio on each test subject. Platelet-poor plasma was obtained by centrifuging each specimen at 2500g for 15 minutes. The specimens were aliquoted into cryovials and stored at approximately –70 °C until ready for testing. Just prior to testing, the samples were thawed rapidly at approximately 37 °C. A mean normal PT (MNPT) was determined for each reagent/instrument combination by assaying 25 normal individuals with no known coagulation abnormalities and who were not on any medication that could influence their coagulation system. The samples from normal donors were assayed three separate times, with each

reagent/instrument system using three different sets of reagents for each combination, over a three-day period. Manufacturer instrument specific ISI values were not available for the STA with TC+, TRS, and I. The CA540 had no ISI value for CI+. The BCS system had no manufacturer assigned ISI values for TC+ and I. Generic photo-optic and mechanical ISI manufacturer values were used for these two systems. The MNPT was determined using a geometric mean. Currently Dade-Behring Inc can now furnish instrument specific ISI values for all of their own reagent/instrument combinations.

A local on-site calibration to determine international sensitivity index (ISI) values for each thromboplastin was performed using a protocol for frozen ISI calibration plasma sets from Precision BioLogic (Dartmouth NS Canada). Calibrators were run in duplicate for five individual runs, with freshly diluted reagents on each reagent/instrument system. A total of 60 PT data points (10 normal and 50 abnormal) for each reagent/instrument combination were derived. The results were sent to Precision BioLogic, which used orthogonal regression analysis to determine the local calibrated ISI of each system.

After the local ISI results had been determined, we assayed 95 patient samples on each reagent/instrument system using the manufacturer ISI and the local calibrated ISI to determine the INR result. The patient samples included five from subjects with a lupus anticoagulant, 30 from heparinized subjects, and 60 from coumadin patients. We then compared the PT/INR results using the manufacturer ISI to the INR results achieved using the locally generated ISI values.

## RESULTS

Table 1 shows the geometric mean results of each reagent/instrument combination MNPT. The times ranged from 8.9 to 13.9 seconds. In Table 2 it is interesting that the percent difference in the ISI did not directly correlate to the sensitivity of the thromboplastin with each reagent/instrument combination. The differences in INR means ranged from 0.9% to 18.9%. We note that some of the reagents were made for mechanical systems while others are directed towards photo-optic instruments. The local ISI calibration is designed to correct the reagent/instrument generated bias that the INR calculation is supposed to correct. Table 3 shows that there can be clinically significant mean differences (>10.0%) between INR results using manufacturer ISI values versus locally calibrated ISI results. The last column (Results >10.0% difference) expresses the number of patient samples (n = 95) that had INR values of >10.0% difference between the different ISI generated values. It was noted that the TRS thromboplastin gave no values that had a >10.0% difference in the subject values with any instrument. Some other reagent/instrument combinations had 64.9% of the patient samples with a >10.0% difference between the vendor assigned ISI and a locally calibrated ISI.

## DISCUSSION

The accuracy and precision of the INR is dependent on the PT assay and the ISI of thromboplastin. Other researchers have noted that, since the ISI is the exponent of the INR equation, the higher the ISI assigned to thromboplastin the greater the imprecision of the INR as a result of the mathematical outcome. The large difference in assigned ISI values is one variable that influences the poor correlation seen in reagent/instrument combinations. Thromboplastins with an ISI less than 1.20 produce a wider range of values in the PT and PT ratio. Consequently, the precision of the INR is improved. Since the calculation of the INR requires using the ISI exponentially, the farther the ISI value is above 1.0, the greater any system inaccuracies will be magnified in terms of INR. Manufacturer assigned ISI not specific to the local reagent/instrument set-up may introduce even more inaccuracy. Instrumentation can also greatly influence the INR values. In our institution we saw serious clinically significant differences in INR results between photo-optic and mechanical reagent/instrument systems using manufacturer generated ISI values particularly at the upper limits of the OAT INR range (>3.0).[20] This could lead to serious errors in OAT treatment decisions. Table 4 demonstrates the large INR differences that resulted for selected patient samples between different reagent/instrument combinations.[20] Because manufacturers provide limited guidelines for all instruments assigned ISI values, the laboratory should be able to validate INR results by performing local on-site ISI calibration. Early researchers in INR studies used the tilt tube method making today's studies comparing photo-optic and mechanical clotting endpoint values suspect, at best. For mechanical endpoint clot detection systems most manufacturers still use the Fibrometer to assign ISI values. With today's

Table 1. Summary of MNPT according to reagent/instrument test system

| Instrument | Reagent | MNPT (sec) |
|---|---|---|
| Diagnostica Stage/STA | Neoplastine CI+ | 13.9 |
| | Thromborel S | 12.9 |
| | Thromboplastin CI+ | 13.9 |
| | Innovin | 9.9 |
| Sysmex 540 | Neoplastine CI+ | 12.7 |
| | Thromborel S | 11.9 |
| | Thromboplastin CI+ | 11.7 |
| | Innovin | 10.6 |
| Dade Behring BCS | Neoplastine CI+ | 13.8 |
| | Thromborel S | 12.6 |
| | Thromboplastin CI+ | 11.0 |
| | Innovin | 8.9 |

Table 2. Summary of mean INR results on 25 normal patient samples before and after local ISI calibration with each reagent/instrument test system

| Instrument | Reagent | Mean INR using vendor assigned ISI | Mean INR using locally calibrated ISI | Difference in INR means (%) |
|---|---|---|---|---|
| Diagnostica Stago/STA | Neoplastine CI+ | 1.24 | 1.29 | -3.9 |
| | Thromborel S | 1.12 | 1.13 | -0.9 |
| | Thromboplastin C+ | 1.96 | 1.76 | 10.1 |
| | Innovin | 0.92 | 1.00 | -8.5 |
| Sysmex CA 540 | Neoplastine CI+ | 1.24 | 1.09 | 11.9 |
| | Thromborel S | 1.06 | 1.02 | 3.4 |
| | Thromboplastin C+ | 1.95 | 1.81 | 7.4 |
| | Innovin | 1.02 | 0.92 | 10.3 |
| Dade Behring BCS | Neoplastine CI+ | 1.24 | 1.16 | 6.9 |
| | Thromborel S | 1.06 | 1.02 | 3.5 |
| | Thromboplastin C+ | 1.77 | 1.57 | 11.1 |
| | Innovin | 0.90 | 1.07 | -18.9a |

Table 3. Summary of mean INR results on 95 patient samples before and after local ISI calibration according to test system

| Instrument | Reagent | Mean INR using vendor assigned ISI | Mean INR using locally calibrated ISI | Difference in INR means (%) | Results >10% difference |
|---|---|---|---|---|---|
| Diagnostica Stago/STA | Neoplastine CI+ | 2.58 | 2.69 | 4.1 | 0.0 |
| | Thromborel S | 2.56 | 2.58 | 0.8 | 0.0 |
| | Thromboplastin C+ | 2.39 | 2.15 | 10.0 | 37.1 |
| | Innovin | 2.45 | 2.67 | 7.9 | 22.1 |
| Sysmex CA 540 | Neoplastine CI+ | 3.46 | 2.90 | 16.1 | 63.9 |
| | Thromborel S | 2.69 | 2.58 | 4.1 | 0.0 |
| | Thromboplastin C+ | 2.82 | 2.60 | 7.8 | 27.8 |
| | Innovin | 2.49 | 2.25 | 9.6 | 39.2 |
| Dade-Behring BCS | Neoplastine CI+ | 2.81 | 2.60 | 7.5 | 20.6 |
| | Thromborel S | 2.67 | 2.57 | 3.7 | 0.0 |
| | Thromboplastin C+ | 2.86 | 2.51 | 12.6 | 52.6 |
| | Innovin | 2.66 | 3.29 | 16.4 | 64.9 |

highly automated mechanical clot-detection systems, this appears to be a less than optimal instrument method for assigning ISI values. Photo-optical coagulation systems use many diverse methods for determining a clot formation that may also greatly influence the ISI of different reagents.

## CONCLUSION

In our protocol we used 12 different reagent/instrument combinations and determined the local ISI by calibration for each coagulation system. We then compared the results using samples from normal subjects and specimens from variety of patients. Our results indicate that this local on-site calibration protocol may help eliminate variability and guesswork between any reagent/instrument sys-

Table 4. Comparison of selected INR differences of OAT subjects plasma on the STA and MLA 900C with thromboplastins CI+, T-D, T-R, and the MLA 1600C with T-D only

| Human Sample | MLA 900C CI+ | MLA 900C T-D | MLA 900C T-R | MLA 1600C T-D | STA CI+ | STA T-D | STA T-R | Difference in INR (%) |
|---|---|---|---|---|---|---|---|---|
| 167 | 2.77 | 3.41 | 3.72 | 3.62 | 2.68 | 3.24 | 3.41 | 38.8 |
| 1181 | 2.53 | 3.20 | 3.65 | 3.36 | 2.64 | 2.85 | 2.91 | 44.2 |
| 1180 | 3.95 | 5.98 | 5.83 | 7.16 | 4.02 | 4.93 | 5.05 | 81.2 |
| 1398 | 2.94 | 3.94 | 3.44 | 4.14 | 3.06 | 3.61 | 3.35 | 40.8 |
| 1393 | 3.57 | 4.98 | 4.48 | 5.00 | 3.57 | 4.39 | 4.18 | 40.1 |
| 1391 | 3.03 | 4.14 | 3.41 | 4.43 | 3.13 | 3.82 | 3.14 | 46.2 |
| 1684 | 3.72 | 5.82 | 4.99 | 6.29 | 3.99 | 5.57 | 4.47 | 69.1 |
| 1688 | 2.95 | 3.94 | 3.93 | 4.44 | 3.12 | 3.70 | 3.79 | 50.5 |
| 1814 | 3.15 | 1.97 | 4.00 | 4.85 | 3.24 | 4.23 | 3.58 | 146.2 |
| 1669 | 3.25 | 2.41 | 3.66 | 3.93 | 3.24 | 4.29 | 3.28 | 78.0 |

CI+ (Neoplastine CI+, Diagnostica-Stago Inc. Parsippany NJ); T-D (Thromboplastin-D, Pacific-Hemostasis, Huntersville NC;, T-R (Recombiplastin, Hemoliance, Pleasantville NY).

tems for ISI values when performing PT/INR assays and potentially improve the clinical accuracy of PT/INR results for patients on OAT.

## ACKNOWLEDGEMENTS

I would like to thank Dade-Behring Inc for their financial and technical assistance in support of this protocol. I would like to thank the following individuals for their personal support: Julie Carlucci, Diane Shafer, Ruben Siller, Tom Hogan, and Sherry Hagman from Dade-Behring Inc for technical and administrative support. Steve Duff from Precision BioLogic was always available for support. Captain Eric Olsen and Rachel Montez gave outstanding editorial assistance.

The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense or other Departments of the U.S. Government.

This article was presented in part at the ASCLS meeting 2000.

## REFERENCES

1. Ens GE. A proposed inter-laboratory INR support system. Clin Hemos Rev 1997;10:3-5.
2. Jensen R. Monitoring anticoagulant therapy. Clin Hemos Rev 1996;10:4-6.
3. Hirsch J, Poller L. The international normalized ratio-a guide to understanding and correcting its problems. Arch Intern Med 1994;154:282.
4. Pi DW, and others. Effect of thromboplastin and coagulometer interaction on the precision of the international normalized ratio, J Clin Pathol 1995;103:358.
5. Poller L, Thomson JM, Taberner DA. Effect of automation on the prothrombin time test in NEQAS Surveys. J Clin Pathol 1989;42:97.
6. Brien WF, Crawford L, Wood DE. Discrepant results in INR testing. Throm Hemost 1994;72(6):985-9.
7. Carraro P, Varagnolo MC, Plebani M. International normalized ratio: merits, limits and improvements. Int J Clin Lab Res 1995;25:56-7.
8. Brophy MT, and others. Comparison of a standard and a sensitive thromboplastin in monitoring low intensity oral anticoagulant therapy. AJCP 1994;102(1):134-7.
9. Cunningham MT, Johnson GF, Pennell BJ, Olson JD. The reliability of manufacturer-determined, instrument-specific international sensitivity index values for calculating the international normalized ratio. AJCP. 1994;102(1):128-33.
10. Ts'ao C, Swedlund J, Neofotistos D. Implications of use of low international sensitivity index thromboplastins in prothrombin testing. Arch Pathol Lab Med 1994;118:1183-7.
11. McGlasson DL. A review of variables affecting PTs/INRs. Clin Lab Sci 1999;12:353-8.
12. Fairweather RB, Ansell J, Anton M, and others. College of American Pathologists Conference XXXI on laboratory monitoring of anticoagulant therapy: laboratory monitoring of oral anticoagulant therapy. Arch Pathol Med 1998;122:768-81.
13. Houbouyan IL, Goguel AF. Procedure of reference calibrated plasmas for prothrombin time standardisation. Thromb Haemost 1993;69:663. Abstract.
14. Stevenson KJ, Craig S, Dufty JMKL, Taberner DA. System ISI calibration: a universally applicable scheme is possible only when coumarin plasma calibrants are used. Br J Haematol 1997;96:435-41.
15. Houbouyan LI, Gouguel AF. Long-term French experience in INR standardisation by a procedure using plasma calibrants. Am J Clin Pathol 1997;108:83-9.
16. Tripodi A, Chantarangkul V, Akkawat B, and others. A partial factor V deficiency in the external quality assessment scheme. Thromb Res 1995;79:283-92.
17. Van den Besselaar AMHP. Field study for lyophilized plasmas for local prothrombin time calibration in the Netherlands. J Clin Pathol 1997;50:371-4.
18. Poller L, Barrowcliffe TW, Anton MH, and others. Minimum lyophilized plasma requirement for ISI calibration. Am J Clin Pathol 1998;109:196-204.
19. Adcock DM, Duff S. Enhanced standardization of the international normalized ratio (INR) through the use of plasma calibrants-A concise review. Blood Coag Fibrin 2000;11:
20. McGlasson DL, Hickman JR, More LE, and others. Discrepancies in international normalized ratios (INR) in swine and humans. Clin Lab Sci 1998;11:156-9.