Editorial

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Manual tilt tube method for prothrombin time: a commentary on contemporary relevance

https://doi.org/10.1515/cclm-2025-0157

Keywords: international normalised ratio; prothrombin time; INR; warfarin; vitamin K antagonist therapy

The prothrombin time (PT) is one of the most often performed routine coagulation tests, along with the activated partial thromboplastin time (aPTT), fibrinogen and D-dimer assays [1]. In addition to its potential utility to broadly assess hemostasis, the PT is often used to monitor vitamin K antagonist (VKA) therapy, for example with warfarin. For this purpose, the PT is typically converted to an international normalised ratio (INR), using the formula:

$$\mathrm{INR} = (\mathrm{PT}/\mathrm{MNPT})^{\mathrm{ISI}}$$

where the PT is derived from the patient, typically from an automated hemostasis or coagulation analyser, the MNPT is the 'mean normal PT', and the ISI is the international sensitivity index [2]. The MNPT and the ISI can be derived using a variety of methods [3], and a generic or instrument specific ISI is often provided by manufacturers of PT reagents. The historical classical method for generating an ISI requires a manual tilt tube method (MTTM) for performing PTs and a reference thromboplastin reagent with a pre-established ISI as the reference, for estimation of an ISI for a new PT reagent [4–6]. Manufacturers of PT reagents tend to use the MTTM to help establish the ISI of their PT reagents prior to commercial release, but often use secondary reference thromboplastin reagents, with some linkage to the official reference thromboplastin reagent, due to limited supply of the latter. However, this process is onerous, requiring MTTM PT values for at least 60 samples from patients stabilised on VKA therapy, plus at least 20 samples from normal individuals. In addition, no modern hemostasis laboratory uses such a process, and instead uses alternate procedures for verification of a manufacturer assigned ISI and generation of a MNPT, largely as per CLSI (Clinical & Laboratory Standards Institute) guidance [3]. Thus, in the age of modern hemostasis analysers, the art of MTTM processing has largely been lost. In the past, our laboratory used to perform MTTM testing when it housed the Australasian Reference Thromboplastin (ART) unit, and later laboratory staff would reflex to a MTTM to check flagged or no clot results from the automated analyser, but we no longer do so in 2025. Indeed, the MTTM is no longer employed throughout the entire 60 laboratory network of NSW Health Pathology (NSWHP). It is doubtful that this represents an isolated case. Accordingly, the continued relevance of the MTTM in 2025 can probably be raised.

In the current issue of this journal, van Rijn et al. describe their experience of MTTM testing for PT in an external quality assessment (EQA) exercise using a total of eight operators [7]. They show improvement in performance of MTTM over time and compared with previous studies. They also show a reasonably low coefficient of variation (CV) amongst the cohort of participants, generally less than 3 %. Thus, in theory (and in practice for the low number of users identified within this study), the MTTM can be reliably used for the purpose of INR standardisation (or ISI calculation).

Of course, this report also leads to several questions: Even if we accept that the MTTM for PT can be used for said purpose, should we continue with this procedure in 2025? The authors cite many valid reasons for continued use of the MTTM in this setting. First, all previous international reference thromboplastins have been calibrated in multicentre studies using the MTTM. Second, it is well known that the value of the ISI can be influenced by the use of various automated coagulation instruments, indeed therefore highlighting the need to establish an ISI for a particular instrument/reagent combination. Thus, the use of the MTTM avoids instrument biases; moreover, replacing the MTTM by one particular automated instrument procedure would require that this particular instrument be available worldwide for the life of the international reference thromboplastin. One the other hand, since no modern hemostasis

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laboratory in the developed world likely uses the MTTM in 2025, there is a large disconnect between what is used by manufacturers to assign an ISI, vs. what is applied in the routine laboratory; for example, does continued use of the MTTM process instead create unintended bias according to the transfer of MTTM generated ISI to the use of associated ISI values generated for automated analysers? Second, an EQA of eight participants using MTTM for PT [7] can be compared to EQA for automated analysers, which often comprise hundreds, perhaps thousands, of participants. An example of EQA for the PT and the INR from the RCPAQAP (Royal College of Pathologists of Australasia Quality Assurance Program) is given in Figure 1 for 2024, being the last year of available data. As noted, overall participant numbers for PT exceed 900 (Figure 1A), with great variation evidenced in PT values according to participant (or reagent/instrument combination used at each participant site). As expected, PT values for optical test systems (green regression lines in Figure 1B) seem to be in general lower than those for mechanical test systems (green regression lines in Figure 1C). However, the INR system, taking into consideration reagent/instrument bias for ISI and MNPT, with ISIs largely assigned by manufacturers, presumably in part using the MTTM, should theoretically fix these discrepant PTs and yield comparatively more uniform results for INR. However, even in 2024, the evidence continues to show problems with INRs, with similar (albeit lower) bias compared to PTs still evident (Figure 1D–F). So, does this imply that the ongoing used of the MTTM is not fixing the problem with INR variation? Well, although manufacturer assigned ISIs are likely, at least in part, to contribute to ongoing INR variation, some of the variation also lies with individual laboratories as they attempt to verify manufacturer ISIs and establish MNPT values. As noted, these laboratories are unlikely to use the MTTM to verify ISIs, instead likely to adopt alternate methods as per CLSI guidance [3]. This may include the use of INR certified (or calibration) plasma sets by participants. Whatever the situation, be it continued application of the MTTM, or laboratory derived problems with verification of ISIs and MNPTs, the current state of play is certainly still not working very well.

Our own laboratory moved away from the established methods for ISI and MNPT estimation/verification in 2008 [9]. At that stage, we identified several problems with the established and recommended procedures [3] and could identify different ISI values for the same PT reagent lot using different INR certified/calibration plasma sets, and different MNPT values were generated for the same PT reagent either using different INR certified/calibration plasma sets or different sets of 20 normal individual plasmas [9]. We therefore established an alternate procedure, based on simple regression analysis, where we established an ISI and a MNPT for a particular PT reagent/instrument combination, and then we used this material as the 'reference thromboplastin' for the next change in PT reagent lot. Subsequently, the new PT reagent/instrument combination became the 'reference thromboplastin' for the next change in PT reagent lot, and so on and so on. We have shown, in many subsequent publications [8, 10–14], the validity of this process to establish/verify ISI/MNPT values for the subsequent 16 years, first within our Westmead laboratory, then as applied to a small network of local Sydney metropolitan labs, then across a network of 27 laboratories within NSWHP. Indeed, the process has most recently been applied to the entire 60 laboratory (85 instruments) NSW Health Pathology network [8, 15]. Evidence via EQA continues to show low between lab variability (inter-lab CVs <5 %) and bias with our novel approach [8]. Disappointingly, our novel approach to harmonisation of the INR, including harmonising the verification of the ISI/MNPT, seems to not as yet been taken up by other laboratories/laboratory networks. The process has most recently been able to identify an incorrect ISI assignment by a leading manufacturer for a PT lot under trial by the NSWHP network. The manufacturer assigned ISI for the ACL TOP analysers for this lot of reagent, 0.96, was substantially different to our estimated ISI of 1.02 using our regression analysis comparing the new lot with the previous reagent lot, and interestingly also differed to the ISI (1.01) the manufacturer had assigned to their ACL 9000/10000 line. Use of the manufacturer assigned ISI of 0.96 evidenced a major shift and bias to INR results compared to that generated by the existing PT reagent lot (from the same manufacturer, used as reference).

Some hemostasis experts may say that the INR system is no longer as relevant in 2025, as it was in the past, given that the newer direct oral anticoagulants (DOACs) are increasingly replacing VKAs [16]. However, although the number of INRs being performed globally may be decreasing, the number of laboratories performing INR testing have not decreased. In other words, although hemostasis laboratories may be performing fewer INRs, they are all still performing INRs, and thus are still required to ensure the accuracy of their test process, including as applied to ISI/MNPT verification.

To conclude, the MTTM for PT, as used by manufacturers to assign ISI values to their reagents/instruments continues to be in use, despite the large disconnect to current universal automated procedures for PT/INR, and laboratory based methods for ISI/MNPT verification. Good concordance in PT values can be shown for experienced MTTM operators [7]. However, the INR system, as in part grounded on MTTM-based ISI assignment remains very problematic in 2025, with vastly different INR values obtained for the same homogeneous plasma cross tested in EQA across different laboratories, even

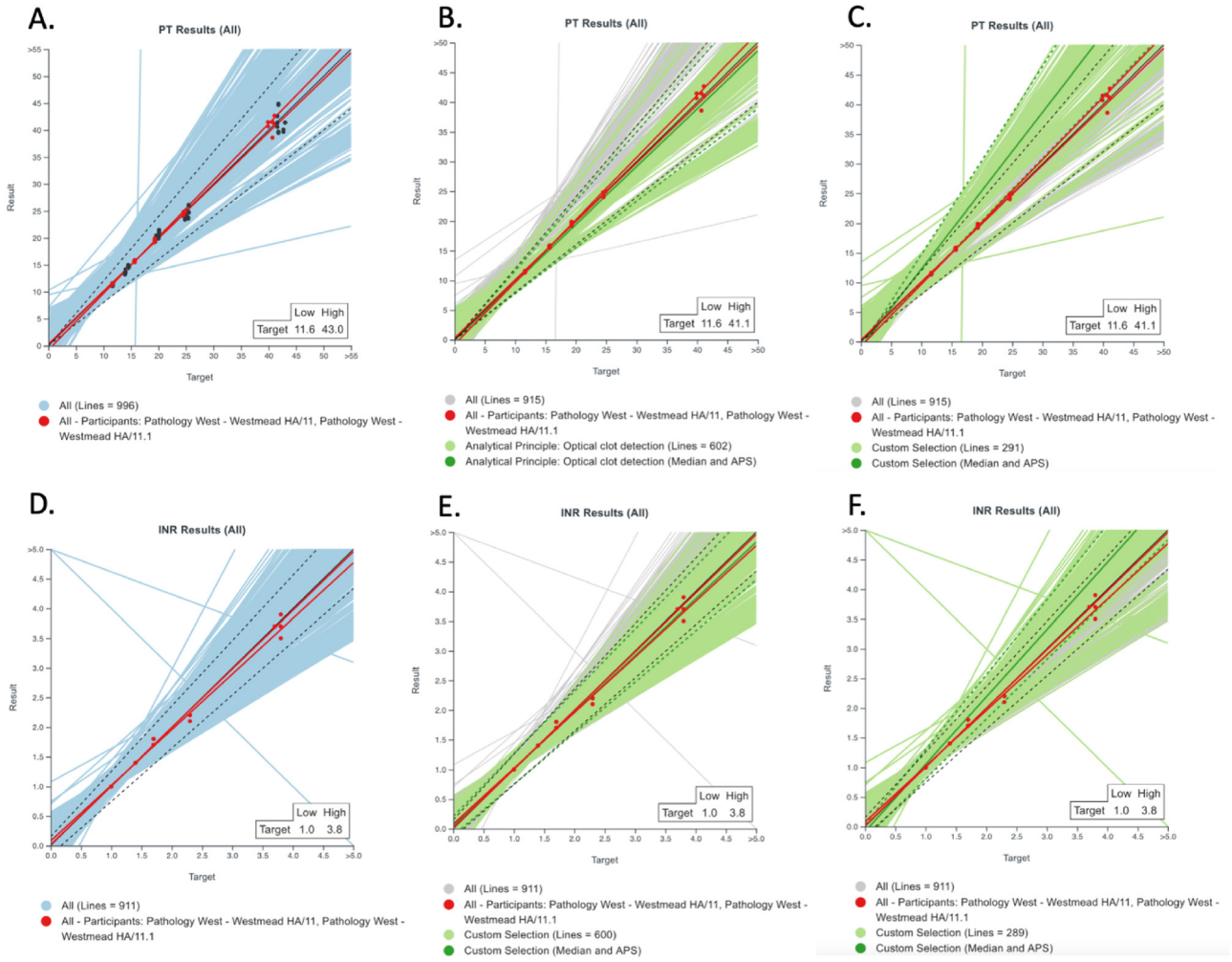

Figure 1: Ongoing variability in prothrombin time (PT) and international normalised ratio (INR) values reported for the same tested homogeneous samples, as evidenced by external quality assessment (EQA). Data shown for 2024, from the RCPAQAP (Royal College of Pathologists of Australasia Quality Assurance Program). (A) Regression lines for all participant submissions (n=996) for PT (participant reported PT value vs. median of reported values = 'target'). The line of identity is shown as a black line from bottom left to top right, but is largely obscured by the red regression lines that identifies data from the Westmead laboratory. Some regression lines bear no relationship to the line of identity, and these identify laboratories that have made significant errors throughout the year. However, almost all regression lines follow the line of identity to some extent, albeit most showing some bias from this line, most likely due to variation in reagent and instrument. (B) As per Figure A, but green regression lines identify optical detection instrumentation, which tend to yield lower PTs than other (mostly mechanical) methods shown as grey lines. (C) As per Figure B, but green regression lines identify mechanical detection instrumentation, which tend to show higher PTs than other (mostly optical) methods shown as grey lines. (D) Regression lines for all participant submissions (n=911) for INR (participant reported INR value vs. median of reported values = 'target'). The line of identity is shown as a black line from bottom left to top right, but is largely obscured by the red regression lines that identifies data from the Westmead laboratory. Some regression lines bear no relationship to the line of identity, and these identify laboratories that have made significant errors throughout the year. However, almost all regression lines follow the line of identity to some extent, albeit most showing some bias from this line. There is lower bias compared to PT (Figure 1A), but there remains considerable bias. In theory, the INR system takes into consideration reagent/instrument variation due to application of the ISI/MNPT; thus, the existing bias is most likely due to variation in, and inaccurate assignment of, ISI and MNPT. The Westmead regression lines, basically superimposed onto the line of identity, is generated using ISI and MNPT values verified according to a novel, simple regression analysis process [8]. (E) As per Figure D, but green regression lines identify optical detection instrumentation, which despite ISI/MNPT 'adjustments' to the PT, still tend to show lower INRs than other (mostly mechanical) methods shown as grey lines. (F) As per Figure E, but green regression lines identify mechanical detection instrumentation. Source of data: RCPAQAP participant portal (https://myqap.rcpaqap.com.au).

when laboratories are using the same PT reagent lot and instrument [17]. My own, admittedly biased, view is that in the age of big data and artificial intelligence [18, 19], we can come up with more modern alternatives to continued application of the MTTM for INR harmonisation, with our own novel process [8, 10–15] potentially being part of the solution.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: The author has accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The author states no conflict of interest.
Research funding: None declared.
Data availability: Not applicable.

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