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ORIGINAL ARTICLE

Paving the way for establishing a reference measurement system for standardization of plasma prothrombin time: Harmonizing the manual tilt tube method

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Abstract

Background: International normalized ratio (INR) is traceable to World Health Organization (WHO) International Standards for thromboplastins. International Standards must be used with a manual tilt tube technique (MTT) for prothrombin time (PT) determination. An important part of the total variability of INR is due to poor harmonization of MTT across WHO reference laboratories.

Objectives: To determine the origins of PT differences between operators performing MTT and to develop a harmonized MTT.

Methods: Two workshops were held where WHO reference laboratory operators could compare their PTs using MTT and the same equipment. A harmonized MTT was used by seven operators in the second workshop.

Results: Differences have been observed in tilting frequency and in the height of pipetting plasma in the test tube. At the beginning of the first workshop, the tilting cycle time varied between 1.1 and 2.7 seconds. The mean PT of normal plasma obtained by pipetting plasma at the top of the tube was 14.3 seconds but was 12.9 seconds when plasma was pipetted at the bottom of the tube. When using the harmonized MTT for WHO International Standard rTF/16, the differences between operators were not greater than 1.1 seconds in normal plasma, and not greater than 1.3 seconds in patient plasma with average INR of 3.0. INR between-operator coefficient of variation was 2.3%.

Conclusion: Application of a harmonized MTT in three reference laboratories resulted in substantial reduction of between-operator variation of PT and INR. The harmonized MTT is proposed as Candidate Reference Measurement Procedure.

KEYWORDS

international normalized ratio, prothrombin time, thromboplastin, reference standards, World Health Organization, metrological traceability

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1 | INTRODUCTION

For monitoring of oral anticoagulation with vitamin K antagonists (VKA), the prothrombin time (PT) is the primary measurement. The PT test requires reagents called thromboplastins. Various types of thromboplastin are prepared commercially and, to be able to interpret the results of the PT test, it is essential that each reagent is correctly calibrated. A procedure for the calibration of thromboplastins has been described in the report of the World Health Organization (WHO) Expert Committee on Biological Standardization.[1] With this procedure, the definition of a calibration parameter called International Sensitivity Index (ISI) became feasible. To achieve standardization of the PT, it is possible to express PT results on a common scale (ie, the international normalized ratio [INR]) if the ISI and the mean normal PT (MNPT) of the thromboplastin is known.

In general, the calibration of a given thromboplastin is more precise if performed against an international reference preparation of similar composition and from the same species. A system of coexisting international reference preparations has been established in which each of these materials is traceable to the first primary international reference preparation of thromboplastin, human, combined (coded 67/40). Two international reference preparations for thromboplastin are currently available from the WHO Laboratory for Biological Standards: the Fifth International Standard for thromboplastin, rabbit, plain (coded RBT/16), and the Fifth International Standard for thromboplastin, recombinant, human, plain (coded rTF/16).[2]

The availability of international reference preparations for thromboplastin alone is not sufficient for standardization of the PT. To define a reference measurement system (RMS) not only a reference thromboplastin is required, but also a reference measurement procedure (RMP).

Routine monitoring of VKA is usually performed with determination of the PT on a fully automated instrument and transformation of the PT into the INR. However, a commercial automated instrument is not universal nor sustainable as a primary reference method. Manufacturers can go out of business or change production. In addition, the ISI of a thromboplastin reagent depends on the type of automated coagulometer.[3] There are several reasons to develop a candidate RMP to determine the plasma PT by manual tilt-tube technique (MTT). MTT has been used from the very beginning for the definition of the INR and the ISI.[4] For continuity of the INR system, the performance of MTT by experienced operators must be continued as well. MTT must be used for primary calibration of replacement International Standards for Thromboplastins, national or regional standards, and commercial manufacturer's standards.[1] Calibration of standards and performance of MTT cannot be confined to a single laboratory as for logistic, workload-related, and scientific reasons multiple calibration laboratories performing MTT across the world are needed. It should be emphasized that the MTT is to be used by specialized expert laboratories, so-called reference laboratories, for calibration of primary and secondary standards and manufacturer's thromboplastin standards. There is no need for end users to perform the MTT.

ESSENTIALS

- International Normalized Ratio (INR) is based on Prothrombin Time and the Manual Tilt Tube method (MTT).
- Standardization management through workshops is instrumental for MTT harmonization.
- INR between-operator variability across WHO reference laboratories is due to lack of a harmonized MTT.
- For adequate implementation of the metrological traceability concept, a harmonized MTT is proposed as Candidate Reference Measurement Procedure.

The coagulation end-point for International Standards for thromboplastins must be determined by MTT.[1] The mean ISI values for the successive International Standards have been established in international multicenter calibration studies. In those studies, each center used an MTT for determination of the PT with each thromboplastin. In all studies, there was considerable between-center variation of the PT in common control plasmas, in the MNPT, and in the ISI for each new thromboplastin. These results suggested that there was considerable between-center variation in the results of the MTT. Only a few authors have provided details on the MTT but these were not in full agreement.[5-7] We organized two workshops to assess the differences in the performance of the MTT between operators. In the first workshop on the MTT held in Leiden, May 8, 2017, we observed that different frequencies of tilting were used by different operators. In the second workshop, we observed that different heights of pipetting the plasma were used. These observations may explain, at least in part, the origin of between-laboratory variation of the PT in multicenter calibration studies.[2]

The purpose of the present paper is to describe the results of the two previously mentioned workshops as well as the measures that were taken to harmonize the MTT.

To achieve harmonization of the MTT among calibration laboratories, full details of a proposed candidate RMP for the MTT are described.

2 | MATERIALS AND METHODS

2.1 | Thromboplastin reagents

RecombiPlasTin 2G was obtained from Instrumentation Laboratory (Bedford, MA). The International Standard for thromboplastin recombinant human (rTF/16) and the International Standard for

thromboplastin rabbit plain (RBT/16) were obtained from the National Institute for Biological Standards and Control (Potters Bar, UK).

2.2 | Deep-frozen and freeze-dried plasma pools

Two deep-frozen plasma pools were prepared. One frozen plasma pool was prepared from 35 healthy adult individual donations (code #140408 and #141202). A second frozen plasma pool (code #130214) was prepared from the plasma remnants of patients receiving VKA, essentially as described previously.[8] The plasma pools were stored in 0.7-mL aliquots in capped 2-mL polypropylene tubes at −70°C. Before use, each sample was thawed in a water bath at 37°C for 4 minutes. The thawed samples were homogenized by three complete top-to-bottom inversions of the tubes. Thawed samples were kept at room temperature and used within 3 hours.

Freeze-dried plasma pools were prepared under the auspices of the Section Coagulation of the Dutch Foundation for Quality Assurance in Medical Laboratories (Nijmegen, The Netherlands). One lyophilized plasma pool was prepared out of 12 healthy adult individual blood donations (code #HNP-20). A second lyophilized plasma pool (code #Cou-28) was prepared out of donations by 33 patients receiving VKA. Each plasma pool was dispensed in 1-mL aliquots into siliconized glass vials. After freeze-drying of the plasma, the vials were closed with rubber bungs under vacuum. The vials were stored at −20°C. Before use, the vials were equilibrated at room temperature, and each vial was reconstituted with 1 mL of water. Reconstituted plasma was kept at room temperature for 20 minutes and gently swirled before use.

2.3 | Equipment

2.3.1 | Water bath

A water bath was used for keeping the test tubes at a constant temperature of 37°C.[4] Dimensions of the water bath were 40 × 30 × 20 cm. The water in the bath is circulated continuously by a pump. An electric thermostat with a power of 1600 to 2000 W was used to keep the temperature at 37°C (tolerance limits: 37 ± 0.5°C). The temperature was controlled with a calibrated thermometer. A light source mounted 20 cm above the water level to illuminate the test tube during tilting facilitated the endpoint clot detection by the operator (Figure 1).

2.3.2 | Test tubes

Nonsiliconized glass tubes (dimensions: 75 × 12 mm and wall thickness 0.8 mm) were used. The test tubes were made of borosilicate glass (eg, disposable culture tubes number 73500-1275, Kimble Chase Life Science and Research Products LLC, Vineland, NJ). Test tubes were discarded after use.

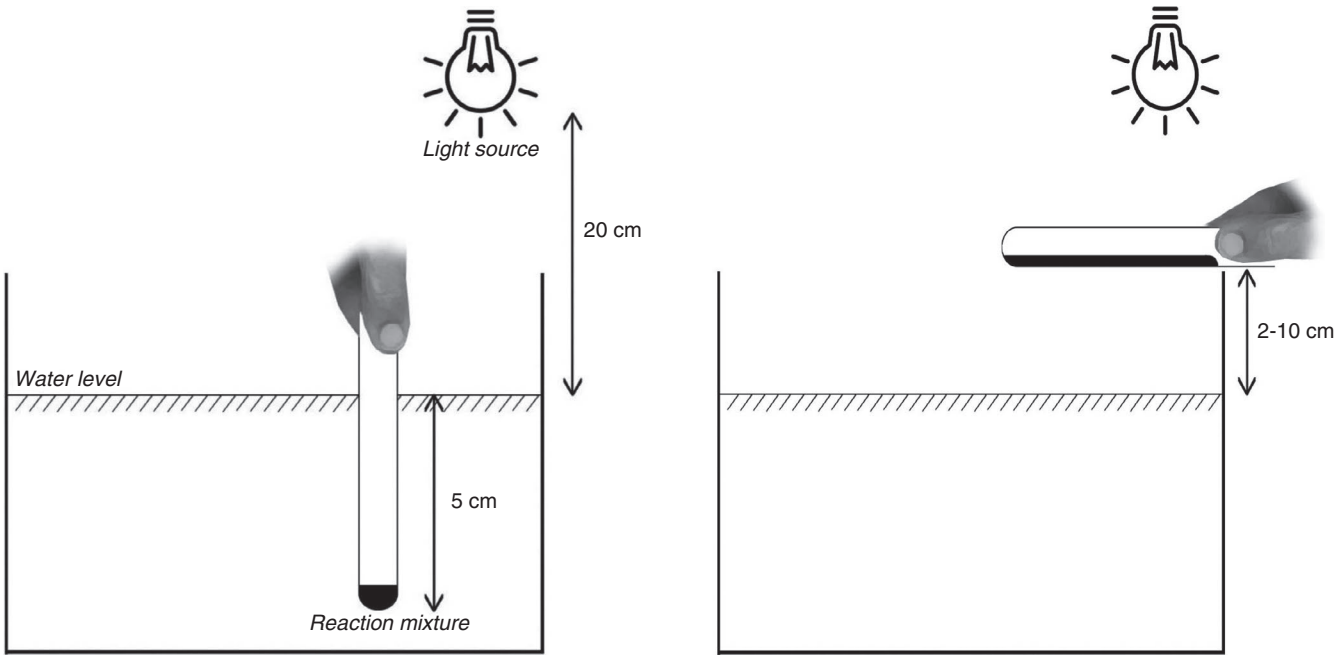

FIGURE 1 Schematic representation of the manual tilt tube technique. Left: test tube in vertical position in water bath. Right: test tube in horizontal position out of water bath. The hand of the operator is resting on the edge of the water bath. Because of variation in the size of the operators' hands, the distance of the tube in horizontal position to the water surface varies between 2 and 10 cm. The dimensions of the picture are not to scale

2.3.3 | Pipettes

Reagents and plasma samples were measured with air displacement micropipettes. The micropipettes were calibrated regularly, at least once per year, according to guideline ISO8655-6. Interchangeable disposable plastic tips were used for the micropipettes.

2.3.4 | Stopwatch

Coagulation times were determined with a digital electronic 60 Memory Stopwatch manufactured by VWR International (Leuven, Belgium). The calibration was traceable to the National Institute for Standards and Technology, in compliance with ISO 9001 ISO/IEC 17025 AND ANSI/NCSL Z540-1.

2.4 | Reconstitution of reagents

Unopened ampoules of lyophilized RBT/16 and rTF/16 should be stored in the dark at −20°C or below.[9,10] Store reconstitution fluids at 2 to 8°C. Equilibrate ampoules at room temperature for 20 minutes before reconstitution. Each ampoule of the lyophilized material must be reconstituted with 1.0 mL of the provided reconstitution fluid (coded 15/316 for RBT/16 and 14/326 for rTF/16). Cover each opened ampoule with a film to prevent loss of material. Leave the ampoule undisturbed for 20 minutes at room temperature and then swirl gently to dissolve the contents. Pool the contents of ampoules if more than one is needed to complete one calibration session. Leave thromboplastin at room temperature and do not use the reconstituted material for longer than 4 hours.

2.5 | Harmonized manual tilt tube technique

The temperature of the room in which the equipment is installed and where the technique is to be performed shall be maintained at 20 to 22°C. Empty test tubes shall be kept in a vertical position in a rack in the water bath at 37°C for at least 4 minutes at a depth of 3.5 cm before reagent and plasma samples are transferred successively with micropipettes. Pipette 200 µL of thromboplastin into glass test tube at 37°C and incubate for 2 minutes. Pipette 100 µL of not prewarmed citrate plasma 1 cm above the level of thromboplastin with the tip resting against the wall of the tube and start the stopwatch with the other hand immediately. Shake gently with the tube immersed in the water to mix the contents. Put the tube in the rack in the water bath. Lay down the pipette. The test tube shall be kept manually in the water covering 5 cm of the tube (Figure 1A). Start manual tilting of the tube 7 seconds after adding the plasma to the thromboplastin and starting the stopwatch. Tilt the tube through an angle of nearly 90° by taking the tube out of the water for 2 seconds and putting it back in the water for 1 second (Figure 1B). The tube should not be stationary during this cycle but continuously tilted with the operator's hand resting on the edge of the water bath. Then the cycle shall be repeated until the clot is formed. In the horizontal position, the tube is kept not more than 10 cm and not <2 cm above the water level (Figure 1B). Before the mixture clots, the operator observes the mixture flowing from the bottom to three-quarters of the length of the tube in the nearly horizontal position and back to the bottom. When clotting commences, the speed of flowing is reduced. At this point, the operator is keen to observe the final stopping of the flow. When flow is stopped, the operator stops the timer and records the clotting time in seconds to one decimal place.

2.6 | External quality assessment

External quality assessment (EQA) is an essential requirement for reference laboratories. The authors of this article are associated with three reference laboratories that participated in an international study for the establishment of WHO International Standards for thromboplastins.[2] The authors decided to organize an EQA survey on the MTT for the three reference laboratories (ie, Milano, Sheffield, and Leiden). To this end, two lyophilized plasmas, rTF/16 + reconstitution fluid, RBT/16 + reconstitution fluid, glass test tubes and a study protocol were dispatched from Leiden to the two other reference laboratories.

2.7 | Calculation of INR

INR was calculated with the formula: $INR = (PT/MNPT)^{ISI}$

MNPT is defined as the geometric mean PT of fresh plasmas obtained from at least 20 healthy adult persons.[1] The operators in the workshop did not use their traditional MNPT for rTF/16 and RecombiPlasTin 2G because it had not been determined with the harmonized MTT. In the workshop, we used the mean PT of frozen normal plasma pool #141202 obtained by each operator as a substitute for the MNPT.

3 | RESULTS

3.1 | First workshop

In the first workshop, the three operators performed the MTT with their own technique they used before in their own laboratory. The results of the clotting times are shown in Table 1. Operator number 3 obtained shorter clotting times with the normal and patient plasmas when compared with the other two operators. The largest relative PT difference was observed with rTF/16 and the normal plasma. Interestingly, operator number 3 used a longer waiting time before tilting and a lower frequency of tilting than the other two operators. The average within-run coefficient of variation (CV) of the clotting times was lower for operator 3 than for the other operators.

3.2 | External quality assessment

In April 2018, a first EQA survey was performed among the three participating laboratories of the workshop. The results are shown in Table 2. The maximum PT differences between the participants were greater than those observed in the workshop (Table 1).

3.3 | Second workshop

In the second workshop, which was held 16 months after the first, the same three operators and four additional operators participated. The additional operators originated from the same centers as the first three. Considering the PT differences observed in the first workshop and the first EQA survey, it was decided to divide the second workshop in two parts. In the first part of the workshop, seven operators performed their usual MTT technique with RecombiPlasTin 2G on normal and patient plasmas. All operators used the same batch of RecombiPlasTin 2G. The results are shown in Table 3. We observed in this session that the operators pipetted the plasma samples at different heights in the test tube. To investigate the effect of different heights, three operators performed the PT test by pipetting the plasma sample either in the upper part of the tube or at the lower part near the bottom. The results are shown in Table 4. The mean PTs of the tests with pipetting normal plasma in the upper part of the tube were approximately 1.4 seconds longer than the mean PTs with pipetting near the bottom of the tube. For patient plasma, the mean difference was approximately 2.1 seconds. In the last part of the workshop, all operators used the same pipetting height (ie, 1.0 cm above the bottom of the tube) as well as the same waiting time before tilting (7 seconds) and the same tilting frequency. The between-operator variations of the PT were much smaller with the harmonized MTT than with the nonharmonized techniques as shown in Table 3.

The operator performing the MTT method lifts the test tube regularly out of the water. Taking the test tube out of the water will result in a temperature drop of the reaction mixture. Immersing the tube back into the water will result in a temperature increase. The average temperature drop in the reaction mixture will depend on the room temperature. Keeping the room temperature at 21 ± 1°C, the average temperature drop in the reaction mixture was approximately 0.4°C (results not shown).

TABLE 1 Mean PT clotting times and within-run coefficients of variation (CV) obtained by three operators in first workshop

Operator	Waiting Time Until Start of Tilting (s)		rTF/16 + Nonstandardized MTT		RBT/16 + Nonstandardized MTT	
			Plasma No. 140408	Plasma No. 130214	Plasma No. 140408	Plasma No. 130214
No. 1	5	Mean PT (s):	13.4	32.0	19.4	46.8
		CV (%):	4.5	4.1	3.8	2.3
		Cycle time (s):	1.7	1.9	1.1	1.5
No. 2	6	Mean PT (s):	13.2	33.5	20.0	46.7
		CV (%):	2.7	2.0	1.9	0.4
		Cycle time (s):	1.5	1.5	1.6	1.6
No. 3	7	Mean PT (s):	12.5	31.9	17.9	44.8
		CV (%):	1.7	0.5	1.0	1.1
		Cycle time (s):	2.8	2.5	2.2	2.7

Note: The International Standards for thromboplastins (rTF/16 and RBT/16) were used with two plasma samples (pooled normal plasma no. 140408 and pooled patient plasma no. 130214). Three repeated determinations were performed by each operator.

TABLE 2 Results of first external quality assessment of the manual tilt tube technique

Control Plasma	Laboratory	Clotting Time With rTF/16		Clotting Time With RBT/16	
		Mean (s)	CV (%)	Mean (s)	CV (%)
HNP-20	No. 1	16.5	1.6	22.6	2.3
	No. 2	14.6	2.6	21.6	2.4
	No. 3	14.4	0.9	21.3	0.8
Cou-28	No. 1	39.9	1.0	50.3	1.1
	No. 2	33.5	2.7	48.2	1.1
	No. 3	35.3	1.2	47.3	0.6

Note: Two lyophilized control plasmas and the International Standards RBT/16 and rTF/16 were shipped to three laboratories for PT testing with the manual tilt tube technique. Each laboratory performed the tests on 5 days (duplicate testing on each day) using the same test tubes and International Standards for thromboplastin. The CV is the between-run coefficient of variation

TABLE 3 Mean PT clotting times, within-run CV, and mean INR obtained by 7 operators in second workshop

	RecombiPlasTin 2G + Nonharmonized MTT					rTF/16 + Harmonized MTT				
	Plasma 141202		Plasma 130214			Plasma 141202		Plasma 130214		
Operator	PT(s)	CV (%)	PT(s)	CV (%)	INR	PT(s)	CV (%)	PT(s)	CV (%)	INR
No. 1	14.1	3.8	35.9	1.5	2.77	12.3	4.2	33.2	2.3	3.01
No. 2	16.9	2.9	39.8	5.7	2.54	12.8	3.3	34.4	2.5	2.99
No. 3	13.2	1.6	35.8	0.7	2.97	12.4	1.2	33.7	0.9	3.03
No. 4	12.6	3.0	35.5	2.0	3.09	13.0	4.0	33.9	2.4	2.91
No. 5	13.4	1.2	35.7	0.8	2.91	12.4	1.2	33.2	0.6	3.00
No. 6	15.0	1.2	37.3	4.5	2.70	11.9	3.4	33.1	1.9	3.13
No. 7	13.4	1.4	35.6	2.8	2.90	12.7	2.3	33.7	1.1	2.95
Mean (n = 7)	14.1	-	36.5	-	2.84	12.5	-	33.6	-	3.00
CV (%), between operator	10.3	-	4.3	-	6.4	3.0	-	1.4	-	2.3

Note: A commercial thromboplastin reagent (RecombiPlasTin 2G; estimated ISI with MTT: 1.09) and the International Standard for thromboplastin rTF/16 were used with two plasmas (pooled normal plasma no. 141202 and pooled patient plasma no. 130214). The PT clotting times using RecombiPlasTin 2G were determined with each operator's usual MTT technique and thereafter PT clotting times were determined with rTF/16 and the harmonized MTT technique (n = 5)

TABLE 4 Mean PT clotting times (± standard deviation) determined with RecombiPlasTin 2G and different pipetting heights in test tube

Pipetting Height in Test Tube	PT (s) of plasma no. 141202	PT (s) of plasma no. 130214
Top of test tube (n = 6)	14.3 ± 1.2	36.9 ± 1.6
Bottom of test tube (n = 6)	12.9 ± 1.0	34.8 ± 0.8

4 | DISCUSSION

Our study has shown that there exist important differences in PT between operators of WHO reference laboratories performing the MTT. The origin of these PT differences is due to differences in the technique. In the first workshop, we observed differences in the waiting time until the start of tilting and in the frequency of tilting. One operator with a lower tilting frequency observed slightly shorter clotting times than another operator who tilted the tube with a higher frequency (Table 1). A potential explanation might be that a lower tilting frequency would permit an earlier clot formation or detection in the tube. The end point may be observed either when the mixture has clotted in the vertical position of the tube and the tube is taken out of the water to the horizontal position, or when the mixture changes from a fluid to a clot in the horizontal phase of the tube. With a longer horizontal phase, it may be possible, on average, to detect the clot earlier. Following the first workshop, an EQA survey was performed among the three participating laboratories of the workshop. In spite of the experience of the first workshop, the PT differences between the operators were not reduced (Table 2). It was decided to organize a second workshop aiming at further harmonization of the MTT. In the second workshop, we observed that one operator pipetted the test plasma in the upper part of the test tube and another in the lower part of the tube. With the former technique, longer clotting times were obtained (Table 4). Obviously, when the plasma is pipetted in the upper part, it takes time for the plasma to run down along the wall of the tube and reach the thromboplastin reagent at the bottom of the tube. Variation in tilting frequency and in pipetting height may explain in part the variation in clotting times observed in multicenter thromboplastin calibration studies and EQA schemes. Another potential source of error in the MTT is the variation in the personal reaction time, which is similar to a measure of error in stellar observations discovered by the astronomer FW Bessel.[11] Bessel's assistant measured the contact of the stars with the threads in the field of view of the eyepiece of his telescope consistently 1 second later than Bessel did. Bessel called this measure of error the "personal equation" (persönliche Gleichung). At present, no attempts have been made to assess the effect of personal reaction time in the MTT. Nevertheless, harmonization of the MTT may reduce the variation in PT. We showed that a harmonized MTT reduced the variation between operators (Table 3). The CV of the INR between seven operators in our second workshop was only 2.3%. This is a much better result than the CV of 6% for the INR of a lyophilized plasma with a mean of 3.0 in the multicenter calibration study of rTF/16 and RBT/16.[2] In the latter study, 20 centers participated and their operators were not trained in a preliminary workshop. It

is not yet possible to estimate the magnitude of the likely effect of MTT harmonization on the variation of the ISI, but we may estimate the magnitude of the effect on the variation of the MNPT. The interlaboratory CV of the MNPT of rTF/16 was 8% in the multicenter study,[2] which can be compared with the interoperator CV of 10.3% observed with the nonharmonized MTT and the CV of 3.0% with the harmonized MTT using pooled normal plasma (Table 3). In the future, it will be essential to run regular ring trials or EQA surveys for specialized expert laboratories performing the MTT, aiming at lowering interlaboratory CVs to meet the analytical performance and measurement uncertainty requirements for use of MTT as an internationally recognized RMP.

Metrological traceability is defined as the "property of a measurement result whereby the result can be related to a stated reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty."[12] The measurement uncertainty of the PT/INR results should remain within allowable specifications in order to be fit-for-clinical use. When the metrological traceability of the INR is considered, we should admit that the INR is not "measured" but is calculated from the patient's PT, the MNPT, and the ISI. Metrological traceability of the MNPT cannot be achieved, because the MNPT is defined with fresh local plasmas and there is no commonly accepted reference.[13] Traceability of the ISI can be achieved because there are commonly accepted references. A proposed traceability chain for ISI determination is shown in Figure 2. In this scheme, a single WHO International Standard for thromboplastin is used with the harmonized MTT as RMS.[14] Secondary standards must be calibrated with the RMS. Each calibration must be performed with fresh plasma samples from healthy ("normal") adults and from patients who are in the steady phase of VKA treatment. In the past, secondary standards have been calibrated in multicenter studies with 20 laboratories participating. It is likely that many participants of the earlier multicenter studies did not use a harmonized MTT. In the future, when the harmonized MTT has been adopted and validated, it may be possible to substantially reduce the number of centers participating in the calibration of secondary standards. It would have the additional advantage of economizing the use of the primary WHO International Standard. The traceability chain of the calibration may be modified by replacing fresh plasma samples by frozen or lyophilized samples if commutability of the latter has been established. One of the contributors to lack of agreement between results among different measurement procedures (MPs) is using noncommutable reference materials as calibrators in the calibration hierarchy for clinical laboratory (end-user) MPs.[15] Recently, new guidelines have been published for assessing commutability of reference materials as calibrators.[15-17] Certified frozen or lyophilized plasmas may be used for local ISI calibration or direct INR determination by end users.[18] The certification (ie, value assignment to calibrator plasmas) is the responsibility of the manufacturer and should be performed by Joint Committee of Traceability in Laboratory Medicine-listed reference laboratories. The measurement uncertainty of the PT/INR results should remain within allowable specifications to be fit for clinical use.

To implement the metrological traceability concept in a sustainable way, an internationally recognized RMP has to be established which is fit for its intended use and is compliant with ISO 15195:2018 and ISO 17511:2020.[19,20] Reaching that status

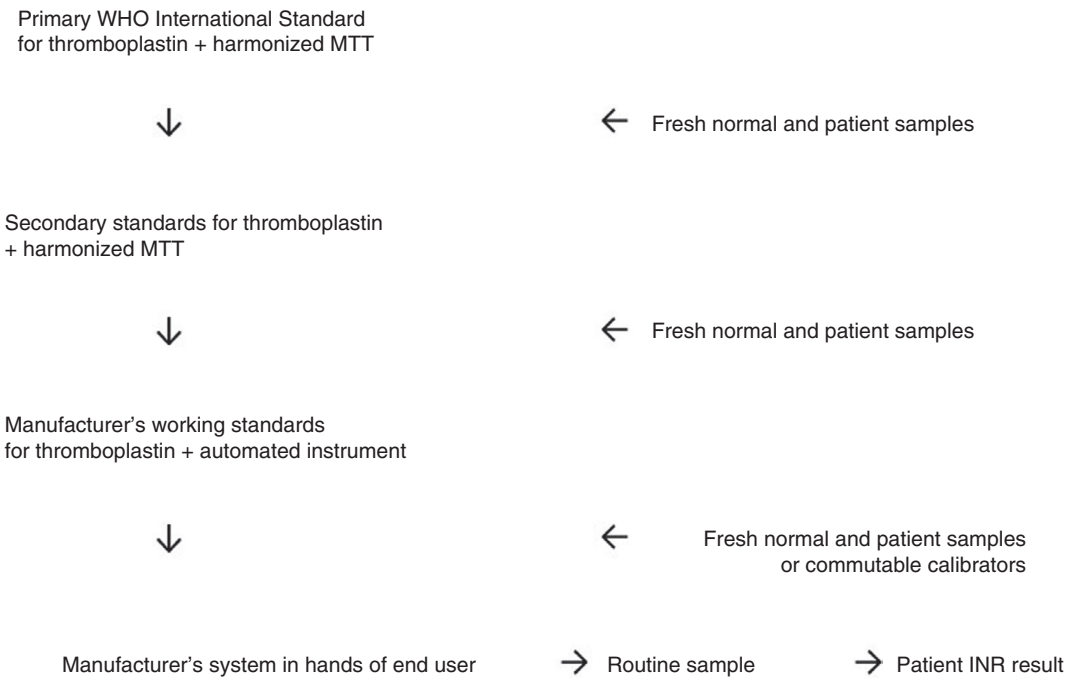

FIGURE 2 Proposed traceability chain for ISI determination. At the top of the chain is the reference measurement system consisting of a primary WHO International Standard for thromboplastin in combination with a harmonized MTT. Vertical arrows indicate calibration activity using the material + procedure. Horizontal arrows indicate the (clinical) samples used for calibration or testing

of RMP can only be accomplished after formal review and approval of the candidate RMP by the International Federation of Clinical Chemistry Scientific Division (IFCC SD), the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (SSC/ISTH) and the responsible Joint Committee of Traceability in Laboratory Medicine review team. This means the harmonized MTT that we describe is considered to become a higher order candidate RMP, which has to be described and controlled in every step. Only a limited number of internationally recognized, dedicated calibration laboratories have the resources to further optimize and harmonize the candidate RMP and develop it in combination with existing reference materials to a higher order RMS. Only then can it be guaranteed that PT/INR test results are and remain traceable to standards of higher order, in time and in space. The establishment of such a global RMS has been formally proposed by the authors of this paper to IFCC SD and SSC/ISTH to enable ongoing PT/INR standardization that guarantees longitudinal accuracy of PT/INR test results and decision limits.

Although the MTT development to the level of a candidate RMP is ongoing by three calibrations laboratories (Leiden, Sheffield, and Milano) the degree of harmonization of test results is monitored by means of evaluating the interlaboratory variability among calibration laboratories at different PT/INR levels. To that end, periodic EQA ring trials are performed among these calibration laboratories and further harmonization attempts are made to reduce the measurement uncertainty to acceptable limits for enabling the role of the MTT as RMP. It is necessary to repeat workshops until the measurement uncertainty of the calibration laboratories is only a fraction of the allowable measurement uncertainty.

In summary, we have shown that the application of a harmonized MTT procedure (as described in the Materials and Methods Section) results in substantial reduction of between-operator variation. We propose to pave the way for further optimization of the harmonized MTT procedure to submit the MTT at some point in time as a candidate RMP. To that end, a joint working group of the IFCC and the SSC/ISTH has to be established to integrate the candidate RMP into a complete RMS for PT/INR standardization.

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CONFLICTS OF INTEREST

All authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Antonius M.H.P. van den Besselaar, Veena Chantarangkul, Claudia J.J. van Rijn, Armando Tripodi, and Christa M. Cobbaert designed and supervised the study. Antonius M.H.P. van den Besselaar, Claudia J.J. van Rijn, Charmane F. Abdoel, Erica Scalambrino, Steve Kitchen, Anita M. Woolley, and Lidia Padovan acquired and analyzed the data.

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