

## Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination

A. M. H. P. VAN DEN BESSELAAR, T. W. BARROWCLIFFE,\* L. L. HOUBOUYAN-RÉVEILLARD,† J. JESPERSEN,‡ M. JOHNSTON,§ L. POLLER¶ and A. TRIPODI\*\* ON BEHALF OF THE SUBCOMMITTEE ON CONTROL OF ANTICOAGULATION OF THE SCIENTIFIC AND STANDARDIZATION COMMITTEE OF THE ISTH

Hemostasis and Thrombosis Research Center, Department of Hematology, Leiden University Medical Center, Leiden, the Netherlands; \*Division of Hematology, National Institute for Biological Standards and Control, Potters Bar, UK; †Service Central d'Immuno-Hématologie, Hôpital Ambroise Paré, Boulogne, France; ‡Department for Thrombosis Research, University of Southern Denmark and Department of Clinical Biochemistry, Ribe County Hospital, Esbjerg, Denmark; §Hamilton Civic Hospitals Research Center, Hamilton, Ontario, Canada; ¶ECAA Central Facility, School of Biological Sciences, The University of Manchester, Manchester, UK; and \*\*A. Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital, University of Milan, Milan, Italy

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**Summary.** Reliable international normalized ratio (INR) determination depends on accurate values for international sensitivity index (ISI) and mean normal prothrombin time (MNPT). Local ISI calibration can be performed to obtain reliable INR. Alternatively, the laboratory may determine INR directly from a line relating local log(prothrombin time [PT]) to log(INR). This can be done by means of lyophilized or frozen plasmas to which certified values of PT or INR have been assigned. Currently there is one procedure for local calibration with certified plasmas which is a modification of the WHO method of ISI determination. In the other procedure, named 'direct' INR determination, certified plasmas are used to calculate a line relating log(PT) to log(INR). The number of certified plasmas for each procedure depends on the method of preparation and type of plasma. Lyophilization of plasma may induce variable effects on the INR, the magnitude of which depends on the type of thromboplastin used. Consequently, the manufacturer or supplier of certified plasmas must assign the values for different (reference) thromboplastins and validate the procedure for reliable ISI calibration or 'direct' INR determination. Certification of plasmas should be performed by at least three laboratories. Multiple values should be assigned if the differences between thromboplastin systems are greater than

10%. Testing of certified plasmas for ISI calibration may be performed in quadruplicate in the same working session. It is recommended to repeat the measurements on three sessions or days to control day-to-day variation. Testing of certified plasmas for 'direct' INR determination should be performed in at least three sessions or days. Correlation lines for ISI calibration and for 'direct' INR determination should be calculated by means of orthogonal regression. Quality assessment of the INR with certified plasmas should be performed regularly and should be repeated whenever there is a change in reagent batch or in instrument. Discrepant results obtained by users of certified plasmas should be reported to manufacturers or suppliers.

**Keywords:** anticoagulant control, international normalized ratio, international sensitivity index, prothrombin time, thromboplastin.

### Introduction

The WHO recommended model for standardization of the prothrombin time (PT) for laboratory control of oral anticoagulant treatment depends upon the accurate determination of the patient's PT and conversion of the PT to international normalized ratio (INR) [1]. The conversion to INR is based on the responsiveness of a thromboplastin measured by its international sensitivity index (ISI).

The INR system of PT standardization was originally based on manual determination of PTs, and envisaged the assignment of a single ISI value for each batch of thromboplastin reagent [2,3]. However, in recent years the manual PT has been almost universally replaced by coagulometers, and many studies have shown that the ISI of thromboplastin reagents differs according

Correspondence: A. M. H. P. van den Besselaar (Chair of working group), Hemostasis and Thrombosis Research Center, Department of Hematology, C2-R, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands.

Tel.: +31 71 526 1894; fax: +31 71 526 6755; e-mail: a.m.h.p.van\_den\_besselaar@lumc.nl

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to the type of instrument used [4–7]. Some manufacturers have introduced ‘instrument specific ISIs’, but this does not overcome the problem completely because of the many possible instrument/reagent combinations and because ISIs often differ with the same thromboplastin even among instruments of the same type. Local PT system (i.e. thromboplastin/coagulometer combination) ISI calibration therefore appears essential when quality assessment of the INR with certified plasmas shows poor performance. ISI calibration using the WHO recommended procedure is not usually possible in routine hospital laboratories for a variety of reasons, including the requirement for manual PT testing with a standard thromboplastin preparation. Standard thromboplastin reagents (either WHO standards or secondary standards) are not readily available to routine hospital laboratories. Furthermore, the WHO procedure requires a sample of 60 fresh plasmas from stabilized oral anticoagulated patients and 20 fresh plasmas from normal subjects. The demand for a large number of fresh plasmas is a considerable constraint on the performance of thromboplastin ISI calibrations at most centers.

To avoid the above-mentioned constraints, laboratories may calibrate their own local system (i.e. instrument/reagent combination) using certified plasmas supplied by manufacturers or reference laboratories. The present guidelines on preparation, certification and use of certified plasmas have been elaborated by a working group of the International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee (ISTH/SSC) Subcommittee on Control of Anticoagulation, and are intended to provide guidance to both manufacturers and users of certified plasmas.

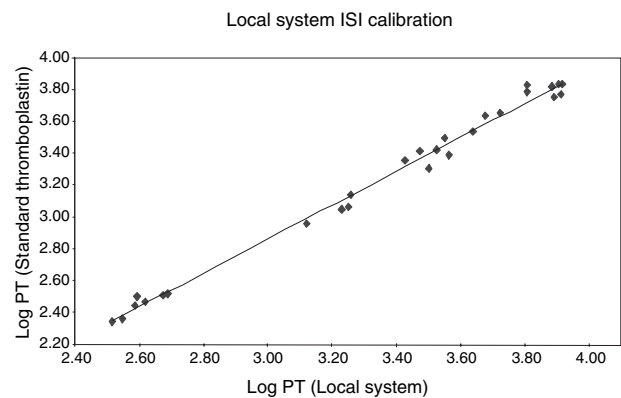
### Purpose of certified plasmas

Definitions and nomenclature used in these guidelines are given in Table 1. Two procedures using certified plasmas have been described:

- 1 One procedure is a modification of the WHO method for ISI determination. In a set of plasmas each plasma is assigned a manual PT value by the manufacturer or reference center using an international standard for thromboplastin. In the local laboratory PTs of each plasma are measured with the local instrument/reagent combination, and the two sets of PTs are plotted on a log/log plot, as illustrated in Fig. 1. The slope of the orthogonal regression line is used to determine the local ISI (see Appendix), which can then be used for

subsequent determination of INRs from the local PTs and mean normal prothrombin time (MNPT) [8–12]. An underlying assumption of the WHO orthogonal regression model is that a single line describes the relationship between  $\log(\text{PT})$  of abnormal and normal plasmas. If there is a significant deviation of the two calibration lines (i.e. abnormal-only and normal/abnormal combined), a correction according to Tomenson should be applied [13,14].

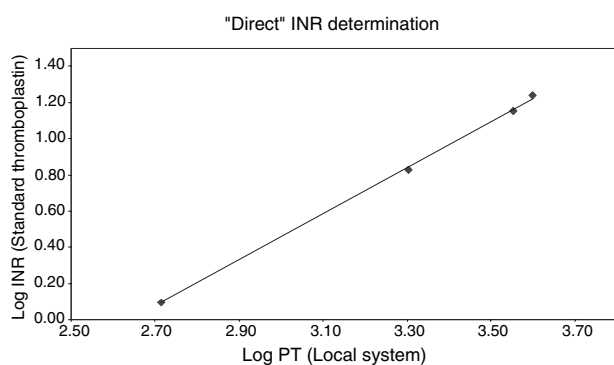
- 2 The other procedure which has been proposed involves assignment of INR values to a set of plasmas with the manual method and an international thromboplastin standard by the manufacturer or reference center. The PTs of these plasmas are measured locally using the local instrument/reagent combination, and the local test system PTs are plotted against the reference INRs on a log/log plot, as illustrated in Fig. 2. An orthogonal regression line is calculated, and INRs of patients' plasmas can be interpolated directly from local PTs using this line, without the need for ISI or MNPT determination. Although many studies of direct INR



**Fig. 1.** Example of a calibration line for determination of local international sensitivity index (ISI), using 20 abnormal and seven normal plasmas. The prothrombin times (PTs) of all certified plasmas are measured with the local instrument/reagent, and the natural logarithms of the local PTs are plotted against the natural logarithms of the PT values assigned by the manufacturer using a reference (standard) thromboplastin and the manual technique. The orthogonal regression line is constructed, and the slope of the line,  $b$ , measured. The local ISI is then calculated as:  $\text{ISI (local)} = b \times \text{ISI (ref)}$  where  $\text{ISI (ref)}$  is the ISI of the reference (standard) thromboplastin. International normalized ratios (INRs) of test plasmas are then calculated from local PTs and local ISI and mean normal prothrombin time (MNPT) according to the formula:  $\text{INR} = (\text{PT}/\text{MNPT})^{\text{ISI}}$ .

**Table 1** Definitions and nomenclature

Certified plasma	Plasma with assigned prothrombin time (PT) (in seconds) or international normalized ratio (INR) value
International sensitivity index (ISI) calibration	Determination of ISI according to 1999 WHO Guidelines
Mean normal prothrombin time (MNPT) according to 1999 WHO Guidelines	The geometric mean of the prothrombin times of the healthy adult population. For practical purposes, the geometric mean of the PT calculated from at least 20 fresh samples from healthy individuals, including those of both sexes, is a reliable approximation of MNPT
Test system	Combination of thromboplastin and instrument for PT determination
Local test system ISI calibration	Determination of local test system ISI using certified plasmas
'Direct' INR determination	Alternative approach to INR determination using certified plasmas without employing an ISI and MNPT



**Fig. 2.** Example of a calibration line for direct determination of international normalized ratio (INR), using one normal pooled and three abnormal pooled plasmas. Prothrombin times (PTs) of the certified plasmas are measured using the local instrument/reagent, and the natural logarithms of the local PTs are plotted against the natural logarithms of the INR values assigned by the manufacturer using a reference (standard) thromboplastin and the manual technique. The mathematical relationship between log PT and log INR should be obtained by orthogonal regression analysis. INRs of test plasmas can be obtained from local PTs by direct interpolation from the graph without the need for mean normal prothrombin time (MNPT) or international sensitivity index (ISI) determination.

determination were performed with linear rather than orthogonal regression, the latter is preferable (see later) [15–22].

The primary aim of the above procedures is to improve INR reliability.

A number of studies have shown that use of either of these procedures can considerably reduce interlaboratory imprecision in INR determination [12,16–18,23,24]. For example, in one study the mean deviation of 95 local systems from the 'true' INR was + 14.4% with the manufacturers' ISI, but reduced to + 1.04% with the local ISI [12]. In another study, the interlaboratory CV of the INR was reduced from 12% with the manufacturers' ISI to 6% using direct INR determination with a certified plasma procedure [16].

It should be recognized that there are a number of different ways in which plasmas can be prepared and certified. The following sections describe the various methods of preparation, certification and use and their advantages and disadvantages.

### Preparation of certified plasmas

*Type of plasma—AVK (from patients on Anti-Vitamin K therapy) or artificially depleted of prothrombin complex factors (ART)*

The intention is for certified plasmas to be as similar as possible to fresh plasmas from patients, thus on theoretical grounds AVK plasmas might be preferred, although for practical reasons these have to be pooled rather than individual donations. In some studies where the two types of plasmas have been compared, AVK plasmas give closer agreement with fresh

plasmas and better interlaboratory agreement than artificially depleted plasmas [25,26]. Artificially depleted plasmas have several advantages over plasmas from patients on oral anticoagulants, including availability of larger volumes, wider selection of PT values across the therapeutic interval, and the possible reduced risk of virus transmission [27]. It can be argued that larger volumes of AVK plasmas could be obtained by pooling donations from patients on antivitamin K therapy, but this procedure would make a spectrum of INR values more difficult to obtain because of the averaging in such a pool.

The European Concerted Action on Anticoagulation (ECAA) has prepared depleted plasmas by artificial depletion of normal human plasma by selective adsorption of vitamin K-dependent clotting factors with barium sulfate to provide a range of values which spanned the therapeutic interval [10]. The ECAA has found that there is a small difference between the results with ECAA lyophilized artificially depleted plasmas and lyophilized AVK plasmas in ISI value assignment, but both of these differed by a similar amount from a conventional fresh plasma ISI calibration [10]. The mean calibration slopes with both types of lyophilized plasma were generally higher than that with fresh AVK plasmas but the differences were not great in clinical terms. It should be noted that the ECAA study was performed with one combination of a human brain international reference preparation (IRP) and recombinant thromboplastin and the manual technique, and that the conclusions may not be applicable to all other reagent-instrument combinations.

The reliability of artificially depleted plasmas and AVK plasmas depends on the method of preparation and certification.

### *Method of preparation—frozen or freeze-dried*

Although lyophilization seems a simple solution to the difficulties associated with storage and shipment of certified plasmas, there are problems associated with lyophilized materials.

Studies have shown that the INR of fresh plasmas is largely unchanged on freezing, whereas on freeze drying the INR may change significantly depending on the method of freeze drying and the thromboplastin/instrument combination used [28–31]. Buffering of plasmas shortly after blood collection can reduce but not eliminate changes after freeze drying. The magnitude of the changes is not the same for all reagents or instruments. The measured INR of lyophilized certified plasmas depends on the thromboplastin reagent and instrument used. The use of RBT/90 presents problems relating to its poor endpoint, particularly with lyophilized plasmas giving long PTs.

The widespread use of frozen plasmas presents logistical difficulties due to the potential instability of frozen plasmas, although in some countries frozen certified plasmas have been used to a limited extent with success regarding the reduction of the interlaboratory imprecision [21,23].

Freeze-dried plasmas represent the most practical approach in general laboratories and their use has been associated with reduced interlaboratory imprecision in several studies.

### *Citrate concentration*

It is well known that citrate concentration can affect the PT, especially of high INR plasmas [32,33]. Furthermore, citrate concentration has a variable effect on the ISI, but the magnitude of the effect is not the same for all reagents and instruments [33–36]. The recommended citrate concentration for the collection of blood (1 volume of citrate solution + 9 volumes of blood) for PT is 0.109 M (3.2%), although concentrations in the range 0.105–0.11 M can be accepted [35], and the citrate concentration of certified plasmas should be as close as possible to that in fresh plasma collected in the above anticoagulant [27]. Citrate concentrations of 0.129 M (3.8%) should not be used for PT tests.

### *Number of plasmas*

The number of plasmas depends on the purpose for which they are used.

*Local test system ISI calibration* According to the WHO Guidelines, to define the ISI of a working thromboplastin, a sufficient number of separate tests should be carried out to obtain a within-laboratory coefficient of variation (CV) for the slope of the orthogonal regression line of 3% or less [1]. In an ECAA study of lyophilized depleted and individual AVK plasmas, it has been shown that the number of 60 lyophilized abnormal samples required for a full WHO calibration can be reduced to 20 if combined with results from seven lyophilized normal plasmas (see Fig. 1); further reductions below this number were associated with decreased precision of the calibration line and hence increased variability of the INR [37]. However, the use of pooled AVK plasmas may reduce the scatter of individual plasmas about the line [38], and with pooled plasmas and repeat testing it is possible that a lower number could be used, e.g. acceptable precision has been achieved with six pooled AVK plasmas containing at least 50 patient samples in each pool and two pooled normal plasmas if these were analyzed on at least 3 days [39].

*'Direct' INR determination* For 'direct' INR determination a smaller number of pooled plasmas can be used. Studies have shown improved interlaboratory variability with as few as six [21], five [17,19], three [15], or two [16] plasmas, but considering that one of the plasmas should be a normal and that at least three plasmas are required to define a line, a set of one normal and at least three abnormal is recommended (see Fig. 2). One study documented the within-laboratory imprecision of the slope of a calibration line (one normal + three abnormal plasmas): the CV ranged from 0.1 to 4.6% [22]. The number of donations in each pool should be at least 10 but higher numbers are preferable to ensure normal levels of factor (F)V.

For both procedures it is important that the abnormal plasmas be chosen to cover the range of 1.5–4.5 INR. The fibrinogen and FV content should be between 60% and 140% of the average content of fresh normal plasma [30].

### **Certification (value assignment) of plasmas**

Manufacturers or suppliers are responsible for certification, i.e. value assignment to the plasmas.

#### *Thromboplastins for certification*

The WHO standard or European Reference thromboplastins should be used directly if possible. Assuming that the certified plasmas are intended for use with the various types and species of thromboplastin, all three types of standard preparations should be used (human, rabbit, and bovine). If insufficient WHO or European standards are available, national or secondary standards can be used provided these have been calibrated against the appropriate WHO or European thromboplastin standards in a multicenter study. If the plasmas are intended for use with only one type of thromboplastin, the appropriate thromboplastin standard preparation should be used (e.g. rabbit for rabbit, etc.). Several studies have shown that the INR value for some lyophilized plasmas obtained with the rabbit standard thromboplastin (RBT/90) is greater than the INR obtained with the human and bovine standard preparations [25,26,40,41], especially for artificially depleted plasmas [42]. It has been suggested that RBT/90 is more sensitive to some changes in the plasmas following lyophilization [42]. For use with one manufacturer's thromboplastin reagent only, certification with the manufacturer's calibrated reagent is acceptable; such 'reagent-specific' value assignments have been shown to be reliable in recent collaborative studies [19,22]. The manufacturer's thromboplastin reagent used for reagent-specific certification of plasmas should be calibrated by at least two independent laboratories using the original WHO procedure [1].

Although thromboplastin standards should be used for the assignment of values, the certified plasmas should be tested for suitability with a variety of commercial thromboplastins before release for general use (see Validation of certified plasmas).

#### *Number of laboratories*

It is recommended that three to five laboratories should be involved in the certification process for each set of plasmas. An individual laboratory's mean value should differ by no more than  $\pm 10\%$  of the overall mean (in terms of INR) obtained with a given thromboplastin reagent. If the difference is greater than 10%, the divergent individual laboratory's value should not be used.

#### *Manual technique or instruments*

The manual tilt tube method should be used for international standard preparations for thromboplastin, as described in the WHO Guidelines [1]. Once certified, the plasmas should be tested for their suitability with various reagent/instrument combinations. Where certification of plasmas is made with one manufacturer's reagent only, an instrument may be used. In

this case the reagent/instrument combination must have been calibrated using the original WHO procedure [1] (see Thromboplastins for certification).

#### *Single or multiple values*

For the local test system ISI calibration, the actual values of the PTs of the certified plasmas will differ according to the species of the standard thromboplastin used, and therefore PT values must be independently certified for the different species. For the direct INR determination approach, the INR values of the plasmas should theoretically be the same whichever reference thromboplastin reagent is used. In practice, differences in INRs using different thromboplastins have been observed with some freeze-dried plasmas; averaging into a single INR should not be performed if the INRs with individual standard reagents differ by more than 10% from the mean. Large discrepancies between INRs with different thromboplastins may indicate that the plasmas are unsuitable for use with thromboplastins of all types. It should be noted that the manufacturer or supplier of the certified plasmas should clearly specify the set of reagent/instrument combinations for which their materials may be reliably used [43] (see Validation of certified plasmas).

#### *Orthogonal regression*

Orthogonal regression is used if each coordinate is subject to independent random error of constant variance [44,45], e.g. PT measurements with two different reagents by the same instrument or operator. Linear regression is used when one of the values is fixed, i.e. essentially without error. The use of certified plasmas does not conform completely to either of these models, but it is important to recognize that apparently 'fixed' values of these plasmas are themselves subject to error. Therefore, orthogonal regression should be used for both procedures, i.e. local system ISI calibration and direct INR determination. The equations for orthogonal regression are given in the Appendix. The orthogonal regression formula for calculation of the ISI is also provided in the WHOCALIB procedure on the ECAA website. The orthogonal regression line equation may be incorporated in the computer of coagulometers.

#### *International Reference Plasmas*

At present there are no established International Reference Plasmas. Work has been initiated towards the development of reference plasmas for 'direct' INR assignment [15,41]. These could then be used for direct certification of batches of commercial plasmas, in the same way as for coagulation factor assays. One difficulty, as mentioned above, is that of preparing lyophilized plasmas with the same properties as fresh plasmas, and it may be that frozen plasmas have to be used. Furthermore, for long-term use, the stability of such reference plasmas would need to be carefully checked. In the meantime, commercial plasmas will continue to have their values assigned as described above.

For local system ISI calibration, ECAA artificially depleted lyophilized plasmas may be used. Sets of 20 are Food and Drug Administration approved to provide calibration of local prothrombin time systems to accord with the WHO PT standardization scheme as substantially equivalent to the latter.

For direct INR determination, a set of EU Certified Reference Materials (CRMs), which consists of a panel of three lyophilized plasmas with assigned INRs, is available from the EU Institute for Reference Materials and Measurements, Geel, Belgium.

It should be realized that the validity of ECAA artificially depleted plasmas and EU CRMs may be limited to certain combinations of thromboplastins and coagulometers, and may not be generalized to all other reagent-instrument combinations.

#### **Validation of certified plasmas**

Each set or batch of certified plasmas intended for either local test system ISI calibration or direct INR determination must be validated before release. The validation should be the responsibility of the manufacturer or supplier who may seek help from expert laboratories. The validation should go through the following process: (i) ten or more fresh plasmas from patients on long-term oral anticoagulation are selected to represent the full therapeutic range of anticoagulation; (ii) the INR of these fresh plasmas shall be determined with an appropriate international standard for thromboplastin, and the mean value ( $INR_R$ ) shall be calculated; (iii) the INR of the same fresh plasmas shall also be determined with a variety of commercial reagent/instrument combinations following the certified plasma procedure (either ISI calibration or direct INR determination). The mean value ( $INR_C$ ) shall be calculated; (iv) finally, paired INR values obtained with the international standard and with the local system are compared to assess their agreement using Bland and Altman's procedure [46]. If the relative difference between the mean values  $INR_R$  and  $INR_C$ , i.e.  $2(INR_R - INR_C)/(INR_R + INR_C)$ , is 0.1 or less, the set of certified plasmas are considered as acceptable and may be released for local ISI calibration or direct INR determination. New batches of the same type of preparation should be validated according to the above procedure.

#### **Use of certified plasmas in clinical laboratories**

##### *Quality assessment*

An important use of certified plasmas is to perform internal or external quality assessment, i.e. to determine whether or not corrective action is necessary [43,47]. For quality assessment, a set of three to five certified plasmas with INR in the range 1.5–4.5 would be required. The INRs of the certified plasmas should be calculated from local PTs and routine ISI, and compared with the certified INR values. If the differences between routine INR and certified

INR are greater than 15%, local ISI calibration or direct INR correction should be performed. In addition, the manufacturer of the reagent and certified plasmas should be informed about the discrepant results. Quality assessment with certified plasmas should be performed regularly at intervals of no more than 6 months and should be repeated whenever there is a change in reagent batch or instrument (e.g. servicing, modification, or new instrument). It should be realized that errors caused by local preanalytical factors (e.g. divergent citrate concentration or contamination of citrate with divalent cations) cannot be corrected by certified plasma procedures [48].

#### To determine local ISI

PTs should be measured in quadruplicate in the same working session, with the local instrument/reagent combination for the full set of normal and abnormal plasmas. It is recommended to repeat the measurements on three sessions or days to control day-to-day variation. Mean local PTs should be plotted on the horizontal axis against the certified PT values on the vertical axis (log scales). Tomenson's test should be performed to test the hypothesis that the mean log(PT) of the certified normal plasmas lies on the same line as the log(PT) of the certified abnormal plasmas [11,13]. If the hypothesis is not confirmed, Tomenson's correction formula should be applied [11,13]. Like-to-like comparison should be used wherever possible, i.e. if the local reagent is a human thromboplastin the certified values should be those determined with a human reference reagent. If the INR difference between the routine ISI and the local ISI calibration procedure is greater than 10%, the calibration should be repeated. If the discrepancy persists, the manufacturer or supplier of the local thromboplastin reagent and coagulometer and certified plasmas should be informed. After consultation with the manufacturer of the certified plasmas and, if possible, an expert laboratory, the clinical laboratory should decide which materials and methods for local ISI calibration should be used.

#### For direct INR measurements

This method is simpler to use as it does not require local ISI or MNPT determinations. PTs should be measured in duplicate with the local instrument/reagent combination for each certified plasma. To allow for day-to-day variation the measurements should be repeated on at least three separate days. Mean PTs should be plotted on the horizontal axis against the certified INR values on the vertical axis (log scales), and an orthogonal regression line derived. The INRs of patients' plasmas should be calculated from the measured PTs. If the INR difference between the routine ISI procedure and the direct determination is greater than 10%, the certified plasma procedure should be repeated. If the discrepancy persists, the manufacturer or supplier of the local thromboplastin reagent and coagulometer and certified plasmas should be informed. After consultation with the

manufacturer of the certified plasmas and, if possible, an expert laboratory, the clinical laboratory should decide which materials and methods for direct INR measurement should be used.

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D. A. Taberner contributed significantly to the earlier draft versions of these guidelines, but retired from the working group in 2003.

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### Appendix

The international sensitivity index of a working thromboplastin/instrument combination ( $ISI_w$ ) is obtained by plotting the prothrombin times of the international reference preparation (IRP) and the working system on logarithmic axes (as shown in Fig. 1), fitting a straight line in the form

$$Y = A + BX \quad (1)$$

and estimating the slope  $B$ . The recommended method involves estimation of a linear structural relation (also called an 'orthogonal regression equation'). With this technique, the slope  $B$  can be estimated as follows.

Consider a set of  $N$  independent prothrombin time measurements  $(x_i, y_i)$ , where  $i = 1, 2, 3, \dots, N$ ; for  $N$  paired results,  $y_i$  represents the natural logarithm of the measured prothrombin time of the IRP, and  $x_i$  that of the working system. Write  $x_0, y_0$ , for the arithmetic means of the  $N$  values of  $x_i, y_i$ , respectively. Write  $Q_1, Q_2$ , for the sums of the squares of  $(x_i - x_0)$  and  $(y_i - y_0)$ , respectively, and  $P$  for the sum of their products  $(x_i - x_0)(y_i - y_0)$ . These quantities are all that is necessary for computing  $a$  and  $b$ , the least-squares estimators for the parameters  $A$  and  $B$  of equation 1. Now define:

$$E = (Q_2 - Q_1)^2 + 4P^2 \quad (2)$$

Then

$$b = \{Q_2 - Q_1 + E^{1/2}\}/2P \quad (3)$$

and

$$a = y_0 - bx_0 \quad (4)$$

are the estimators that minimize the sum of the squares of the perpendicular distances of the  $N$  points from the line represented by equation 1. The variance of  $b$  is given by:

$$\text{Var}(b) = \{(1 + b^2)P + NbV\}bV/P^2 \quad (5)$$

where  $V$  is defined as

$$V = (Q_2 - bP)/(N - 2) \quad (6)$$

The standard error of  $b$  ( $s_b$ ) is the square root of  $\text{Var}(b)$ . If  $t$  is a deviate from the  $t$ -distribution, with  $(N - 2)$  degrees of freedom and at a chosen probability, approximate confidence limits for  $B$  can be obtained by setting an interval  $t \times s_b$  on either side of  $b$ . The residual standard deviation is the square root of  $V$ . Outlying points should be rejected if their perpendicular distance from the calibration line is greater than  $3 \times V^{1/2}$ .

The  $\text{ISI}_w$  is calculated as follows:

$$\text{ISI}_w = \text{ISI}_{\text{IRP}} \times b, \quad (7)$$

where  $\text{ISI}_{\text{IRP}}$  is the ISI of the international reference preparation.

For the 'direct' INR determination, the same equations 1, 2, 3, 4, 5, 6 can be used to estimate the line relating the natural logarithm of the prothrombin time of the local system ( $x_i$ ) to the natural logarithm of the certified INR ( $y_i$ ).