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REVIEW

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Standardization of Prothrombin Time/International Normalized Ratio (PT/INR)

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Abstract

The prothrombin time (PT) represents the most commonly used coagulation test in clinical laboratories. The PT is mathematically converted to the international normalized ratio (INR) for use in monitoring anticoagulant therapy with vitamin K antagonists such as warfarin in order to provide test results that are adjusted for thromboplastin and instrument used. The INR is created using two major PT 'correction factors', namely the mean normal PT (MNPT) and the international sensitivity index (ISI). Manufacturers of reagents and coagulometers have made some efforts to harmonizing INRs, for example, by tailoring reagents to specific coagulometers and provide associated ISI values. Thus, two types of ISIs may be generated, with one being a 'general' or 'generic' ISI and others being reagent/coagulometer-specific ISI values. Although these play a crucial role in improving INR results between laboratories, these laboratories reported INR values are known to still differ, even when laboratories use the same thromboplastin reagent and coagulometer. Moreover, ISI values for a specific thromboplastin can vary among different models of coagulometers from a manufacturer using the same method for clot identification. All these factors can be sources of error for INR reporting, which in turn can significantly affect patient management. In this narrative review, we provide some guidance to appropriate ISI verification/validation, which may help decrease the variability in cross laboratory reporting of INRs.

KEYWORDS

coagulation factors, harmonization, hemostasis, INR, prothrombin time, standardization

1 | INTRODUCTION

The prothrombin time (PT) represents the most commonly used coagulation test and was first introduced into use by Dr Armand Quick and colleagues in 1935.[1] The PT is a single-stage screening test used to evaluate the tissue factor (TF) and common coagulation pathways, and thus is affected by the activity of coagulation factors II (FII), V (FV), VII (FVII), X (FX), and fibrinogen.[2] The test measures the time (in seconds) required for clot formation after thromboplastin reagent (a mixture of TF, lipids, and calcium chloride) is added to platelet-poor plasma (PPP). The classic model of coagulation, as reflected by the coagulation cascade, was first proposed scientifically in the 1960's, and divided into two pathways, one reflected by tissue factor activation of FVII (PT), and the other reflecting activation of the contact pathway (APTT, activated partial thromboplastin time). These pathways converge to a common pathway, becoming linked through the production of thrombin by prothrombinase complex and the conversion of fibrinogen to fibrin by thrombin.[2-4]

Recent studies indicate that the TF pathway reflects the major driver of in vivo coagulation.[2,5-7] A prolonged PT might be due to one or more coagulation factor defects in the TF or common pathways, or might indicate the presence of inhibitors against these factors.[2,8]

In order to differentiate between coagulation factor deficiency and the presence of inhibitors, a mixing study is performed. If the PT is corrected after the addition of normal plasma, congenital, or acquired coagulation factor(s) deficiency is implied, whereas, if the PT remains prolonged after the addition of normal plasma raises the possibility of inhibitors.[8] Acquired defects of coagulation factors prolonging the PT are commonly observed in people with vitamin K deficiency.[9] Alternatively, vitamin K antagonists (VKAs) such as warfarin, as prescribed to prevent thromboembolic events, hamper gamma-carboxylation of vitamin K-dependent coagulation factors (VKDCFs) and thus reduce their activity and also prolong PT.[9,10] However, in the case of VKAs, prolongation of the PT into a therapeutic range is desired. Given that the synthesis of most coagulation factors is restricted to the liver, disorders of this organ can be associated with a decrease of most coagulation factors, resulting in prolonged PT and/or APTT. Prolonged PT may also occur in patients with acquired FX deficiency due to amyloidosis, especially amyloid light-chain (AL) amyloidosis.[10,11]

Thromboplastin reagent is an essential component of the PT test. Thromboplastin reagent is a combination of TF, in complex with lipid surface of procoagulant membrane and calcium chloride ions. Thromboplastin reagents may be derived from human or animal tissues (brain or placenta), or through the production of recombinant human TF within phospholipid vesicles.[12,13] Use of different TF sources leads to variation in PT results reported by different laboratories. The significance of this emerged when North American countries used rabbit thromboplastin to monitor anticoagulation; this was less sensitive than the human thromboplastin reagents used in the UK and elsewhere.[13,14] Due to the lower sensitivity of the rabbit thromboplastin, North American physicians prescribed higher doses of VKAs than the UK, as based on PT testing. However, the number of patients with bleeding in North American countries was about 5 times that in Britain.[13,14] For this reason, attempts to better standardize results of PT testing began in 1962, leading to the introduction of British Comparative Thromboplastin (BCT) as the reference thromboplastin in 1969.[15] Following the standardization process, the World Health Organization (WHO) introduced the International Normalized Ratio System (INR) in 1983.[16] This standardization system provides a simple way to interpret PT results independently of the thromboplastin used.

1.1 | International normalized ratio (INR)

Over the past few decades, anticoagulation has seen tremendous and undeniable developments. Decades ago, patients in need of anticoagulant therapy had only a few options, including VKAs, and unfractionated heparin (UFH) to help manage and prevent thrombosis.[17] Nowadays, there are much broader options for improving treatment and quality of life in these patients. A variety of low molecular weight heparins (LMWHs), heparin analogs and heparin-like compounds are available. In addition, direct oral anticoagulants (DOACs) have now been marketed for nearly a decade. Like VKAs, DOACs are also taken orally, but unlike VKAs, DOACs generally do not require regular laboratory monitoring of anticoagulation, and thus reflecting one of their main strengths.[17,18] DOACs include factor Xa inhibitors (eg, apixaban, edoxaban, and rivaroxaban) and direct thrombin inhibitors (dabigatran).[19,20] Although various studies indicate general acceptance of these drugs, VKAs remain widely used, in part due to lack of experience in, and knowledge of, the newer drugs. In addition, some patients may not be good candidates for DOACs,[17,20,21] and also because in some countries DOACs are seen as prohibitory expensive compared to VKAs. Nevertheless, due to differences in the preparation, source, and constituents of thromboplastin reagents, their sensitivity to monitoring and controlling VKAs - particularly warfarin - varies greatly. This difference can be large enough to require a change in patient management. Significant increases or decreases of VKA dose can increase the risk of thrombosis or bleeding if not appropriate to the situation.[1,8,22]

Initial efforts to standardize PT test results by reporting PT results as PT ratios were unable to completely resolve the issue of variability.[13] The PT ratio, which is the patient's PT over a mean normal PT ratio, did not fully eliminate discrepancies between results: the test was still affected by variations in thromboplastin and could not be used to harmonize results for patients receiving VKAs.[13] The INR instead reflects a mathematical calculation using a PT ratio as further adjusted with a correction factor called the international sensitivity index (ISI).[1]

$$\text{International Normalized Ratio} = \left[\frac{\text{Patient PT}}{\text{Mean Normal PT}}\right]^{\text{ISI}}$$

According to WHO standards, an acceptable ISI value for thromboplastin reagents for manual methods is between 0.9 and 1.7.[20] However, due to a more severe decrease in FVII levels than other vitamin K-dependent coagulation factors, INR is not an appropriate index to evaluate the patient's condition at the onset of anticoagulation with VKAs.[23] Although INR is recommended for patients on VKAs, it is cannot harmonize PT results in patients with chronic liver disease. A modified INR, called INR_{liver} is suggested by some authors for this purpose. In INR_{liver}, plasma from patients with cirrhosis is used to calculate ISI of thromboplastin used.[24]

Although the INR tries to harmonize PT results according to process differences, the INR value is also influenced by other factors, including the type of coagulometer used and the value of the mean normal PT (MNPT) or geometric mean normal PT (GMNPT). Manufacturers of reagents and coagulometers have made some efforts to improve standardization, tailoring reagents to specific coagulometers. The reagents may designate two types of ISIs, the general (or 'generic') ISI and the reagent/coagulometer-specific ISI.[25] Commercial thromboplastins should be assessed and calibrated by their manufacturers against the WHO thromboplastin international reference preparation (IRP). For this purpose, manual-tilt PT is typically performed, using a new or manufactured thromboplastin and the reference thromboplastin, on 20 healthy subjects and 60 patients with stable conditions undergoing VKA therapy.[26] An

individual is considered to be therapeutically stable when treated with VKAs for at least 6 weeks.[27] The values obtained from the manufactured thromboplastin are plotted on the *X*-axis and the values from the reference thromboplastin on the *Y*-axis. The logarithm of these values is calculated and a line drawn with the best fit of the points obtained. The slope of the orthogonal regression line provides the ISI value (Figure 1).

In vitro monitoring of VKAs is required for many reasons, and the aim is to keep the INR in the range of 2-3 for most patients. However, a higher INR range may be required (eg, 2.5-3.5) for some patients at high risk of venous thromboembolism (VTE), and patients with mechanical heart valves. For this reason, clinically stable patients are usually monitored every 4-6 weeks and patients with unstable status are monitored by PT/INR tests at shorter time intervals - every week or every few days.[28,29]

1.2 | Problems with monitoring of vitamin K antagonists

As mentioned earlier, a significant decrease in INR can increase the risk of thrombosis, mandating higher doses of VKAs. In contrast, a significant increase in INR represents an increased risk of bleeding, thus potentially indicating a reduction in the dose, temporarily discontinuing the drug, or if associated to very high bleeding risk (eg, INR > 10), reversing its effect by use of an antidote.[30] This issue, and the need for regular drug monitoring, is among the accepted limitations in the use and administration of VKAs. However, one less appreciated, but important, issue is the accuracy or inaccuracy of the INR as reported by laboratories.[31,32]

Success or failure of treatment with warfarin is sometimes determined by estimating the "time in therapeutic range" or TTR. The TTR index determines how clinically stable a patient is when receiving such anticoagulants, and thus could approximately determine whether VKAs are appropriate for any given patient.[33-35]

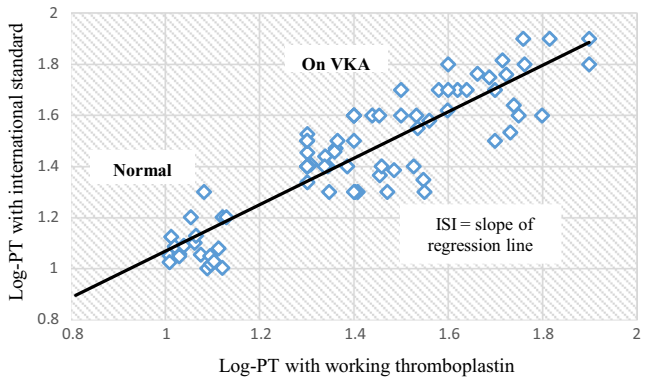

FIGURE 1 Calibration of the working thromboplastin solution (*X*-axis) against the international standard thromboplastin (*Y*-axis). Each dot represents the mean PT results (normal individuals and patients on VKA) interpolated on logarithmic paper. The slope of the regression line represents the ISI value

Although determination of reagent/coagulometer-specific ISI has played a large role in improving INR standardization, INR values reported by two laboratories may differ even with the same thromboplastin reagent and coagulometer. Moreover, the ISI value of a specific thromboplastin can vary among the different models of coagulometers of a particular manufacturer using the same method for clot identification.[36,37] All these factors comprise sources of error for INR reporting, which in turn can significantly affect patient management.[38] However, proper ISI verification and validation can decrease the rate of errors.

2 | THROMBOPLASTINS WITH A 'GENERAL' ISI VALUE

A general (or 'generic') ISI refers to an ISI value that is not specific to a particular coagulometer. This type of ISI is usually used identified for manual methods or coagulometers that use the same end-point clot detection method, for example, a manufactured thromboplastin for optical-based coagulometers or mechanical clot detection methods.[39] Use of this type of ISI may lead to greater variability than reagent/coagulometer-specific ISI values because even coagulometers that use the same clot detection method do not necessarily use the same principles (eg, differing mathematical algorithms). The error scale of the generic ISI, if not validated in the laboratory, is typically higher than that of reagent/coagulometer-specific ISIs. General ISI values should never be used clinically without prior validation in the laboratory.[39-42]

3 | THROMBOPLASTINS WITH REAGENT/COAGULOMETER-SPECIFIC ISI VALUES

This type of ISI value is thromboplastin/coagulometer-specific, with reagents having individual ISI values for different coagulometer models. The ISI may differ more significantly between coagulometers with different clot detection methods. Sometimes this difference can be noted across a company's coagulometers, even with the same clot detection method.[42-44] Thus, different laboratories using identical reagents and coagulometers may identify significant differences in reporting INR values. For this reason, if the same sample is sent to different laboratories, different INR values will be reported. Although the difference in INR value is less than the PT value, even the existing level of variation in INR value can affect the physician's decision to change the treatment dose.[44-46]

If the thromboplastin manufacturer does not designate a reagent/coagulometer-specific ISI for the model used in a laboratory, the laboratory should not permit use of that specific thromboplastin for its own coagulometer without determination and verification of the instrument-specific ISI. In addition, each laboratory is obligated to verify even the asserted reagent/coagulometer-specific ISI of the manufacturer before using the PT reagent for clinical tests. In each case, if the verification process cannot confirm the asserted ISI, the

reagent cannot be used for patient testing without ISI calibration and verification of calibrated ISI.[26,42]

4 | LABORATORY ISI CALIBRATION

4.1 | World Health Organization (WHO) recommended method

The World Health Organization (WHO) recommends this method for estimating ISI and GMNPT values for a new PT reagent by using a reference thromboplastin. To determine ISI and GMNPT of a new PT reagent, the laboratory should test the plasma of at least 20 healthy subjects and 60 patients undergoing VKA therapy with stable clinical conditions.[47] To determine MNPT, the plasma PT value of 20 to 40 normal individuals is determined and its geometric mean is calculated (ie, GMNPT).[48] As in Figure 2, the logarithm of these numbers is plotted against the logarithm of the new PT results. The ISI is calculated as 1/slope of the regression line. Alternatively, one can replace the *X*- and *Y*-axes and then calculate the ISI based on the slope of the regression line.[49]

4.2 | Laboratory calibration of test system using certified plasma

Laboratory calibration can be performed using certified plasmas one of two different methods:

1. Calculation of laboratory ISI
2. Direct determination of INR (calculation of PT/INR calibration line)

Proper use of either of these methods can improve the accuracy of the INR report.

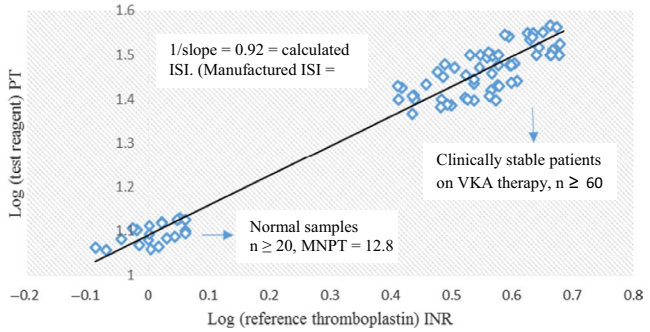

FIGURE 2 How to achieve the ISI and MNPT values for a new reagent, based on the WHO method. The MNPT value is obtained by calculating the geometric mean of at least 20 normal individuals' plasma PT results. In this example, MNPT value is 12.8. The INR numbers are calculated by reference thromboplastin and are used for deriving reference INR. The ISI value is calculated by the slope of regression line, which in this example is 0.92

4.2.1 | Method of laboratory ISI calculation

This is a modification to the WHO-recommended method, and can be used in most laboratories.[50] The PT value of each certified plasma is determined according to the reagent and the coagulometer in the laboratory and plotted on logarithmic paper against the determined INR for each citrated plasma. The slope of the regression line is used to determine the laboratory ISI. Orthogonal regression is the appropriate regression analysis.[50,51]

4.2.2 | Recommended method of the European Concerted Action on Anticoagulation (ECAA)

Another method is to use commercial plasma with a specified INR value. In this procedure, the INR of several commercial plasma samples is determined by the recommended WHO method or other, alternative methods.[52] These commercial plasmas can sometimes be used to determine the ISI, and perhaps even the GMNPT, for the new reagent/coagulometer combination, or to verify or reject a manufacturer's asserted ISI. One alternative that is also approved by the FDA is that recommended by the ECAA Committee - the simplified WHO method.[32] In this procedure, 27 artificially reduced lyophilized plasmas, including 20 factor-deficient plasmas and 7 normal plasmas were suggested. Indeed, because of the unavailability of stabilized patients under treatment with VKAs, the number of plasmas used in this procedure is greater than in other methods that use these patients for commercial plasma preparation. This is one of the limitations of the ECAA's proposed method, which is performed manually with only one type of recombinant thromboplastin and a WHO human reference thromboplastin, and which may not to be suitable for all PT methods or all laboratories. In addition, one of the most important considerations when using certified or commercial plasma is that one should read the plasma guidelines before purchase to ensure that plasmas are suitable for the coagulometer and reagent used in the desired laboratory. Accordingly, the certified plasma specifies which commercial plasmas are usable for which devices. Moreover, if a laboratory uses recombinant human thromboplastin, it should ensure that certified commercial plasma is also verified for use with this type of thromboplastin.[32,53]

In this procedure, similarly to the WHO-recommended method, the logarithm of the INR values obtained by commercial calibrant plasma is plotted against the PT values obtained by the reagent/coagulometer combination. The ISI is calculated as 1/slope of the regression line, as in the WHO method. The MNPT value is calculated as antilog of the y value.[52,53]

An alternative way to calculate ISI is by using the slope of the regression line, which can be used when replacing the *X*- and *Y*-axes is possible (Figure 3).

This alternative is also applicable and can be used in laboratories, but it should be considered that the accuracy of this method depends on the accuracy of the INR values set for commercial calibrators by the manufacturers.

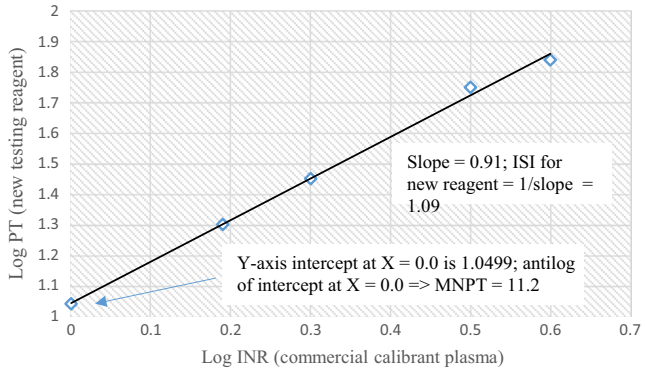

FIGURE 3 Demonstrates how to use commercial calibrators to determine ISI and MNPT for a new PT reagent. The INR values set for commercial plasma are considered as the reference INR values. Logarithm of these values is plotted against the logarithm of the PT number obtained by the reagent/coagulometer combination. ISI, which is 1.09 in this example, is calculated as 1/slope of the regression line. The MNPT is calculated as the antilog of the Y-axis with the X value considered to be zero. In this example, with INR = 1.0, the MNPT is 11.2

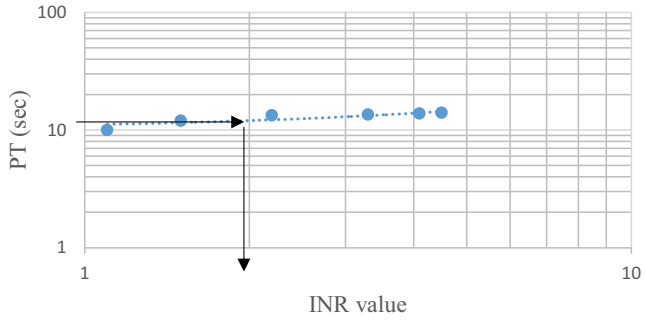

FIGURE 4 How to obtain INR directly using commercial certified plasma. In this method, PT is determined for commercial certified plasma having an INR specified by the PT reagent and the laboratory coagulometer. The mean PT obtained for each verified commercial plasma is then plotted on the *Y*-axis on logarithmic paper and the assigned number corresponding to each verified plasma is plotted on the *X*-axis. The reference line is then drawn. For each patient, a PT test is performed, and from the *Y*-axis, the PT number line is connected first to the reference line, then perpendicular to the *X*-axis. The number that represents the perpendicular line on the X-axis is the patient's INR

5 | DIRECT DETERMINATION OF INR

This method of determining INR, first proposed by researchers from France, and latter modified in Clinical and Laboratory Standards Institute (CLSI) document,[50,54] is independent of ISI and MNPT.[54] The PT test for commercially certified plasma is performed by a laboratory coagulometer and reagent.[54]

This should be done within 3 days or three separate runs to minimize day-to-day and between-runs variations. The difference between the PT of a certified plasma must not exceed the permissible CV of the laboratory. If this number exceeds the designated CV, the test must be repeated for the certified plasma(s). The mean PT of each plasma is plotted on the *Y*-axis and the specified INR value of each certified plasma is plotted on the *X*-axis of logarithmic paper. The reference line is then drawn. This reference line helps to obtain a patient INR based only on a patient's PT values, without the need for ISI and MNPT values [54] (Figure 4).

6 | VERIFICATION OF INR RESULTS

Specific reagent/coagulometer ISIs should be verified prior to clinical use in all laboratories using that ISI. For all laboratories using a generic ISI, verification is required and laboratory calibration is strongly recommended.[42]

7 | USAGE OF CERTIFIED PLASMAS FOR VERIFICATION

It is recommended that at least three certified plasmas, covering the therapeutic dose of INR ranging from 1.5 to 4.5, be used to verify ISI. For some of these commercial certified plasmas, it is not possible to determine a single INR for all coagulometers. Before using these plasmas, one should read the instructions for these reagents to check the possibility of using these plasmas for their own test system (combined set of reagent and coagulometer). If a new laboratory ISI is designated or a new calibration line is created for PT/INR, the test system must be validated prior to reporting patient results.[50,51]

8 | VERIFICATION METHOD

In the verification process, one can use certified or lyophilized plasma. The certified plasma INR value must be determined by the reagent and device used in the laboratory and by the ISI designated by the manufacturer.[51] When certified plasma is used for verification, it should be used in duplicate tests for at least 2 days.[50] According to the International Society on Thrombosis and Haemostasis (ISTH) standards, the INR obtained by the laboratory system with the INR determined by the company should not differ more than ± 15%.[51] All coagulometers used, even as support for PT testing, must be verified.[52]

9 | REQUIRED TIME INTERVAL FOR VERIFICATION

The verification process should be performed for each thromboplastin with a general ISI. It is also recommended for thromboplastins with reagent/coagulometer-specific ISI. The approval process should be re-performed following any changes to the reagent, lot

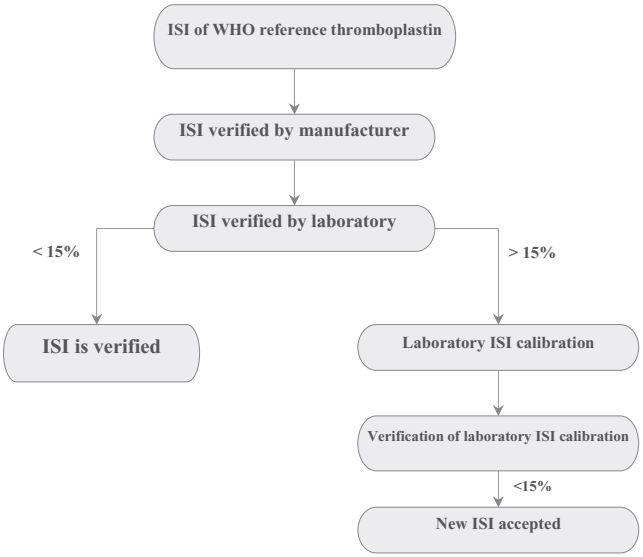

FIGURE 5 ISI Calibration and Verification Process in the Laboratory (Adopted from reference 42 with some changes)

number, coagulometer, or any major overhaul of the coagulometer, all of which can change the PT, MNPT, and ISI values. If a significant change in quality control results, or a severe deviation is observed during external quality control testing, and the cause of the problem is not identified by standard and determined methods, the verification process should be initiated,.[42,51]

10 | INTERPRETATION OF VERIFICATION RESULTS AND CORRECTIVE ACTIONS

Corrective measures should be considered if the results obtained for the INR by the laboratory differ from those determined by the commercial plasma manufacturer (actual values) by more than ± 15% [42,51,52] (Figure 5).

Firstly, one must ensure that the coagulometer is working properly and that the verification process has been done correctly.[42,50] If the verification process was not achieved, laboratory calibration of PT/INR should be performed. The verified plasma used for calibration should be different from the verified plasma used for the verification process. After calibration, the verification process must be performed again, and if the results of the verification process are acceptable, patient tests can be performed and reported.[50,51]

If the verification process still had unacceptable results, these corrective actions should be taken:

1. Evaluation of coagulometer performance
2. Evaluation of the test system with another thromboplastin
3. PT/INR calibration using a set of different verified plasmas
4. Contact with the certified plasma manufacturer and institution of recommended corrective actions.

11 | IMPACT OF GOOD LABORATORY PRACTICE (GLP) ON INR RESULTS

Good laboratory practice (GLP) can be considered an optimal laboratory performance and ensures that INR results are correct. Best practices should also include[50]:

1. Ensuring that the coagulometer is maintained and used in accordance with the conditions specified by the manufacturer.
2. Ensuring that the laboratory method used to perform PT is fully controlled. It is important to make sure that the reagents used have not expired and all reagents are from one lot. Additionally, the laboratory must ensure that ISI and MNPT values have been correctly identified and incorporated into the INR formula.
3. Ensuring that reagents are maintained, prepared, and used in accordance with company instructions.

12 | CONCLUSIONS

Accurate reporting of PT/INR results has a direct effect on the management of patients undergoing VKAs therapy. An appropriate standardization process, can significantly improve the accuracy of reported results. Verification and if necessary, calibration of reagent/coagulometer-specific, and generic ISIs, prior to clinical use is mandatory, and play a crucial role in improving PT/INR results.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

A. Dorgalaleh, F. Rad, and M. Bahraini wrote the manuscript. EJ. Favaloro revised the manuscript. All the authors approved the submission.

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