Prothrombin Time Testing

spectra laboratories

Introduction

The Prothrombin Time (PT) was first described by Quick et al. in 1935. Oral anticoagulants were initially introduced for clinical use in the 1940's, and since then a variety of laboratory tests have been used to monitor therapy. Five decades later, PT testing is the most commonly used test to monitor oral anticoagulants. Prothrombin Time measures the effect of a reduction in the vitamin K-dependent coagulation Factors II, V, VII and X, and it measures the integrity of the extrinsic system.

Prothrombin Time is performed by adding a mixture of calcium and thromboplastin to the test plasma. The length of time, in seconds, it takes for the sample to clot is the Prothrombin Time.

The following information describes critical considerations for ensuring safe, effective Prothrombin Time testing, including:

- International Normalized Ratio (INR) reporting on PT assays
- Adsorption techniques for Heparin contaminated samples
- Important cautions when comparing test results from different laboratories

Oral Anticoagulant - Coumadin

Coumadin and other coumarin anticoagulants act by inhibiting the synthesis of vitamin K-dependent clotting factors which include Factors II, V, VII, X and the anticoagulant proteins C and S. Coumadin (sodium warfarin) is primarily used to treat many thromboembolic (clot forming) conditions. The treatment is monitored closely because the anticoagulant response to fixed dosages varies among individuals. The efficacy and safety of warfarin are highly dependent on maintaining the anticoagulant effect within a defined therapeutic range. Coumadin is considered a “narrow therapeutic index drug,” meaning that there is a very narrow window where coumadin is considered safe and effective. The most serious risk associated with anticoagulant therapy with sodium warfarin is hemorrhage in any tissue or organ. The risk of hemorrhage is related to the level of intensity and the duration of anticoagulant therapy.

Sample Collection

Buffered citrate is the standard anticoagulant for collection of samples for coagulation studies. Spectra Laboratories provides the BD light blue hemogard top tube, 2.7 mL draw—a sterile tube with siliconized interior, with 0.109M buffered sodium citrate (equivalent to 3.2% sodium citrate). The tube is labeled with distinct yellow stripes on a white background.

The optimal volume ratio of anticoagulant to blood is 1:9. It is extremely important that this ratio is maintained when obtaining blood for PT or Activated Partial Thromboplastin Time (APTT). The amount of anticoagulant to blood increases if the tube is not filled to vacuum capacity, causing falsely prolonged PT and APTT. It is important that the tube be mixed by gentle inversion soon after blood collection. Inadequate mixing causes the sample to clot, rendering it unsuitable for testing. Do not shake; shaking can cause activation of some of the clotting factors.

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INR Imprecision Factors Contributing to Total Imprecision of INR:

- Poor quality sample
- Error in establishing "normal population" PT
- Incorrect International Sensitivity Index (ISI)
- Incorrect transformation of Prothrombin Time Ratio (PTR) to INR value

Prothrombin Time Testing Interference

One of the perplexing problems in coagulation testing is heparin contamination of blood samples. Heparin is used prophylactically during dialysis to maintain the patency of the extracorporeal circuit. It is also instilled in the ports of central venous catheters intradialytically to prevent clotting and occlusion.

It is recommended that at least 10 mL of blood be removed from each port of the central venous catheter prior to the collection of blood for PT analysis, to avoid the pitfalls of heparin contamination.

Specimens contaminated with residual heparin produce PT results of questionable value. It also delays reporting of accurate results and additional costs are incurred in performing repeat testing.

Heparin Extracted Prothrombin Time

To better serve dialysis patients on oral anticoagulant therapy, Spectra Laboratories has validated the use of heparin extraction reagent to neutralize heparin-contaminated samples for PT testing. An anion exchange resin is added to the plasma and binds the heparin. The heparin-cellulose complex is removed from plasma by centrifugation.

Prothrombin Time results equal to or greater than 60 seconds on initial testing, will be retested utilizing the heparin extraction procedure. This procedure effectively absorbs and removes up to 2U of heparin per mL of plasma, producing accurate PT results without residual heparin interference.

International Normalized Ratio

Recognizing the inherent variables and limitations of the PT assay as an effective monitor of oral anticoagulant therapy, the World Health Organization (WHO), along with the International Committee on Thrombosis and Haemostasis (ICTH) and the International Committee for Standardization in Haematology (ICSH), developed a protocol to standardize the PT. The protocol is based upon the establishment by WHO of one of the ICTH thromboplastin preparations as the international reference against which all other thromboplastins would be calibrated. The end result was the creation of the International Normalized Ratio (INR) calculation that effectively equates any source of thromboplastin to the WHO "gold standard."

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

The INR is derived in the following way: the patient PT, in seconds, is divided by the mean PT of the normal population. This ratio, referred to as the Prothrombin Time Ratio (PTR), is then raised to the power of a value known as the International Sensitivity Index (ISI). The result of the PTR taken to the power of the ISI value is called the International Normalized Ratio, or INR.

The ISI value represents the responsiveness of the PT to the reduction of vitamin K-dependent coagulation Factors II, V, VII and X, measured with a given thromboplastin reagent used in the assay. The WHO reference reagent was assigned an ISI of 1.0. Therefore, thromboplastins as sensitive as the WHO reference reagent will also have an ISI of 1.0. Relatively less sensitive thromboplastins will have an ISI greater than 1.0. The higher the ISI, the less sensitive the thromboplastin. The less sensitive the thromboplastin, the shorter the PT result. The INR value is then used by the physician to manage the drug dosage, relative to the clinical situation of the patient.

INR Value

The standardized INR system is intended only for patients on stable oral anticoagulant therapy. The PT values from patients with other coagulation defects often do not follow the relationship predicted by the ISI and INR. The Sixth American College of Chest Physicians (ACCP) Consensus Conference on Antithrombotic Therapy (2000) provided recommended therapeutic ranges for therapy with oral anticoagulants. (Table 1)

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Table 1

Recommended Therapeutic Ranges for Oral Anticoagulant Therapy

Level of Therapy	Indications	Target
Standard Dose	•Prophylaxis of venous thrombosis (high risk surgery) •Treatment of venous thrombosis •Treatment of pulmonary embolism •Prevention of systemic embolism •Recurrent systemic embolism	2.0-3.0
High Dose	Mechanical prosthetic valves	2.5-3.5

Hirsh J, Dalen JE, Deykin D, Poller L. Oral anticoagulants: mechanism of action, clinical effectiveness, and optimal therapeutic range. Chest. 1992; 102 (suppl): 312S-326S

Normal Population Mean

Normal population range (reference range) consists of data generated from a group of individuals judged to be free of any known abnormalities. The establishment of the normal population mean must be accurate because it plays an important part in establishing the equivalency of different laboratories' INR values. An error of only one second can affect the INR as illustrated in Table 2. This normal PT mean is used to calculate the PTR.

Table 2

Effect of Mean Population PT on INR

	Example 1	Example 2	Example 3
PT	19.5	19.5	19.5
Mean	9.5	10.5	11.5
ISI	1.0	1.0	1.0
INR	2.1	1.9	1.7

ISI – International Sensitivity Index

For many years, after the introduction of the PT assay, clinical laboratories in the United States prepared their own thromboplastin. Those preparations varied markedly in their responsiveness to the anticoagulant effects of warfarin. Modern thromboplastin formulation, based on recombinant DNA technology, is highly reproducible from lot to lot, with a clearly defined chemical matrix, and is highly sensitive to deficiencies of Factors II, V, VII and X. Each batch of thromboplastin reagent is calibrated against the WHO standard and assigned an ISI value. Spectra Laboratories is currently using recombinant thromboplastin for the performance of the PT assay. It is an ultra sensitive reagent with an ISI close to 1.3.

Prothrombin Time measurements made with thromboplastin with a low ISI value provide a wider range (in seconds) to make adjustments in the dosage of warfarin. Table 3 illustrates the fact that as the ISI of the thromboplastin reagent increases (decreased sensitivity), the relative PT in seconds decreases as well.

Table 3

Therapeutic INR ranges

INR		ISI	
		1.0	2.0
	PT	12.9	11.3
1.0	PT Ratio	1.0	1.0
	PT	25.8	16.0
2.0	PT Ratio	2.0	1.4
	PT	32.3	17.9
2.5	PT Ratio	2.5	1.6
	PT	38.7	19.6
3.0	PT Ratio	3.0	1.7
	PT	45.2	21.1
3.5	PT Ratio	3.5	1.9

Although the INR is designed to make PT measurements from different laboratories be comparable, experience has demonstrated that different instrument-reagent combinations can occasionally affect the equivalency of INR values.

Summary

In summary, INR values are intended to assess stable patients on long-term oral anticoagulant therapy. Caution should be used when comparing test results from different laboratories. In dialysis patients, residual heparin contamination of the sample can cause questionable results and require additional testing. Spectra Laboratories has instituted a heparin adsorption procedure to alleviate the problems of heparin interference.

Call 800-433-3773 today for more information on how a renal-specific laboratory can benefit your dialysis facility.

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