

Guidelines for the Laboratory Monitoring of Oral Anticoagulants

Background

Therapeutic control of oral anticoagulation with Vitamin K antagonists such as Warfarin sodium (Coumadin R) requires monitoring of the dosage by means of the prothrombin time (PT). The PT is technically a simple test to perform but until recently has been difficult to standardize because of the variability in the responsiveness of PT reagents to warfarin-induced reduction of Vitamin K-dependent clotting factors. The INR (International Normalized Ratio) system has provided an impetus to achieve better clinical results and this guideline is intended to assist laboratory personnel in understanding and implementing good laboratory practices in this area.

Analysis and Interpretation

a) Prothrombin Time Analysis

The procedure consists of adding a thromboplastin reagent with calcium to citrated plasma and measuring the time it takes for a fibrin clot to form. "Thromboplastin" refers to a reagent which contains phospholipids and "tissue factor" (TF). TF is a membrane protein which forms a complex with Factors VII and VIIa. This promotes conversion of Factors VII to Factor VIIa and the TF-VIIa complex in the presence of phospholipids to activate Factor X. Thromboplastin reagents are produced from human brain, rabbit brain, human placenta and, most recently, by recombinant production of TF. Warfarin acts by interference in the Vitamin K dependent post-translational carboxylation of certain blood coagulation factors Factors II, VII, IX and X, as well as the coagulation inhibitors Protein C and Protein S. The speed at which the active factor levels fall is dependent upon their synthesis rates in the liver. These rates range from a half-life of 6 hours for Factor VII to 72 hours for Factor II. The responsiveness or sensitivity of a reagent refers to the ability to detect minor changes in factor levels; hence the less Factor Xa generated, the longer the PT and the more sensitive the reagent. During the first few days of Warfarin therapy the PT prolongation is largely due to the rapid fall of Factor VII but later on the levels of Factor II (Prothrombin) and Factor X also have an influence and there is some evidence that the antithrombotic effect is related more to the level of Factor II and X than to Factor VII ¹.

b) Factors which influence the Prothrombin Time

Clinical

Significant variations in PT results occur for clinical reasons including drugs, diet, disease states and compliance. Many drugs have well described influences on Warfarin metabolism. Potentiation of the Warfarin effect and hence an increase in the PT may occur as a result of inhibition of metabolic clearance as with, for example, phenylbutazone, cotrimoxazole and cimetidine. Potentiation can also result from a variety of other mechanisms such as inhibition of synthesis of coagulation factors or even

an increased clearance of these factors. Alcohol may be a potentiator in the presence of liver disease but on its own may not affect the Warfarin effect. Inhibitory effects of barbiturates, rifampicin and cabamezapine occur due to induction of hepatic enzymes and increased clearance of Warfarin. Dietary intake of Vitamin K contained in green vegetables can have significant effects on PT results as can Vitamin K deprivation through fat malabsorption or restricted oral intake and antibiotics.

Patient compliance or understanding of intended dosage schedules should also be considered when anticoagulation control appears difficult to achieve. For a more detailed account the reader should refer to Hirsh et al ¹.

Laboratory

Therapeutic Ranges

Numerous studies have been used to determine the therapeutic ranges currently being recommended (see Table 1) and these are discussed elsewhere in great depth. For many years it has been evident that there was an inability to compare clinical studies. In North American trials Warfarin dosage levels were considerably higher as was the incidence of bleeding complications despite similar therapeutic targets. The explanation has largely been due to the wide variation in sensitivity of thromboplastin reagents in use. After European initiatives, World Health Organization (WHO) and relevant international agencies adopted a system to enable standardization of results to enable valid clinical comparisons. The International Normalized Ratio (INR) is obtained by raising the PT ratio (PT result in seconds/a normal PT) to the power of the International Sensitivity Index (ISI) of the Thromboplastin reagent:

$$INR = \left(\frac{\text{Patient PT in secs}}{\text{Mean of normal PT's in secs}} \right)^{ISI}$$

Table 1. Recommended Therapeutic Range for Oral Anticoagulant Therapy

(The ranges quoted in Table 1 are taken from the Fourth Consensus Conference of the American College of Chest Physician in April 1995.¹)

Indication	INR
Prophylaxis of venous thrombosis (high risk surgery)	2.0 - 3.0
Treatment of venous thrombosis	2.0 - 3.0
Treatment of pulmonary embolism	2.0 - 3.0
Prevention of systemic embolism	2.0 - 3.0
Tissue heart valves	2.0 - 3.0
Acute myocardial infarction (to prevent systemic embolism)*	2.0 - 3.0
Valvular heart disease	2.0 - 3.0
Atrial fibrillation	2.0 - 3.0
Mechanical prosthetic valves (high risk)	2.5 - 3.5

*If anticoagulant therapy is elected to prevent recurrent myocardial infarction, an INR of 2.5 to 3.5 is recommended, consistent with FDA recommendations. Source: Hirsh *et al.*, Chest/108/4/October, 1995 231S

Thromboplastin reagents in use in private laboratories in Ontario are listed in Table 2 and demonstrate the same widely varying sensitivities to deficiencies of Vitamin K as reported in the hospital sector.^{2,3}

Table 2. Reagents currently in use in Ontario Private Laboratories

Reagent	Manufacturer	Source of reagent	ISI
Innovin	Dade	Human recombinant	0.96
Thromboplastin C Plus	Dade	Rabbit brain	2.0
PT Fibrinogen HS	IL Test	Rabbit brain	1.47 or 1.834
Simplastin Excel	Organon Teknica	Rabbit brain	2.07
Simplastin Excel	Organon Teknica	Rabbit brain	1.14
Ortho Brain Thromboplastin	Ortho	Rabbit brain	1.92 or 1.88
Recombiplastin	Ortho	Human recombinant	1.0
Thromborel S	Behring	Human placenta	1.0

Results obtained with different reagents cannot be compared, **when reported in seconds**. This is particularly critical in the transition between initiation of therapy in hospital and stabilizing control in the community setting. Not only are different reagents likely to be used but rapidly changing levels of Factors are to be expected. By adopting the INR system of reporting of results, comparability can be obtained. Table 3 illustrates these differences:

Table 3. PT reported as 23 seconds, assuming the same median of the normal range (10.5 secs)

Reagent	Source of reagent	ISI	INR	Therapeutic decision
Recombiplastin	Human recombinant	1.0	2.1	Lower limit of control
PT Fibrinogen	Rabbit brain	1.47	3.2	Upper limit of control
Dade C	Rabbit brain	2.7	4.8	Out of control, requires review

It is important to note that even though normal (reference) ranges for the PT in seconds may be similar among reagents of different sensitivities, the PT results in seconds on abnormal samples may differ substantially.

Commercial reagents now have ISI values assigned to each lot number by the manufacturer and in some cases this is instrument specific. The lower the ISI value the more 'sensitive' the reagent. With increasing use of the INR system it has become clear that although the system is a major step forward there are several problems which should be understood and these have been well summarised¹.

- **Lack of reliability at the onset of warfarin therapy**

Factor levels fall at differing rates and the reagent response in the first 3-5 days is mainly due to declining levels of Factor VII; only later do lower Factor II and X levels start to exert their influence. Use of reagents with lower ISI's that are more responsive to Factor II and X are likely to minimize this problem as well as improve the precision of INR measurements.

- **Loss of accuracy and precision with insensitive Thromboplastins (ISI's >1.5)**

The INR calculation raises the PT ratio to the power of the ISI; a variation in the time of clot detection will result in amplification in the INR calculation by a higher ISI value. For example a 2 second

difference at the level of the example (Table 3) given above (PT increasing from 23 secs to 25 secs) will increase the INR from 2.1 to 2.4 (ISI 1.0) and from 4.8 to 5.7 (ISI 2.0).

- **Inaccuracies in determination of ISI**

The ISI system is based on calibration using manual clotting techniques. Differing methodologies using clotting detection instruments significantly influence the accuracy of this system but this will be overcome in the future by the adoption of further international standardisation and, in the mean time, by using the instrument specific ISI's provided by manufacturers, which are becoming more accurate. ISI calibrants may soon become commercially available however methodological variance can be minimised by use of reagents with low ISI values.

- **Incorrect calculations of INR's.**

The denominator in the INR calculation is the mean of the normal range which has to be locally determined using the same reagent and a minimum of 20 plasma samples from healthy volunteers. Determination of the mean ideally requires log transformation of the data due to a skewed distribution (NCCLS- LPTP) however the median can also be used with little loss of accuracy. The traditional use of a control plasma is no longer valid. In early quality assurance surveys inaccurate calculation of the INR was a major source of error in determining the INR.²

c) Reporting of Prothrombin Time Assays as an INR

The INR system was originally intended for use in monitoring patients on oral anticoagulants and some believe that it should continue to be restricted for this purpose.⁴ There are significant concerns with this practice, not the least being confusion resulting from using results in seconds as well as the INR. A study of patients with liver disease found that comparisons of INR's between reagents was not valid, probably because of the differing reagent sensitivities to factor deficiencies.⁵ However, the authors considered using the INR equally valid as using PT results reported in seconds. Easily remembered therapeutic ranges for INR seem to be a safer practice than using a system which is prone to inaccurate calculations and variable results between reagents, reagent lots and instruments.

It is the feeling of many in Ontario including the members of the OAML that it is less confusing and safer laboratory practice to report *only* the INR.

d) Sample Integrity Issues Affecting PT/INR Results

It has been good laboratory practice to reject for coagulation testing the first few mls. of blood aspirated because of the potential presence of thromboplastin released by the venipuncture, which would result in shortened PT's.⁶ However, this has been challenged in a recent publication⁷ in which no difference could be demonstrated. Traumatic or 'difficult draws' should possibly be rejected for analysis and the sample be recollected. Due to the necessity of an accurate ratio of anticoagulant to plasma the correct volume of blood added is essential. All laboratories should have procedures for ensuring correct filling of coagulation tubes.

Sample integrity is of significant concern in the community setting. Some laboratories require that the sample be tested within 12 hours of collection however there is some evidence that centrifuged but not separated samples, if maintained at 4 degrees or room temperature, retain their integrity for up to 24 hours. Obviously transportation of all specimens in a controlled manner is part of good laboratory practice and other tests such as the PTT may demonstrate more variance due to delay and temperature variation.

Tube type has become an issue recently due to changes in material. Siliconized glass and plastic tubes are not interchangeable and laboratories should be conscious of the differences before making changes usually for economic reasons. Due to evaporation of fluid anticoagulant from plastic tubes after the container is unsealed there is a 30 day period during which these tubes can be used reliably, making inventory control another variable requiring monitoring.

e) Response to prolonged INR's

Community laboratories have a responsibility to communicate results to ordering physicians in a timely manner. There may be for practical rather than clinical reasons a necessity to phone or transmit all results as soon as available. Problems arise when trying to decide at what level a result is sufficiently abnormal that the well-being of the patient will be compromised if the result is not communicated to a physician immediately. Useful levels of INR that require clinical intervention agreed to at the consensus conference are listed in Table 4. From this chart any level in excess of 6.0 clearly requires clinical consideration and must be communicated.

Bleeding incidents increase with the level of INR but it should be remembered that many bleeding incidents occur when levels are in the therapeutic range and a more appropriate gauge of clinical effectiveness comes from the time a patient's results are in the therapeutic range. Evidence from one laboratory in Ontario suggests that patients are more likely to be underdosed with 75% of all results in the community falling in the subtherapeutic range.

Table 4. Management of patients with high INR values

INR	Action
INR > 3.5 but < 6.0 patient not bleeding	rapid reversal not indicated; omit Warfarin for a few doses
INR > 6.0 but < 10.0 patient not bleeding	if rapid reversal required give 1-2 mg sc Vit K <ul style="list-style-type: none"> • reduction of INR will occur in 8 hrs • back in therapeutic range in 24 hrs
INR > 10.0 patient not bleeding	give 3 mg sc Vit K <ul style="list-style-type: none"> • INR will be reduced substantially by 6 hrs • check INR at 6 hrs
patient is bleeding or INR > 20	give 3 mg sc Vit K give 10 mg sc Vit K supplemented with FFP or prothrombin complex

f) Communication of Results by Laboratories

Computer programmes are being introduced which make therapeutic suggestions based upon previous results and dosage schedules⁸. It is likely that in the environment of outcome-based medicine and managed care that these therapeutic devices will become more widely accepted in North America.

Summary and Recommendations:

The OAML feels that the evidence is available to make certain recommendations better to serve patients who require oral anticoagulation. These are as follows:

- **Thromboplastin reagents should be sensitive with ISI < 1.5;**

- **Results should use the INR system and seconds should not be reported;**
- **INR calculations should use instrument specific ISI's;**
- **All results greater than an INR of 6.0 should be communicated urgently.**

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The Ontario Association of Medical Laboratories

The Ontario Association of Medical Laboratories (OAML) represents the community-based laboratory sector in Ontario.

Its mission is to promote excellence in the provision of laboratory services and, as an essential component of the health care system, to contribute to shaping the future of health care in Ontario.

The OAML encourages the highest level of professional and ethical integrity and technical excellence among laboratory owners, operators and staff in the provision of laboratory services for the benefit of the people of Ontario.

Guidelines for Clinical Laboratory Practice

The OAML, through its Quality Assurance and Clinical Laboratory Practice Committee, co-ordinates the development and dissemination, implementation and evaluation of Guidelines for Clinical Laboratory Practice.

A **proposed Guideline** is developed by a working group of the Committee with the participation of outside experts. The proposed guideline is then submitted to the Committee as a whole and to a Professional Advisory Group who provide an overall review of the document. The comments of the Committee and the Professional Advisory Group are incorporated into a revision of the guideline and this draft is submitted to laboratory Medical Directors, professional associations and other representatives of end users for additional comment. The document is revised in light of these comments and submitted to the OAML Board of Directors for approval.

Approved guidelines are distributed to Community-based Laboratories and by them to their client physicians.

There may be additional educational materials produced, if it is thought that they might be useful, and these are distributed with the guideline.

The comments of end users are essential to the development of guidelines and will encourage adherence. You are strongly encouraged to submit your comments on this or on any other OAML Guideline to:

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