De fining a metrologically traceable and sustainable calibration hierarchy of international normalized ratio for monitoring of vitamin K antagonist treatment in accordance with International Organization for Standardization (ISO) 17511:2020 standard: communication from the International Federation of Clinical Chemistry and Laboratory Medicine - SSC/ISTH working group on prothrombin time/international normalized ratio standardization

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ISTH SSC COMMUNICATION

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Abstract

Calibration of prothrombin time (PT) in terms of international normalized ratio (INR) has been outlined in "Guidelines for thromboplastins and plasmas used to control oral anticoagulant therapy" (World Health Organization, 2013). The international standard ISO 17511:2020 presents requirements for manufacturers of *in vitro* diagnostic (IVD) medical devices (MDs) for documenting the calibration hierarchy for a measured quantity in human samples using a specified IVD MD. The objective of this article is to define an unequivocal, metrologically traceable calibration hierarchy for the INR measured in plasma as well as in whole blood samples. Calibration of PT and INR for IVD MDs according to World Health Organization guidelines is similar to that in cases where there is a reference measurement procedure that defines the measurand for value assignment as described in ISO 17511:2020. We conclude that, for PT/INR standardization, the optimal calibration hierarchy includes a primary process to prepare an international reference reagent and measurement procedure that defines the measurand by a value assignment protocol conforming to clause 5.3 of ISO 17511:2020. A panel of freshly prepared human plasma samples from healthy adult individuals and patients on vitamin K antagonists is used as a commutable secondary calibrator as described in ISO 17511:2020. A sustainable metrologically traceable calibration hierarchy for INR should be based on an international protocol for value assignment with a single primary reference thromboplastin and the harmonized manual tilt tube technique for clotting time determination. The primary international reference thromboplastin reagent should be used only for calibration of successive batches of the secondary reference thromboplastin reagent.

KEYWORDS
anticoagulants, calibration, international normalized ratio, metrological traceability, prothrombin time, thromboplastin

1 | INTRODUCTION

Treatment with vitamin K antagonists (VKAs) is usually monitored with 1-stage prothrombin time (PT) measured in a citrate plasma sample in a laboratory or in a whole blood sample in a point-of-care (POC) test procedure [1]. The scientific name for PT is tissue factor–induced coagulation time [2], but the name PT is still used in daily practice. PT is always measured by means of a tissue factor (thromboplastin) reagent and a technique or instrument to determine the clotting end point, ie, using an *in vitro* diagnostic (IVD) medical device (MD). PT is measured in seconds and depends on the thromboplastin reagent and measurement technique in the device. The PT measured in seconds should be transformed to the international normalized ratio (INR) by means of a mathematical formula to achieve equivalence between various reagents and techniques. In many cases, the mathematical formula is based on the international sensitivity index (ISI) and the mean normal prothrombin time (MNPT): $INR = (PT/MNPT)^{ISI}$. The definition of ISI and the procedures to determine the ISI and MNPT have been described in the World Health Organization (WHO) guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with VKAs [3]. PT systems have been based on the use of thromboplastin reagents from 3 different species: human, bovine, and rabbit. Some reagents have been prepared with addition of plasma depleted of vitamin K–dependent factors, ie, providing factor V and fibrinogen, making the assay more specific [4]. The latter are referred to as combined reagents (Owren methods), while reagents without depleted plasma addition are referred to as plain reagents (Quick methods) [5]. The standardization of these thromboplastin reagents involved 3 different international reference preparations (IRPs), one for each of the 3 species of thromboplastins in use (Figure 1) [6]. The first IRP, coded 67/40, was a human combined thromboplastin and had an ISI value of 1.0 by definition.

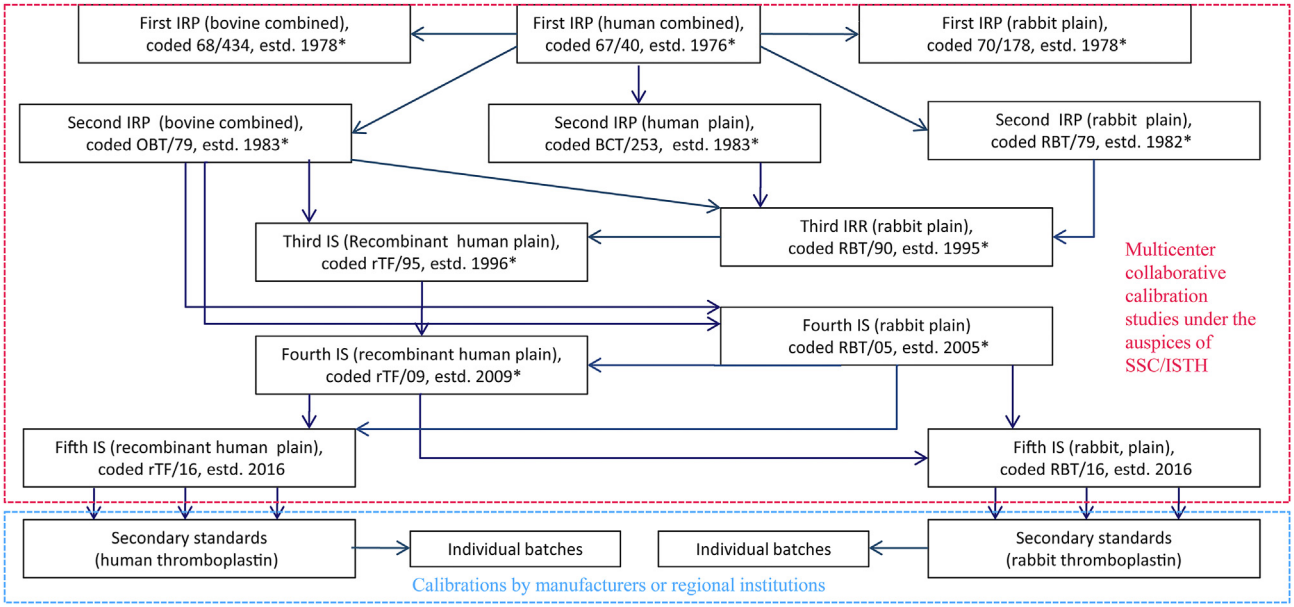

FIGURE 1 Current World Health Organization (WHO) calibration hierarchy. The first international reference preparation (IRP) of thromboplastin (human, combined [coded 67/40]) was established by the WHO Expert Committee on Biological Standardization (ECBS) in 1976. Its international sensitivity index value was set at 1.0 by definition. Successive IRPs of thromboplastin (either from human, rabbit, or bovine brain tissue) were established by the WHO ECBS following calibration studies performed under the auspices of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH). It should be noted that ECBS replaced the original term IRP by the term international standard (IS). The third, fourth, and fifth IS were established by comparison to 2 or 3 predecessor IS and by averaging the international sensitivity index values obtained from the predecessors. Currently, there are 2 coexistent ISs of thromboplastin—5th IS [recombinant human plain] coded rTF/16 and 5th IS [rabbit, plain] coded RBT/16). These preparations are used to calibrate so-called secondary standards, which include regional, national, and manufacturers' working standards. Working standards are used for routine calibration of individual batches of thromboplastin. Working standards should have been calibrated with the appropriate IS (adapted from the study by Poller [5]). *Now discontinued. estd., established; IRR, international reference reagent.

Reagents prepared from bovine tissue are no longer used and the last IRP for bovine combined thromboplastin (coded OBT/79) has been discontinued [3]. The agreement among results is greatest when similar thromboplastin reagents and methods are compared. For this reason, thromboplastins of human origin were to be calibrated against the current international standard for human thromboplastin (human, recombinant, plain, coded rTF/16). Thromboplastins of rabbit brain and rabbit lung were to be calibrated against the current international standard for thromboplastin (rabbit, plain, coded RBT/16). The calibration of the IRPs for thromboplastins and their replacements were carried out as part of international multicenter collaborative studies using fresh local plasma samples. Each collaborative study for replacement of an IRP included all existing IRPs irrespective of the human or rabbit source of thromboplastin. The ISI value assigned to the replacement reagent was the mean of the ISI values obtained by calibration with all existing IRPs [3]. A major limitation of the above calibration procedure of IRPs is that any measurement bias between different thromboplastin reagents used in the calibration hierarchies is propagated to future IRPs and to results for clinical samples [7]. This problem may be solved by establishing a single primary IRP based on human recombinant thromboplastin that will be available only for the calibration of successive secondary IRPs also based on human recombinant thromboplastin. The secondary IRP is intended to be used for calibration of commercial end-user PT/INR measurement procedures (MPs) (both human and rabbit thromboplastin) used in medical laboratories.

A working group of stakeholders with an interest in INR measurements was formed under the auspices of the International Federation of Clinical Chemistry and Laboratory Medicine and the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. The members of the working group were recruited from calibration laboratories, Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (Subcommittee on Control of Anticoagulation), WHO Expert Committee on Biological Standardization, National Institute of Biological Standards and Control, external quality assessment organizations, and manufacturers of reference materials (RMs) and MPs. The primary purpose of the working group is to develop a harmonized reference measurement procedure (RMP) based on the manual tilt tube technique (MTT) as well as a complete higher-order reference measurement system for global standardization of the PT/INR test [8] (https://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-ptinr/).

Equivalent results for human samples for a measurand such as PT/INR can be achieved by establishing metrological traceability of the values assigned to the calibrators for a MP to the highest available reference system component for the measurand [9]. Metrological traceability describes the calibration hierarchy and the sequence of value assignments, demonstrating an unbroken chain of calibrations

between the measurement result for a human sample up to the highest available reference system component in the calibration hierarchy [9]. The point at which metrological traceability begins (ie, the highest level of metrological traceability in the calibration hierarchy) depends on the availability of higher-order RMPs and RMs for the stated measurand [9].

The international standard ISO 17511:2020 presents requirements for manufacturers of IVD MDs for documenting the calibration hierarchy for a measured quantity in human samples using a specified IVD MD [9]. The document includes 6 model calibration hierarchies offering potential technical solutions for different kinds of measurands in establishing metrological traceability of assigned values for human samples, calibrators, and trueness control materials.

We noticed differences between the terms and definitions used in the WHO guidelines [3] and those used in ISO 17511 [9]. The purpose of this article is to describe the differences and to propose a model calibration hierarchy for the INR that conforms to ISO 17511. Some PT IVD MDs are designed for INR measurement in citrate plasma samples and others for measurement in whole blood without citrate. The plasma IVD MDs and whole blood IVD MDs cannot be included in a single model and, therefore, require different calibration hierarchies.

The WHO guidelines [3] and ISO 17511 [9] were reviewed and mutually compared with respect to their strengths and limitations. The terms used in the WHO guidelines were interpreted against the terms used in ISO 17511. The proposed calibration hierarchy of the plasma INR is defined in line with the ISO 17511 standard.

2 | COMPARISON OF TERMS AND DEFINITIONS

The terms and definitions used in ISO 17511 are different from those used in the WHO guidelines. For example, a "standard" in ISO 17511 is defined as the "realization of the definition of a given quantity, with stated quantity value and associated measurement uncertainty, used as a reference". In the WHO guidelines, the expression "International Standard" is used to refer to an international reference thromboplastin, ie, a reagent. Obviously, the definition of a "standard" in ISO 17511 does not conform to the designation used in the WHO guidelines. The term "International Reference Preparation for thromboplastin" would be a procedure for defining the measurand and assigning INR values to be consistent with the terminology used in ISO 17511. Table 1 shows a list of expressions used in the WHO guidelines and the corresponding designations used in ISO 17511. INR is considered as a measurand, but there is a limitation. INR is influenced by several different coagulation factors, each of which cannot act alone in the PT system. The INR is a calculated quantity from 3 measurement results, ie, the measurement of the human sample's PT, the mean of measurements of healthy individual samples' PTs (MNPT), and the assigned value of the ISI. In the WHO guidelines, the expression "thromboplastin calibration" refers to the determination of the ISI value for an IRP or for any other thromboplastin used with any technique or instrument. In the light of ISO 17511, it would be better to replace "thromboplastin calibration" with "PT-INR calibration".

TABLE 1 Expressions and definitions used in WHO guidelines and corresponding designation in ISO 17511.

WHO guidelines	Designation in ISO 17511:2020
Prothrombin time, reported in seconds	Measured quantity [4.4.2]
International normalized ratio	Measurand [3.26]
Thromboplastin calibration	Calibration [3.4]
International standard for thromboplastin (international reference preparation)	Component of reference measurement system [3.41]
International sensitivity index for international reference preparation	Component of reference measurement system [3.41]
Prothrombin time system	Measurement system [3.29]
Certified plasma	Measurement standard [3.28]

The numbers in square brackets refer to the appropriate paragraph in ISO 17511:2020 standard.

ISO, International Organization for Standardization; WHO, World Health Organization.

3 | SELECTION OF CALIBRATION HIERARCHY

In the WHO guidelines, it is recommended that calibration of PT MPs should be performed primarily with a set of freshly prepared plasma samples obtained from 20 healthy individuals and 60 patients on VKA therapy. The set of fresh plasma samples are used as commutable calibrator materials. In ISO 17511, 6 calibration hierarchy models are described, depending on the availability of higher-order RMs and MPs. The calibration hierarchy shown in ISO 17511:2020 clause 5.3 when the higher-order MP defines the measurand is the most appropriate for INR. For INR, there is no International System of Units of measurement, no primary pure substance RM, and no international conventional calibrator material available. Figure 2 shows a calibration hierarchy suitable for metrological traceability of INR measurements in medical laboratories. The notation used in ISO 17511:2020 designates a sequence of procedures (p.x) and materials (m.x) used in the metrological traceability chain and is used here to refer to the sequence of the calibration hierarchy steps shown in Figure 2 for INR and how they relate to the ISO clause 5.3. The WHO procedures for preparing the primary IRP and assigning its ISI are the p.1 primary procedures conforming to the diagram used in ISO 17511:2020. The primary IRP used with the manual tilt tube measurement of PT in position p.3a defines the measurand and assigns values to a panel of human samples with specified characteristics. Separate panels of human samples are used in positions m.3a and m.3b (see the next paragraph) as commutable secondary calibrators. A panel of freshly prepared normal and patient samples is commutable by definition and represents different amounts of the measurand. Note that individual samples could have specimen-specific effects that could affect a measurement result. Individual samples with results influenced by significant specimen-specific effects are typically removed as outliers in the value transfer procedure.

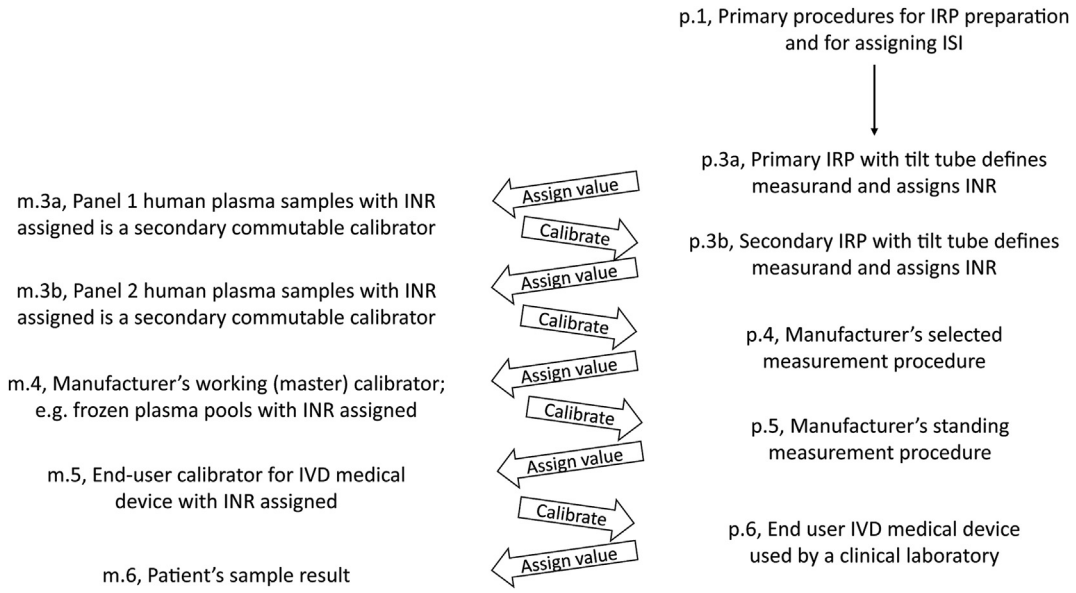

FIGURE 2 Proposed calibration hierarchy of plasma international normalized ratio (INR) measurement. The measurand (INR) is defined by the primary international reference preparation (IRP) for thromboplastin established by the World Health Organization and the harmonized manual tilt tube technique. There is one secondary reference preparation (human). The secondary reference preparation (human) with the harmonized manual tilt tube technique is calibrated directly against the primary IRP. The secondary reference preparation is available to commercial manufacturers for calibration of their selected measurement procedures. ISI = international sensitivity index; IVD MD, *in vitro* diagnostic medical device.

We propose to establish a secondary IRP that is calibrated against the primary IRP using a separate panel of fresh plasma samples as secondary commutable calibrators. The main reason for introducing a secondary IRP is to maintain longevity of the primary IRP (see section "Primary and secondary reference thromboplastin reagents"). The calibration hierarchy steps shown as p.3a, m.3a, and p.3b in Figure 2 represent calibration of the secondary IRP in position p.3b. A different panel of fresh plasma samples, m.3b, is value-assigned by the p.3b secondary IRP and tilt tube procedure and used as secondary commutable calibrators to calibrate the IVD MD manufacturer's selected MP in position p.4, which assigns INR values to the m.4 manufacturer's working (master) calibrator. For calibration of sequential lots of m.5 end-user calibrator used by medical laboratories, the m.4 manufacturer's master calibrator is used to calibrate the p.5 manufacturer's standing MP, which assigns INR values to the m.5 end-user calibrator. In most cases, the p.4, p.5, and p.6 MPs are the same for a given manufacturer, but p.4 and p.5 are used with more stringent calibration, eg, added replication to reduce uncertainty, for their functions in the calibration hierarchy of the p.6 end-user IVD MD.

4 | INTERNATIONAL PROTOCOL FOR VALUE ASSIGNMENT

The international protocol for value assignment of INR is described in the WHO guidelines, where international standard thromboplastin reagents (ie, IRPs for thromboplastin) and harmonized MTT for clotting time determination define the INR. Thus, the IRP for thromboplastin and harmonized MTT are components of the international protocol for value assignment in position p.1 in Figure 2. The IRP for thromboplastin is characterized by a value for the ISI and MNPT, which is used to calculate the INR of a patient's plasma sample. Therefore, ISI and MNPT can also be considered as components of the international protocol for value assignment.

5 | USE OF FRESH PLASMA SAMPLES AS SECONDARY COMMUTABLE CALIBRATORS

The international protocol for value assignment shall specify the process used to assign values (arbitrary units, not traceable to International System of Units) to the calibrator material. In the WHO guidelines, fresh plasma samples obtained from at least 20 healthy adults and from at least 60 patients on long-term VKA treatment are used as secondary commutable calibrators. Preanalytical variables may affect the results of routine coagulation assays such as PT [10]. It is therefore essential that the correct sodium citrate concentration (0.109 mol/L) is used for blood collection and that centrifugation of blood should be such that the plasma is rendered poor in platelets [3]. In PT measurements, fresh citrate plasma is homogeneous and stable for 5 hours after blood collection if stored at room temperature in a closed container made of inert material (eg, plastic) [11]. Although it is possible to calculate the relative combined value assignment uncertainty of each of the fresh plasma samples, this is usually not done in the calibration of manufacturer's selected MPs. The main reason is that all plasma samples are used simultaneously with the RMP and with the selected MP in the same run. Repeated PT measurements on each plasma sample are not required in the WHO guidelines. Instead

TABLE 2 International normalized ratio measurement uncertainty budget based on different models.

Component of measurement uncertainty budget	Based on clinical outcome, Milan model 1	Based on biological variation for patients on VKA, Milan model 2; minimum imprecision specification (0.75 CV-I upper)	Based on biological variation for patients on VKA, Milan model 2; desirable imprecision specification (0.5 CV-I average)
Reference measurement system CV (%ru_{ref})	3.3	3.6	1.7
Manufacturer's calibrator CV	5.8	6.3	3.0
End-user result CV (% $ru_{end\ user}$)	7.4	8.1	3.9
Total standard uncertainty CV (%*ru*[y])	10	0.75 × 14.5% = 10.9%	0.5 × 10.3% = 5.2%
Total expanded uncertainty (%U)	20	22	10

CV-I is the within-subject biological variation expressed as a coefficient of variation in %. The reference measurement system's uncertainty budget (% ru_{ref}) represents 33% of the total standard uncertainty (% *ru*[y]). It is calculated according to the following formula:

$$\%ru_{ref} = \left(\left[\%ru_{\text{primary reference measurement system}} \right]^2 + \left[\%ru_{\text{secondary reference measurement system}} \right]^2 \right)^{½}$$

The relative percent combined value assignment uncertainty of the *in vitro* diagnostic medical device (IVD MD) calibrator is calculated according to the following formula:

$$\%\text{rucal} = (\%\text{ruref}]2 + [\%\text{ruselected measurement procedure}]2 + [\%\text{rustanding measurement procedure}]2)½,$$

where %ru_{cal} represents 67% of the total standard uncertainty.

The relative percent combined standard measurement uncertainty for reported INR values with the end-user IVD MD is calculated according to the following formula:

$$\%\text{ru(y)} = (\%\text{rucal}]2 + [\%\text{ruend user}]2)½,$$

where %$ru_{end\ user}$ is the relative percent standard uncertainty of the end-user IVD MD based on long-term imprecision (reproducibility conditions of measurements).

The relative percent expanded uncertainty (%U) = 2 × %*ru*(y).

CV, coefficient of variation; VKA, vitamin K antagonist.

of calculating the PT/INR value uncertainty of each of the fresh plasmas, the uncertainty of the ISI of the manufacturer's selected MP is calculated using orthogonal regression analysis on the PT measurement results of all fresh plasma samples. In this way, the PT value uncertainties of all individual plasmas are contained in the ISI value uncertainty. The ISI value uncertainties of the primary IRP and the secondary reference preparations are to be calculated and provided by the group of reference laboratories participating in the multicenter calibration study of the preparations. Likewise, the uncertainties of ISI values of the manufacturer's selected MP, the standing MP, and the end-user IVD MD are combined to calculate the standard measurement uncertainty for the reported values of the measurand with the end-user IVD MD.

6 | ANALYTICAL PERFORMANCE SPECIFICATION

Correct INR measurements are essential for appropriate monitoring of patients on VKA therapy and appropriate dosage adjustments if necessary. This underscores the requirement for an appropriate analytical performance specification (APS) for INR measurements. The First Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine held in Milan in 2014 identified 3 models to define APS: the effect on clinical outcome, biological variation, and the state of the art [12]. Model 1 (outcome-based performance specification) should be applied when the measurand has a central and well-defined role in the decision making of a specific disease or a given clinical situation, and test results should be interpreted through established decision limits [13]. Therapeutic ranges and decision limits for INR have been established for several clinical indications [14]. These may be used for the derivation of APS. For the most common indications, a therapeutic range of 2.0 to 3.0 has been established for INR. For an INR therapeutic target value of 2.5, the measurement error should not exceed ±0.5, amounting to a relative total expanded error of ±20% (0.5/2.5).

Model 2 (biological variability–based APS) should be applied when the measurand is in a steady state status when a subject is in good health [13]. It has been suggested that model 2 should be used for the PT [13]. Model 2 should be used where a measurand has to be kept at a certain concentration level; otherwise, the body will suffer (ie, the measurand is under strict homeostatic control) [15]. However, there is some doubt about the usefulness of model 2 for PT/INR because PT/INR is not under strict homeostatic control when the patient is treated with VKA. Several studies have shown that the biological variation of the INR in adult nonanticoagulated subjects is much smaller than that in patients on VKA therapy [16–20]. Therefore, the biological variation in adult nonanticoagulated subjects cannot be used for INR APS

in patients on VKA therapy. Following the induction phase of VKA therapy, many patients reach a steady state in the therapeutic range with a constant dose of VKA. It should be realized that the biological variation of the INR depends on the type of VKA drug and on compliance with medication regimen by patients. The biological variation of the short-acting acenocoumarol was higher than the variation of the long-acting phenprocoumon [18]. Furthermore, the biological variation depends on the type of reagent used for INR measurement: the biological variation observed with recombinant human thromboplastin was greater than the variation observed with a rabbit thromboplastin combined with adsorbed plasma as a source of factor V and fibrinogen [19]. Taking the upper value of the within-subject biological variation (commonly known as CV-I) observed in steady-state patients on a constant dose of warfarin and a uniform therapeutic range of INR of 2.0 to 3.0 (ie, 14.5% CV-I) [20], we may derive a value for the minimum imprecision specification of INR (Table 2). Alternatively, we may estimate a value for the desirable imprecision specification based on the average within-subject biological variation in patients on warfarin, acenocoumarol, and phenprocoumon, ie, 10.3% CV-I [18–20]. Evidently, the latter specification is much stricter than the minimum specification based on the upper value of the biological variation. In fact, the total standard uncertainty of 5.2% associated with the desirable imprecision specification of the INR cannot be achieved in practice (see the section below). For this reason, we reject the desirable imprecision specification for the INR based on biological variation. We conclude that minimum APS for the INR in Milan model 2 is in good agreement with those observed in Milan model 1 (Table 2). We recommend Milan model 1 in defining APS for INR because it has a central and well-defined role in the decision making of the dose in VKA therapy.

7 | ESTIMATING UNCERTAINTY OF ASSIGNED VALUES FOR END-USER IVD MD CALIBRATORS

According to ISO 17511, the combined standard measurement uncertainty of the value assigned to an IVD MD calibrator (designated u_{cal}) shall be estimated and made available to end users by the manufacturer. The method of statistical calculation of the u_{cal} shall be documented and maintained in the technical file of the IVD MD calibrator at least during the life cycle of the product. For each IVD MD calibrator identified by a manufacturer for use in calibration of a specified IVD MD, the u_{cal} has to be estimated and provided by the

TABLE 3 ISI and between-PT system variation of INR in selected measurement systems.

Thromboplastin reagent, coagulation instrument	Reagent manufacturer	Calibration laboratory (calibration year)	IRP, manual tilt tube method	*n*	ISI model			Tomenson's model	
					ISI_{SMS}	*M*	CV_{CAL} (%)	*M*	CV_{CAL} (%)
Neoplastin CI Plus (rabbit, plain), STA-R	Diagnostica Stago	LUMC (2013)	RBT/05	53	1.47	2.49	2.8	2.51	2.4
			rTF/09	53	1.46	2.45	5.2	2.42	5.2
HemosIL PT Fib HS Plus (rabbit, plain), ACL 300	Werfen	LUMC (2013)	RBT/05	60	1.13	2.54	3.9	2.53	3.7
			rTF/09	60	1.12	2.48	7.4	2.43	6.7
Recombiplastin 2G (human, rec.), ACL Advance	Werfen	LUMC (2013)	RBT/05	60	0.98	2.53	5.7	2.53	4.7
			rTF/09	60	0.97	2.44	3.4	2.43	3.4
Thromborel S (human, plain), Sysmex CA-1500	Siemens Healthineers	LUMC (2013)	RBT/05	60	1.03	2.52	4.3	2.53	4.2
			rTF/09	60	1.02	2.46	4.2	2.43	3.7
Neoplastin R (human, rec.), STA-R	Diagnostica Stago	LUMC (2013)	RBT/05	60	0.96	2.50	6.1	2.53	5.4
			rTF/09	60	0.96	2.45	4.4	2.43	4.3
Hepato Quick (rabbit, combined), STA-R	Diagnostica Stago	LUMC (2011)	RBT/05	58	0.84	3.04	3.5	3.05	3.4
			rTF/09	58	0.81	2.91	5.7	2.88	5.6
SPA Plus (rabbit, combined), STA-R	Diagnostica Stago	LUMC (2011)	RBT/05	55	0.96	2.68	3.7	2.70	3.1
			rTF/09	55	0.92	2.53	6.1	2.51	6.3
Owren's PT (rabbit, combined), STA-R	MediRox	LUMC (2011)	RBT/05	55	1.01	2.68	3.6	2.70	3.4
			rTF/09	55	0.97	2.53	6.2	2.51	6.2
Thrombotest (bovine, combined), STA-R	Axis-Shield	LUMC (2011)	RBT/05	55	0.99	2.69	5.9	2.70	5.2
			rTF/09	55	0.94	2.53	4.6	2.52	4.6
Innovin (rec. human), Sysmex CA-1500	Siemens Healthineers	LUMC (2012)	RBT/05	59	1.04	2.57	5.8	2.62	3.3

(Continues)

TABLE 3 (Continued)

Thromboplastin reagent, coagulation instrument	Reagent manufacturer	Calibration laboratory (calibration year)	IRP, manual tilt tube method	*n*	ISI model			Tomenson's model	
					ISI_{SMS}	*M*	CV_{CAL} (%)	*M*	CV_{CAL} (%)
			rTF/09	59	1.03	2.51	3.1	2.52	2.8
PT Fib recombinant (rabbit, rec.), ACL 300	Werfen	LUMC (2012)	RBT/05	59	1.01	2.57	6.3	2.62	4.7
			rTF/09	59	1.01	2.52	4.8	2.52	4.8
HemosIL Recombiplastin 2G (human, rec.), ACL Advance	Werfen	LUMC (2013)	RBT/05	59	0.96	2.59	4.7	2.62	3.3
			rTF/09	59	0.96	2.53	2.8	2.53	2.8
PT Fibrinogen (rabbit, plain), ACL TOP	Werfen	RHH (2017)	RBT/16	59	1.51	2.81	5.0	2.84	4.3
PT Fib HS Plus (rabbit, plain), ACL TOP	Werfen	RHH (2017)	RBT/16	59	1.09	2.85	3.0	2.83	2.7
Recombiplastin 2G (human, rec.), ACL TOP	Werfen	RHH (2018)	rTF/16	57	1.00	3.11	2.4	3.09	2.3
TriniCLOT Excel S (rabbit, plain), Destiny MAX optical	Tcoag	RHH (2019)	RBT/16	59	1.19	2.80	4.6	2.79	4.6
TriniCLOT HTF (human, plain), Destiny MAX mechanical	Tcoag	RHH (2020)	rTF/16	60	1.05	2.97	3.6	2.94	3.1
CoaguChek XS (human, rec.), whole blood sample[a]	Roche	LUMC (2011)	RBT/05[a]	55	1.04	2.69	4.0	2.70	3.6
			rTF/09[a]	55	1.00	2.55	5.0	2.52	4.8
CoaguChek INRange (human, rec.), whole blood sample[a]	Roche	LUMC (2022)	rTF/16[a]	60	1.05	2.58	3.8	2.58	3.8

Calibrations were performed using fresh plasma samples collected with 0.109-mol/L sodium citrate solution according to World Health Organization guidelines [3]. CV_{CAL} is calculated with equation 5 (see Supplementary Method). Two models were used for calibration of reagent/instrument combinations (PT-selected measurement systems) with IRP and the manual tilt tube technique— either the ISI model or Tomenson's model. All calibrations were performed by experienced operators in selected laboratories. Previous studies showed good agreement between results obtained by LUMC and RHH [26]. There were minor differences in the within-operator variability between operators of the 2 calibration sites, and therefore, these did not have a significant effect on CV_{CAL}. *n* is the number of patient samples with INRs in the range of 1.5 to 4.5. *M* is the mean INR of the patient samples determined with the PT test system and the IRP (equation 6 in Supplementary Method). ISI_{SMS} is not the value stated by the manufacturer but is the ISI of the selected measurement system obtained by calibration with the indicated IRP.

INR, international normalized ratio; IRP, international reference preparation; ISI, international sensitivity index; LUMC, Leiden University Medical Center; PT, prothrombin time; RHH, Royal Hallamshire Hospital.

[a] Split sample (see Figure 3).

manufacturer of the IVD MD calibrator. It shall be determined by statistically combining the uncertainties associated with each of the sequential value assignment steps under the control of the manufacturer with the combined uncertainty of higher-order steps in the calibration hierarchy used to calibrate the manufacturer's standing MP. Braga et al. recommended limits for combined measurement uncertainty across the entire metrological traceability chain [21]. These authors focused on the performance specification for combined uncertainty associated with patient results and recommended that the higher-order references should display at most equal to 33% of the total uncertainty [21]. In the case of the plasma INR measurement, several comments to the ISO 17511 requirements should be made. First, in each calibration step, not a single calibrator but approximately 80 fresh clinical samples are used as commutable secondary calibrators. In the calibration of an end-user IVD MD, a reduced number of frozen or freeze-dried plasmas may be used as calibrator [22–24]. Second, the measurement uncertainty for each of the frozen or freeze-dried plasma calibrators may be calculated by statistically combining the uncertainties of the PT, MNPT, and ISI of the MP used for assigning the INR value [25].

We attempted to estimate the uncertainty of the INR in distinct steps of the calibration hierarchy and to estimate the combined uncertainty at the level of the end user. The first step is an estimation of the relative uncertainty of the INR determined with an international reference measurement system. In the multicenter calibration study of the current IRP for thromboplastin (coded rTF/16 and RBT/16), the INR between-laboratory variation was estimated at distinct INR values [26]. At an INR level of approximately 2.0, the coefficient of variation (CV) was 5.3% and 5.6%, respectively [26]. At an INR level of approximately 3.0, the CV was 6.5 and 6.1%, respectively [26]. This is greater than the proposed CV for the reference measurement system based on clinical outcome (see Table 2), but it is expected that harmonization of the MTT will result in lower CV values [8]. In the next step of the calibration hierarchy, a manufacturer's selected MP is

calibrated with a reference measurement system. We estimated the uncertainty of the INR in this step of the hierarchy using a method described in the Supporting Information. Table 3 shows the between-thromboplastin CV of the INR for a number of commercial systems calibrated with distinct IRPs. In general, the CV was slightly lower with Tomenson's calibration model than with the ISI model [27,28]. Nevertheless, the ISI model seems to be acceptable for PT/INR procedures and systems and should be the preferred model unless the total allowable standard uncertainty of the INR is exceeded due to inadequacy of the model. The CV in this step depends on the similarity of the compositions of the manufacturer's PT system and the IRP. With similar compositions, the between-system CV is lower than with unlike compositions. The upper limit of the CV in this step is approximately 7% (Table 3). The CV of the end-user INR result may be estimated from within-laboratory precision studies [29,30]. Using CVs of 6% and 7% for ru_{ref} and $ru_{selected\ MP}$, respectively, the CV of the end user ($ru_{end\ user}$) should be less than 5% when the allowable total standard uncertainty is set to 10%, ie, Milan model 1 (Table 2).

8 | USE OF FRESH WHOLE BLOOD SAMPLES FOR CALIBRATION OF WHOLE BLOOD IVD MD

Most commercial POC PT/INR coagulometers are designed for the measurement of whole blood samples without addition of citrate as an anticoagulant (noncitrated blood). There are a few devices that can use citrated plasma in addition to noncitrated blood. Calibration of the POC devices is performed by manufacturers, with the specific parameters encoded in the test cartridge or in a batch-specific code chip [31]. Measurement of recalcified citrated plasma in whole blood POC coagulometers may result in biased results because the plasma may not be commutable with whole blood clinical samples [32].

Some commercial POC PT/INR coagulometers function only with whole blood and not with plasma. Therefore, fresh whole blood samples—collected as mentioned in the instructions for use from the manufacturer—are the only materials for reliable unbiased calibration of whole blood coagulometers. Fresh whole blood samples should be considered as RMs in the calibration hierarchy for whole blood PT/INR coagulometers (Figure 3). An essential step in the calibration hierarchy is the use of split samples. Each recruited individual, either a healthy individual (normal) or a patient treated with VKA, is requested to donate 2 samples in the same session, ie, a whole blood sample without addition of anticoagulant and a whole blood sample collected in a container with sodium citrate for the preparation of plasma. The blood sample without addition of anticoagulant can be obtained with a finger prick and is applied immediately to the appropriate point of the coagulometer, or can be collected with a plastic syringe from a vein and is then applied to the appropriate point of the coagulometer. The syringe method has the advantage that successive samples of venous blood may be applied to several coagulometers. Validation of measurements with venous whole blood by comparison to measurements

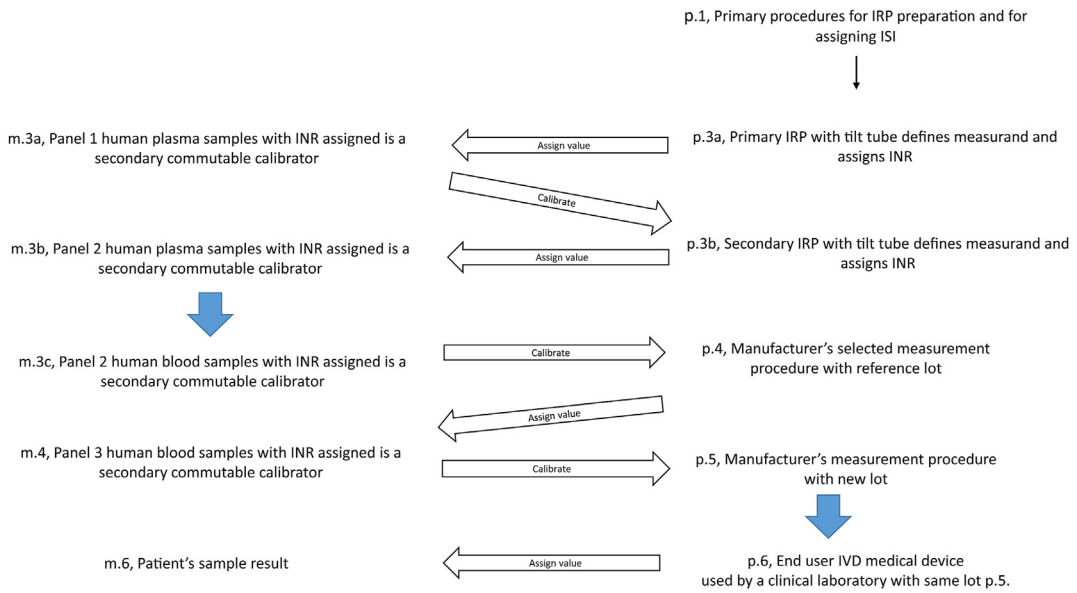

FIGURE 3 Proposed calibration hierarchy of whole blood international normalized ratio (INR) measurement. The calibration of a manufacturer's selected whole blood measurement procedure against a (secondary) reference thromboplastin (human) is performed with split samples, ie, plasma samples (m. 3b) are used with the (secondary) reference thromboplastin (human) with the harmonized manual tilt tube technique and whole blood samples (m. 3c) obtained from the same individuals at the same time are used with the manufacturer's selected measurement procedure. The manufacturer's reference lot (p. 4) refers to a lot of single-use test strips or test cartridges. The manufacturer's new lot (p. 5) refers to any other lot of test strips or cartridges for dispatch to end users. It is assumed that the manufacturer's calibration can be applied directly to the end-user measurement procedure (p. 6). In this calibration hierarchy, there is no end-user calibrator (human blood samples) for calibration of the end-user *in vitro* diagnostic (IVD) medical device. IRP = international reference preparation; ISI = international sensitivity index.

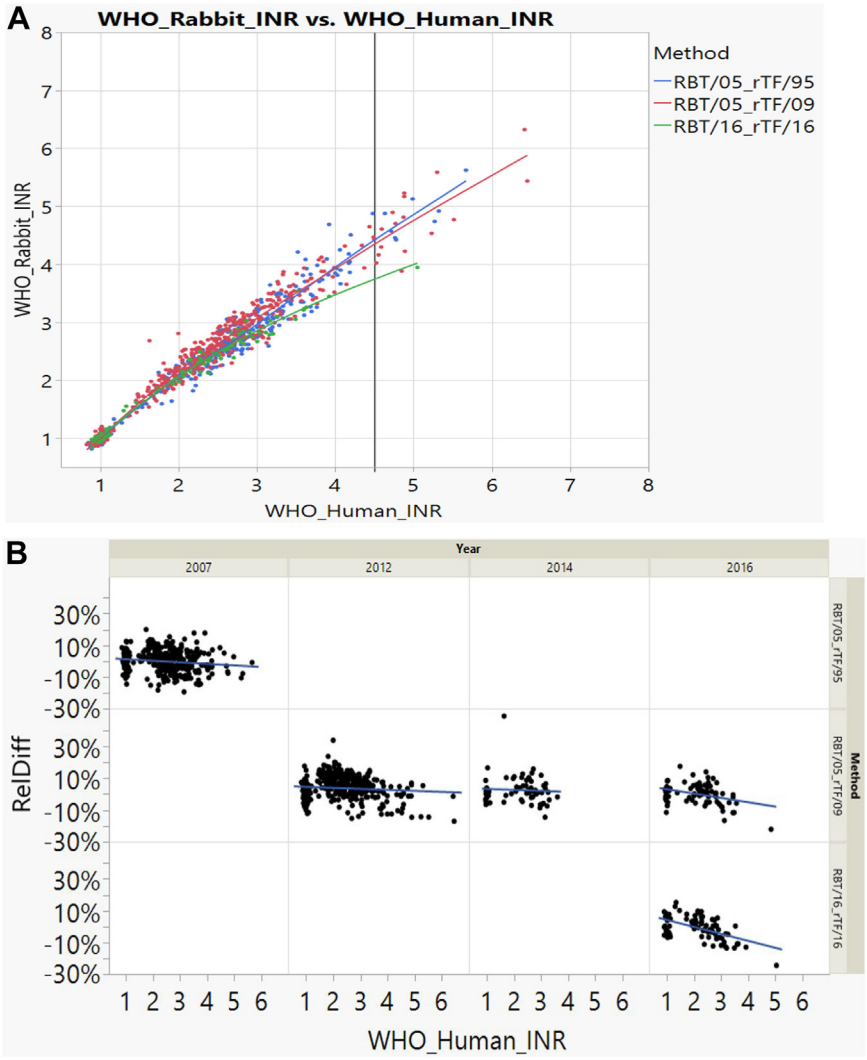

FIGURE 4 Comparison of international normalized ratio (INR) obtained with World Health Organization (WHO) international reference preparations (IRPs) for human recombinant and rabbit brain thromboplastin by a calibration laboratory using the manual tilt tube technique. Three comparisons are shown: RBT/05 vs rTF/95 (performed in 2007), RBT/05 vs rTF/09 (performed in 2012, 2014, and 2016), and RBT/16 vs rTF/16 (performed in 2016). (A) INRs were calculated with the stated international sensitivity index and the local mean normal prothrombin time. (B) For each individual sample, the relative difference (RelDiff) in INR between the rabbit IRP and the human IRP is plotted against the INR obtained with the human IRP. A regression line is shown in each difference plot.

with capillary blood has been reported for the CoaguChek S (Roche Diagnostics, Mannheim, Germany) and the CoaguChek XS (Roche Diagnostics, Mannheim, Germany) systems [33,34]. It should be noted that the step from a secondary reference thromboplastin to a manufacturer's selected whole blood MP makes use of split samples, ie, human plasma samples for the reference thromboplastin and whole blood samples obtained from the same individuals at the same time for the manufacturer's selected whole blood MP. Most whole blood POC INR MPs utilize recombinant human thromboplastin in the devices, but rabbit tissue thromboplastin is used in some types [31]. It should be realized that it is unrealistic to assume that the end user's coagulometer would give the same measurement result as the manufacturer's in-house coagulometer. The manufacturer should estimate the variation between individual POC coagulometers of the same series or production line and use this estimate to provide the combined standard measurement uncertainty for the INR values reported by the end user's instrument.

9 | PRIMARY AND SECONDARY REFERENCE THROMBOPLASTIN REAGENTS

The current WHO guidelines refer to "International Standards" (ie, IRP) and "secondary standards" for thromboplastin. National reference preparations and manufacturers' working standards are referred to as secondary standards. Currently, there are no primary or secondary IRPs. All IRPs have equal status. In the current WHO guidelines, it is stated that each collaborative study for replacement of an IRP should include the testing of all existing IRPs. Furthermore, it is stated that the ISI assigned to the replacement should be the mean of the ISIs obtained by calibration with all existing IRPs (ie, human and rabbit). This requirement was based on the expectation that averaging of the ISIs would reduce any bias between different calibration routes [35]. There are 2 problems with the calibration approach of the WHO guidelines. The first problem is that the WHO guidelines allow the existence of more than 1 IRP, which could result in significant bias in

the measurand value at the highest level of the calibration hierarchy. INRs obtained with the human IRP and with the rabbit IRP are similar but not identical (Figure 4). In other words, the 2 IRPs are not equivalent and cannot both define the highest level of metrological traceability. The second problem is that successive replacements of IRPs could result in a drift of the assigned ISI value [7]. The ISI drift was observed in the latest replacement of the IRP for thromboplastin (human, recombinant) [26]. In the calibration exercise, the replacement IRP (coded rTF/16) was tested against rTF/09 and RBT/05. The ISI for rTF/16 against rTF/09 was 1.09 and against RBT/05 was 1.14. After implementing the WHO requirement to use the mean of ISIs against the 2 existing IRPs, an ISI of 1.11 was assigned to rTF/16 [26]. The changeover from rTF/09 to rTF/16 was associated with small and clinically irrelevant differences in median INRs obtained in routine use for patient management [36].

To avoid these problems, we propose the establishment of a single primary IRP for thromboplastin defining the measurand. To avoid rapid use of the primary IRP, we propose that secondary IRPs be established for calibration of the manufacturer's selected MPs (see Figures 2 and 3). The primary IRP should be used only for calibration of successive batches of secondary IRP and calibration of a replacement primary IRP when required.

In general, the calibration of a given thromboplastin is more precise if performed against a reference preparation of similar composition and from the same species [35]. However, the availability of 2 secondary reference preparations (ie, human and rabbit) is not strictly necessary. A manufacturer's selected procedure with a rabbit thromboplastin (either Quick or Owren) can be calibrated against a secondary reference preparation of human thromboplastin, and the number of unlike calibrations (ie, rabbit against human) would be the same as in an alternative route using a secondary reference preparation of rabbit thromboplastin (Figure 5). The combined uncertainty of the INR would be the same irrespective of the route of calibration.

In the next year, the stock of the current primary IRP for thromboplastin (recombinant, human, plain) [coded rTF/16]) will be depleted. It will be replaced by a new IRP and the value for its ISI will be assigned by a group of calibration laboratories using the harmonized MTT [8]. The ISI value for the new primary IRP should be assigned by calibration with the current human IRP rTF/16 only and not by simultaneous calibration with rabbit RBT/16. For this reason, the current WHO guidelines should be amended to no longer assign the ISI to the replacement IRP as the mean of the ISIs obtained by calibration with all existing IRPs.

We recommend that the replacement IRP for thromboplastin (recombinant, human, plain) should be established as the primary IRP defining the INR measurand. This recombinant human thromboplastin with synthetic phospholipids has the advantage that it can be defined in chemical terms. Establishing a single primary IRP (human) and 1 secondary reference thromboplastin (human) calibrated against the primary IRP will enable full implementation of metrological traceability according to the international protocol for value assignment described in ISO 17511:2020 and will prevent further bias increases with every new replacement of thromboplastin reference preparations. Solutions have to be found for reliable calibration within allowable measurement uncertainty of rabbit thromboplastins in Quick and Owren reagents to produce equivalent INR results to other human reagent–based MPs. This can only be accomplished if it can be unequivocally proven that the combined measurement uncertainty of PT/INR will stay within allowable measurement uncertainty and will not deteriorate if using only 1 reference thromboplastin.

10 | CONCLUSION

For establishing a PT/INR reference measurement system, the selected calibration hierarchy is based on the ISO 17511:2020 model

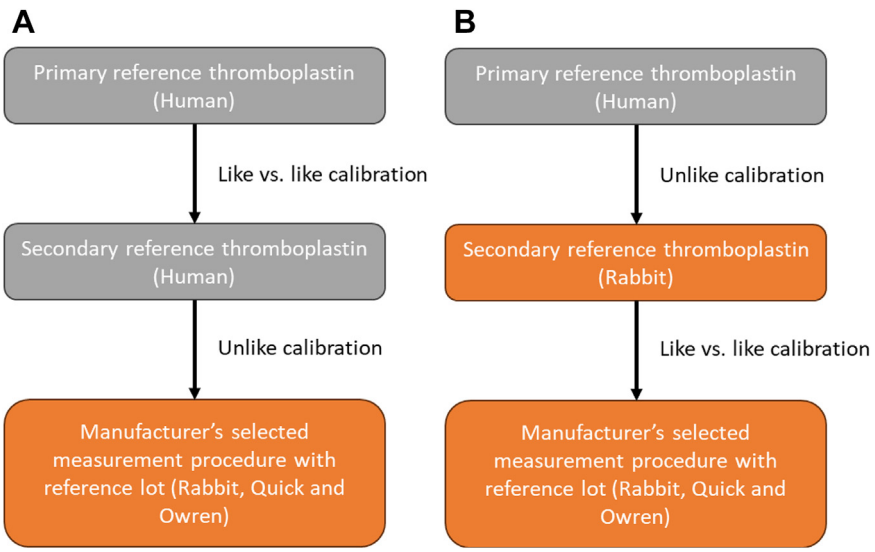

FIGURE 5 The calibration of a manufacturer's selected measurement procedure based on a rabbit thromboplastin, either Quick or Owren. (A) Based on the availability of only a single secondary reference thromboplastin (human), as proposed in this article. (B) Based on the availability of 2 secondary reference thromboplastins—1 human and 1 rabbit. In both situations, there is one like vs like calibration (human-to-human or rabbit-to-rabbit) and 1 calibration of unlike thromboplastin preparations. Like vs like calibration results in better precision, but the overall uncertainty of the international normalized ratio measured with the manufacturer's selected procedure with rabbit thromboplastin will be the same irrespective of situation (A) or (B). Therefore, a single secondary reference thromboplastin (human) is sufficient.

with a higher-order primary reference reagent and MP that defines the measurand. Only one primary and one secondary reference thromboplastin reagents (both human recombinant) are recommended. In following these recommendations, the current WHO guidelines, in which the ISI to be assigned to the replacement IRP is the mean of the ISIs obtained by calibration with all existing IRPs, would need to be amended. The secondary reference thromboplastin serves to prevent further drifting of the ISI with every thromboplastin IRP lot replacement.

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AUTHOR CONTRIBUTIONS

A.M.H.P.v.d.B. wrote the first draft of the manuscript. All authors contributed to revision of the intellectual content and approved the final version of the manuscript.

DECLARATION OF COMPETING INTERESTS

A.C. has served as a consultant for Synergy, New York Blood Center, and MingSight Pharmaceuticals and has received authorship royalties from UpToDate. S.K. has received consultancy payments from Werfen. N.Z. is an employee of Siemens Healthineers. P.H., A.J., and S.M.d.D. are employees of Roche Diagnostics GmbH. C.K. and Z.C. are employed by Werfen, Orangeburg, NY. Y.I. is employed by Sysmex Corporation. F.D. is employed by Diagnostica Stago. All other authors declare no relevant conflicts of interest.

REFERENCES

[1] Biedermann JS, Leebeek FWG, Buhre PN, De Lathouder S, Barends JPF, de Maat MPM, van der Meer FJM, Kruip MJ. Agreement between CoaguChek XS and STA-R Evolution (Hepato Quick) INR results depends on the level of INR. *Thromb Res*. 2015;136:652–7.

[2] Blombäck M, Abildgaard U, van den Besselaar AMHP, Clementson KJ, Dahlbäck B, Exner T, Francis CW, Gaffney P, Gralnick H, Hoyer LW. Nomenclature of quantities and units in thrombosis and haemostasis (Recommendation 1993). A Collaborative project of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH/SSC) and the Commission/Committee on Quantities and Units (in Clinical Chemistry) of the International Union of Pure and Applied Chemistry-International Federation of Clinical Chemistry (IUPAC-IFCC/CQU(CC)). *Thromb Haemost*. 1994;71:375–94.

[3] World Health Organization Expert Committee on Biological Standardization. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists, Annex 6, TRS No 979. https://www.who.int/publications/m/item/annex-6-trs-no-979; 2013. [accessed January 8, 2024].

[4] Owren PA. Thrombotest. A new method for controlling anticoagulant therapy. *Lancet*. 1959;2:754–8.

[5] Poller L. International normalized ratios (INR): the first 20 years. *J Thromb Haemost*. 2004;2:849–60.

[6] Hermans J, van den Besselaar AMHP, Loeliger EA, van der Velde EA. A collaborative calibration study of reference materials for thromboplastins. *Thromb Haemost*. 1983;50:712–7.

[7] Van den Besselaar AMHP, van Rijn CJJ, Hubbard AR, Kitchen S, Tripodi A, Cobbaert CM. Requirement of a reference measurement system for the tissue factor-induced coagulation time and the international normalized ratio. *Clin Chem Lab Med*. 2019;57:e169–72.

[8] Van den Besselaar AMHP, van Rijn CJJ, Abdoel CF, Chantarangkul V, Scalambrino E, Kitchen S, Tripodi A, Woolley AM, Padovan L, Cobbaert CM. Paving the way for establishing a reference measurement system for standardization of plasma prothrombin time: harmonizing the manual tilt tube method. *J Thromb Haemost*. 2020;18:1986–94.

[9] International Organization for Standardization. ISO 17511:2020. In vitro diagnostic medical devices—requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples. https://www.iso.org/standard/69984.html; 2020. [accessed January 10, 2024].

[10] Gosselin RC, Marlar RA. Preanalytical variables in coagulation testing: setting the stage for accurate results. *Semin Thromb Hemost*. 2019;45:433–48.

[11] Van Geest-Daalderop JHH, Mulder AB, Boonman-de Winter LJM, Hoekstra MMCL, van den Besselaar AMHP. Preanalytical variables and off-site blood collection: influences on the results of the prothrombin time/international normalized ratio test and implications for monitoring of oral anticoagulant therapy. *Clin Chem*. 2005;51:561–8.

[12] Sandberg S, Fraser CG, Horvath AR, Jansen R, Jones G, Oosterhuis W, Petersen PH, Schimmel H, Sikaris K, Panteghini M. Defining analytical performance specifications: consensus statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine. *Clin Chem Lab Med*. 2015;53:833–5.

[13] Panteghini M, Ceriotti F, Jones G, Oosterhuis W, Plebani M, Sandberg S. Task Force on Performance Specifications in Laboratory Medicine of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM). Strategies to define performance specifications in laboratory medicine: 3 years on from the Milan Strategic Conference. *Clin Chem Lab Med*. 2017;55:1849–56.

[14] Keeling D, Baglin T, Tait C, Watson H, Perry D, Baglin C, Kitchen S, Makris M, British Committee for Standards in Haematology. Guidelines on oral anticoagulation with warfarin—fourth edition. *Br J Haematol*. 2011;154:311–24.

[15] Ceriotti F, Fernandez-Calle P, Klee GG, Nordin G, Sandberg S, Streichert T, Vives-Corrons JL, Panteghini M. Criteria for assigning laboratory measurands to models for analytical performance specifications defined in the 1st EFLM Strategic Conference. *Clin Chem Lab Med*. 2017;55:189–94.

[16] Chen Q, Shou W, Wu W, Guo Y, Zhang Y, Huang C, Cui W. Biological and analytical variations of 16 parameters related to coagulation screening tests and the activity of coagulation factors. *Semin Thromb Hemost*. 2015;41:336–41.

[17] de Maat MPM, van Schie M, Kluft C, Leebeek FWG, Meijer P. Biological variation of hemostasis variables in thrombosis and bleeding: consequences for performance specifications. *Clin Chem*. 2016;62:1639–46.

[18] Van Geest-Daalderop JHH, Péquériaux NCV, van den Besselaar AMHP. Variability of INR in patients on stable long-term treatment with phenprocoumon and acenocoumarol and implications for analytical quality requirements. *Thromb Haemost*. 2009;102:588–92.

[19] Van Geest-Daalderop JHH, Kraaijenhagen RJ, van der Meer FJM, van den Besselaar AMHP. Intraindividual variation of the

international normalized ratio in patients monitored with a recombinant human thromboplastin. *J Thromb Haemost*. 2010;8:1641–2.
[20] Van den Besselaar AMHP, Fogar P, Pengo V, Palareti G, Braham S, Moia M, Tripodi A. Biological variation of INR in stable patients on long-term anticoagulation with warfarin. *Thromb Res*. 2012;130:535–7.
[21] Braga F, Infusino I, Panteghini M. Performance criteria for combined uncertainty budget in the implementation of metrological traceability. *Clin Chem Lab Med*. 2015;53:905–12.
[22] van den Besselaar AMHP, Barrowcliffe TW, Houbouyan-Réveillard LL, Jespersen J, Johnston M, Poller L, Tripodi A. Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the ISTH. Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination. *J Thromb Haemost*. 2004;2:1946–53.
[23] Lindahl TL, Egberg N, Hillarp A, Ødegaard OR, Edlund B, Svensson J, Sandset PM, Rånby M. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. *Thromb Haemost*. 2004;91:1223–31.
[24] Hillarp A, Egberg N, Nordin G, Stigendal L, Fagerberg I, Lindahl TL. Local INR calibration of the Owren type prothrombin assay greatly improves the intra- and interlaboratory variation. A three-year follow-up from the Swedish national external quality assessment scheme. *Thromb Haemost*. 2004;91:300–7.
[25] Van den Besselaar AMHP, Witteveen E, van der Meer FJM. Uncertainty of international sensitivity index and international normalized ratio. *J Thromb Haemost*. 2013;11:1615–7.
[26] van den Besselaar AMHP, Chantarangkul V, Angeloni F, Binder NB, Byrne M, Dauer R, Gudmundsdottir BR, Jespersen J, Kitchen S, Legnani C, Lindahl TL, Manning RA, Martinuzzo M, Panes O, Pengo V, Riddell A, Subramanian S, Szederjesi A, Tantanate C, Herbel P, et al. International collaborative study for the calibration of proposed international standards for thromboplastin, rabbit, plain, and for thromboplastin, recombinant, human, plain. *J Thromb Haemost*. 2018;16:142–9.
[27] Tomenson JA. A statistician's independent evaluation. In: van den Besselaar AMHP, Gralnick HR, Lewis SM, eds. *Thromboplastin calibration and oral anticoagulant control*. the Hague: Martinus Nijhoff; 1984:87–108.
[28] Poller L, van den Besselaar AMHP, Jespersen J, Tripodi A, Houghton D. Correction for lack of coincidence of normal and abnormal calibration slopes in ISI determination. *Thromb Haemost*. 1999;81:935–9.
[29] Yis OM, Bugdayci G, Pehlivan MB, Yildiz RN, Alisik M. Analytical performance evaluation of Sysmex CS-2500 and Stago STA Compact. *Blood Coagul Fibrinolysis*. 2020;31:324–9.
[30] Kitchen S, De Maat M, Nagler M, Jones R, Lowe A, Burden J, Groneberg K, Rozsnyai G. System performance evaluation of the cobas t 711 and cobas t 511 coagulation analyzers in routine laboratory settings. *Blood Coagul Fibrinolysis*. 2020;31:459–68.
[31] Wool GD. Benefits and pitfalls of point-of-care coagulation testing for anticoagulation management: an ACLPS critical review. *Am J Clin Pathol*. 2019;151:1–17.
[32] Van den Besselaar AMHP, Abdoel CF, van Rijn CJJ, van der Meer FJM, Cobbaert CM. Accuracy assessment of consecutive test strip lots for whole blood INR point-of-care instruments: clarifying the role of frozen plasma pools. *Clin Chem Lab Med*. 2019;57:1349–57.
[33] Taborski U, Plesch W. Equivalence of capillary versus venous INR results and patient- versus professional-determined INR values using the CoaguChek S system. *Clin Appl Thromb Hemost*. 2002;8:187–9.
[34] Plesch W, van den Besselaar AMHP. Validation of the international normalized ratio (INR) in a new point-of-care system designed for home monitoring of oral anticoagulation therapy. *Int J Lab Hematol*. 2009;31:20–5.
[35] Tripodi A, Poller L, van den Besselaar AMHP, Mannucci PM. A proposed scheme for calibration of international reference preparations of thromboplastin for the prothrombin time. On behalf of the Subcommittee on Control of Anticoagulation. *Thromb Haemost*. 1995;74:1368–9.
[36] Kitchen S, Jennings I, Kitchen DP, Reilly-Stitt C, Walker ID. Laboratory international normalized ratios determined with commercial thromboplastins in >450 centers before and after the establishment of the international standard for human thromboplastin-rTF 16: data from United Kingdom National External Quality Assessment Scheme for Blood Coagulation. *J Thromb Haemost*. 2023;21:1385–7.

SUPPLEMENTARY MATERIAL

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