Plasma Prothrombin Time And The International Normalized Ratio (INR)

This LabGuide discusses traditional plasma prothrombin time testing methods, not waived whole blood methods in popular use in point-of-care settings.

Requirements for good laboratory practice and COLA Laboratory Accreditation programs are underlined.

INTRODUCTION

Since it's introduction in 1935, the prothrombin time (PT) or "protime" has been the preferred laboratory test for monitoring stabilized patients undergoing oral anticoagulant therapy. Although well established in many clinical laboratories, the prothrombin time has seen dramatic advancements in recent years with the development of more sensitive thromboplastin reagents and with the introduction of the International Normalized Ratio (INR) as the reporting vehicle.

THEORY

The prothrombin time is useful for demonstrating the effects of oral anticoagulants upon the vitamin K-dependent clotting factors. It is important to realize that the test does not measure the actual levels of these clotting factors, but demonstrates the effect of these factors upon the clotting time. Oral anticoagulants block the transformation of Vitamin K into a form necessary for the synthesis of functional coagulation factors. When this occurs, non-functional clotting factors are produced. With anticoagulant therapy, the goal is to reduce the levels of functional clotting factors to a point at which undesired clot formation is inhibited without putting the patient in danger of uncontrolled bleeding. It is the effect of a decrease in these functional factors that is measured by an increase in the prothrombin time.

REAGENTS

Thromboplastin is a tissue extract. When combined with calcium and added to plasma it activates the extrinsic coagulation pathway. For many years, thromboplastin was derived from human brain tissue and was widely employed internationally. This reagent was very sensitive and responsive in demonstrating diminished levels of clotting factors. But, due to social pressures in the United States, human tissue sources fell out of favor and manufacturers began to produce thromboplastins from animal sources. These thromboplastins turned out to be less responsive than the human brain thromboplastins that continued to be in use in other parts of the world. Highly responsive recombinant preparations of human tissue thromboplastins are now available and have greatly improved our ability to monitor patients on anticoagulant therapy.

INTRODUCTION OF THE INR

For years, routine anticoagulant therapy theory called for a patient prothrombin time to be compared to the normal control time with therapeutic target ranges defined as a patient/control ratio (PT ratio). Due to the relative insensitivity of the animal thromboplastins to decreases in the levels of clotting factors, physicians were found to administer greater doses of medication in their efforts to achieve the desired patient PT ratio. These patients then experienced an increase in undesired bleeding episodes.

It was recognized that the problems in monitoring a patient's coagulation status was complicated by the abundance of different thromboplastin reagent preparations as well as instrumentation variations. The World Health Organization (WHO) addressed the lack of standardization of prothrombin time testing with the introduction of international thromboplastin standards.

The first International Reference Preparation (IRP) thromboplastin was established in 1977. The sensitivity of other reagent thromboplastin preparations are compared to this standard. The relative sensitivity of these reagent thromboplastins is reported as an International Sensitivity Index (ISI). The IRP is defined as having an ISI of 1.0. Reagent preparations that are more sensitive than the IRP will be reported to have an ISI of less than 1.0, while those preparations less sensitive than the IRP will have an ISI greater than 1.0.

Further complicating the matter of anticoagulant therapy is the variation in the manner of reporting the patient test results, including the time in seconds, ratios, and % activity. To obtain a uniform scale of anticoagulant intensity, the WHO recommended that the prothrombin time should be reported as an International Normalized Ratio (INR). This value is described as the prothrombin ratio that would have been obtained if the WHO International Reference Preparation had been the reagent used in the test.

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This patient INR value is determined using the prothrombin ratio and the ISI in the following calculation:

$$INR = \left[\frac{\text{patient prothrombin time in seconds}}{\text{mean of the normal reference interval in seconds}} \right]^{ISI}$$

Therapeutic ranges are then expressed as target INRs.

Many of the laboratory instruments in use today have the capability to perform this calculation and report the patient INR in conjunction with the patient's prothrombin time in seconds.

For those laboratorians using instrumentation without this capability, manual calculation of the INR can be tedious. Reagent manufacturers may be able to assist their customers by preparing a conversion chart. Using the reagent ISI and the mean of the normal reference interval (supplied by the laboratory) the manufacturer will be able to calculate the INR that correlates to any prothrombin time in seconds. The user simply needs to locate their patient protime on the chart and report the corresponding INR value. A new conversion chart must be prepared with each change in thromboplastin lot number.

TEST PROCEDURE

Described simply, the prothrombin test is performed by incubating an aliquot of citrated patient plasma with a thromboplastin-calcium chloride reagent. The time from reagent addition to clot formation is measured. The test is frequently performed in duplicate with the duplicate values accepted if they are within 5% of each other. Clot detection is accomplished visually (tilt-tube method), photo-optically (MLA Electra models), or mechanically (fibrometer). The ISI will vary depending upon the test method in use. Therefore, the reagent manufacturer may publish several different ISI values that reflect a variety of popular instrument applications.

THE MEAN OF THE NORMAL REFERENCE INTERVAL

Thromboplastin sensitivity varies from lot number to lot number as indicated by the ISI. As seen in the above formula for INR, the INR is the result of a calculation that takes the patient's protime in seconds divided by the mean of the normal reference interval raised to the power of the ISI. The laboratorian must determine the mean of their normal reference interval for each new lot number of thromboplastin employed, following any changes in instrumentation, and/or at least once each year.

The mean of the normal reference interval is not the same as the mean of the normal control plasma. The mean of the normal reference interval is determined by calculating the mean of prothrombin times (in seconds) obtained when testing a pool of normal healthy subjects. These normal subjects should be free of any known illness and should not be taking any medication that is known to alter coagulation processes. The donor pool should consist of at least 20 subjects, equal numbers of males and females, and should span the adult age range of patients tested by the laboratory. Testing should be performed in the same manner as patient specimens. A separate reference interval should be determined for pediatric populations. Use Worksheet A as a convenient tool to document your values when establishing a new normal reference interval.

CONTROLLING THE PROTHROMBIN TIME TEST

The laboratorian needs to assay quality control (QC) plasmas with known expected clotting times as a method of monitoring the testing process. Normal and abnormal (prolonged) control plasmas should be assayed daily at the beginning of patient testing, after eight hours of operation, and when there is a change of reagent. Additionally, if the manual tilt-tube method is employed, two levels of controls need to be assayed by each laboratorian performing patient tests each day of testing and with each change of reagents, and patient tests must be performed in duplicate. Refer to your manufacturer's handbook for any additional instrument-specific QC requirements.

Commercially prepared quality control plasmas are available, but the manufacturer can not routinely provide target quality control ranges for every possible instrument/reagent combination. It falls upon the user of these "unassayed" control products to establish a quality control expected range. This is accomplished by repeatedly assaying the control plasmas over a period of time, allowing for normal variation of operator and testing conditions. These

tests should be performed in the same manner as routine patient tests.

For example, if your normal procedure includes performing patient tests in duplicate with an average value reported, follow this same protocol with your QC. A minimum of 20 test values, run concurrently with the existing QC material, are compiled for each new control lot number. The mean and 2 standard deviation range can then be calculated from the accumulated data to prepare an expected range of performance for each level of new quality control product. Worksheet B may be employed to record your values. It should be noted that some reagent manufacturers may be able to assist the user with this data reduction.

For those laboratorians who must perform these calculations manually, Worksheet C is designed to guide the user through the steps involved in the calculation of a mean and standard deviation (SD).

This process must be repeated with each change in lot number of control material or thromboplastin reagent. It is prudent for the laboratorian to plan to use the same lot number of thromboplastin reagent and QC material for an extended period of time. Manufacturers will often be able to sequester a specific lot number of thromboplastin and/or QC product to be shipped in stages over the period of a year or more.

The laboratorian also needs to plan for some period of "overlap" in which the new lot numbers of quality control products can be tested parallel to the current lot numbers for the purpose of acquiring the required 20 (or more) values that will be used to calculate the new target QC ranges. This must be accomplished prior to the exhaustion or expiration of the current lot of QC materials in use.

Other factors to be controlled for reliable prothrombin time results include reagent storage conditions and incubation temperature of the instrument. Timing devices should be verified periodically, and pipettes should be calibrated for accurate dispense volumes. Reagents should be reconstituted with approved diluents, and handled according to manufacturers recommendations. Some instruments may require calibration. Refer to your specific instrument manufacturer's calibration recommendations.

The prothrombin time is a regulated analyte and, as such, requires participation in an approved proficiency testing program.

LIMITATIONS OF THE PROTHROMBIN TIME TEST

Pre-testing variables have significant potential for affecting clotting times and should be considered when establishing and monitoring laboratory policy and procedure for prothrombin time testing. The ratio of blood to anticoagulant is critical, requiring that the evacuation tube be filled properly with less than 10% deviation from the target volume. To correct for an elevated hematocrit (above 55%) the volume of anticoagulant must be adjusted to avoid false prolongation of the prothrombin time. The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) document H21-A2 includes a nomogram for determining the volume of anticoagulant required for such specimens.

Specimens for prothrombin time testing must be thoroughly mixed immediately after collection, and centrifuged promptly and effectively. Delays in testing should be avoided. CLSI standards detail that specimens held at room temperature (22 - 24°C) should be tested within 2 hours, refrigerated (2 - 4°C) if testing will be completed in 2 - 4 hours, or frozen. Specimens stored at -20° C may be held up to two weeks while specimens stored at -70° C are stable for 6 months. Frozen specimens should be thawed rapidly at 37°C and tested immediately, or refrigerated for up to 2 hours after thawing.

The order of draw is another important consideration for the phlebotomist. When collecting a series of tubes, the citrate tube must not follow any tube with an additive in order to prevent contamination of the specimen with additive "carryover." CLSI recommends that a plain (red stoppered) vacuum tube be collected first and discarded to eliminate any effect that tissue fluid may have on the clotting time, but recent studies indicate that this practice may not be necessary.[6] Possible heparin contamination should be considered when collecting a specimen from an indwelling catheter.

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Furthermore, care must be taken to avoid variations in the type of anticoagulated collection tubes used by the phlebotomy staff. Collection tubes with sodium citrate are manufactured in two concentrations, 3.2% and 3.8%. These seemingly small variations in citrate concentration may result in a significant increase or decrease in the patient prothrombin time. It is imperative that the laboratorian employ a consistent practice of which specimen tube is accepted for testing, as well as establish the normal reference interval with donor specimens collected in the same type of tube as that used for collecting patient specimens. The 3.2% sodium citrate concentration is preferred when utilizing a highly responsive thromboplastin. To avoid unreliable results, hemolyzed, clotted, lipemic, or icteric specimens should be rejected.

With an informed laboratory staff alert to the many influences upon prothrombin time testing, the highest levels of quality and confidence may be achieved.

REFERENCES

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SUPPLEMENTAL READING

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Worksheet A:

Determination of Normal Reference Interval

Procedure: Assay prothrombin time for a minimum of 20 normal, healthy subjects. Employ even numbers of males and females, free of known illnesses and medications known to alter coagulation processes. Record below. Determine the mean and standard deviation of the data. Worksheet C may be employed by those who perform these calculations manually.

Thromboplastin Lot Number _____ Expiration Date _____

Test Date		Normal Patient Value	Age	Sex
_____	1.	_____	_____	_____
_____	2.	_____	_____	_____
_____	3.	_____	_____	_____
_____	4.	_____	_____	_____
_____	5.	_____	_____	_____
_____	6.	_____	_____	_____
_____	7.	_____	_____	_____
_____	8.	_____	_____	_____
_____	9.	_____	_____	_____
_____	10.	_____	_____	_____
_____	11.	_____	_____	_____
_____	12.	_____	_____	_____
_____	13.	_____	_____	_____
_____	14.	_____	_____	_____
_____	15.	_____	_____	_____
_____	16.	_____	_____	_____
_____	17.	_____	_____	_____
_____	18.	_____	_____	_____
_____	19.	_____	_____	_____
_____	20.	_____	_____	_____
_____	21.	_____	_____	_____
_____	22.	_____	_____	_____
_____	23.	_____	_____	_____
_____	24.	_____	_____	_____
_____	25.	_____	_____	_____

Mean _____

Standard Deviation _____

+/- 2 SD Range _____

Technologist _____ Date _____

Reviewed by _____ Date _____

(Director or Technical Consultant Signature)

Worksheet B

Establishing Ranges for Unassayed Controls

Procedure: Assay the new lot(s) of control product in the same manner as patient tests, repeatedly, over several days to acquire a minimum of 20 test values. Record below. Determine the mean and standard deviation of the data. Worksheet C may be employed by those who complete these calculations manually.

Thromboplastin Lot Number _____ Expiration Date _____

Normal Control Lot Number _____ Abnormal Control Lot Number _____
Expiration Date _____ Expiration Date _____

Test Date		Normal Control Value	Abnormal Control Value
_____	1.	_____	_____
_____	2.	_____	_____
_____	3.	_____	_____
_____	4.	_____	_____
_____	5.	_____	_____
_____	6.	_____	_____
_____	7.	_____	_____
_____	8.	_____	_____
_____	9.	_____	_____
_____	10.	_____	_____
_____	11.	_____	_____
_____	12.	_____	_____
_____	13.	_____	_____
_____	14.	_____	_____
_____	15.	_____	_____
_____	16.	_____	_____
_____	17.	_____	_____
_____	18.	_____	_____
_____	19.	_____	_____
_____	20.	_____	_____
_____	21.	_____	_____
_____	22.	_____	_____
_____	23.	_____	_____
_____	24.	_____	_____
_____	25.	_____	_____
	Mean	_____	Mean _____
	Standard Deviation	_____	SD _____
	+/- 2 SD Range	_____	+/- 2SD Range _____

Technologist _____ Date _____

Reviewed by _____ Date _____
(Director or Technical Consultant Signature)

Calculating a Mean and Standard Deviation

This procedure is used to establish a quality control range for unassayed quality control materials or to establish a normal reference interval. The calculation is performed in the same manner.

1. Transfer the values documented on Worksheet A or Worksheet B to Column A of Worksheet C. Any individual value is called *x*. The number of values recorded is *n.*

2. Find the mathematical mean of all of the values. Add together all of the values in Column A and then divide the sum by *n*. The mean is denoted as ***x***.

3. Subtract each individual value (*x)* from the mean *(**x**)* and enter the difference into Column B. This is denoted as (*x*-***x**)*

4. Find the square of each difference (multiply each value in Column B by itself) and enter into Column C. This is denoted as $(x-\boldsymbol{x})^2$.

5. Add together all of the values in Column C. This is denoted as $\sum (x-\boldsymbol{x})^2$.

6. Divide the sum from step 5 by one less than the number of original QC values from Column A. This is denoted as $\frac{\sum (x-\boldsymbol{x})^2}{n-1}$

7. Find the square root of the value determined in step 6. Your result equals 1 Standard Deviation (SD).

8. A typical quality control range will be determined from the mean (***x**) +/- 2 SD.*

Worksheet C

Calculating a Mean and Standard Deviation

Please mark one of the following:

New normal reference interval _____ New lot number of control product _____

	Column A (x)	Column B (x-x)	Column C (x-x)²
1.			
2.			
3.			
4.			
5.			
6.			
7.			
8.			
9.			
10.			
11.			
12.			
13.			
14.			
15.			
16.			
17.			
18.			
19.			
20.			
21.			
22.			
23.			
24.			
25.			
x =		Σ (x-x)² =	
n=			

Control Manufacturer _____
Control Level _____
Control Lot Number _____
Control Expiration Date _____

Thromboplastin Lot _____
Expiration Date _____

Step 6: $\frac{\Sigma (x-x)^2}{(n-1)}$ = _____

Step 7: 1 SD = $\sqrt{\frac{\Sigma (x-x)^2}{(n-1)}}$ = _____

Step 8: QC lower limit = (x - 2SD) = _____

QC upper limit = (x + 2SD) = _____

Technologist _____ Date _____

Reviewed by _____ Date _____

(Director or Technical Consultant Signature)