

Protime / INR Testing Update – January 2019

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Please note: This update clarifies “interfering substances” for Protime and INR testing. Please see section 3.

Millions of Americans take oral anticoagulant therapy (e.g., Coumadin). For each of these patients, the prothrombin Time (PT) and international normalized ratio (INR) are laboratory tests performed to achieve and monitor the appropriate medication dosage. Additionally, these tests are used to assess unexplained bleeding or clotting.

This article will discuss five basic areas:

1. basic coagulation terms,
2. basic requirements for changing lot numbers,
3. some examples of interfering substances,
4. common problems in the collection and processing of coagulation testing, and
5. miscellaneous information.

Note: For single-use devices (e.g. Roche Diagnostics CoaguChek, ITC Prothrombin Time Microcoagulation System, etc.), the laboratory should refer to the manufacturer’s instructions for specimen requirements, procedures and quality control requirements.

1) Basic Coagulation Terms

Prothrombin time (PT): The PT/INR measures how long it takes for the patient’s blood specimen to clot, and monitors oral anticoagulant therapy (e.g., Coumadin). This test may be ordered to determine the cause of unexplained

bleeding or blood clots, DIC, liver failure and Vitamin K deficiencies.

The PT test evaluates the extrinsic “tissue factor” pathway and common pathways of the coagulation cascade VII, X, V, II, and I (fibrinogen). The PT test is reported in seconds.

INR: Differences in thromboplastin reagents have caused problems when comparing PT results across laboratories because of varying sensitivities of different thromboplastin reagents used in the PT test. This lack of comparability is of special concern for patients who may use more than one laboratory for PT testing.

The World Health Organization has recommended standardization of oral anticoagulant monitoring, based on expressing PT results in terms of an international normalized ratio (INR). INR calculations are intended to yield identical INR results when a single specimen is tested by two laboratories; one using a more sensitive thromboplastin.

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Practice Guidelines

The following practice guidelines have been developed by the Clinical Laboratory Advisory Council. They can be accessed at the [LQA website](#).

Acute Diarrhea	Lipid Screening
Anemia	PAP Smear Referral
ANA	Point-of-Care Testing
Bioterrorism Event Mgmt	PSA
Bleeding Disorders	Rash Illness
Chlamydia	Red Cell Transfusion
Diabetes	Renal Disease
Group A Strep Pharyngitis	STD
Group B Streptococcus	Thyroid
Hepatitis	Tuberculosis
HIV	Urinalysis
Infectious Diarrhea	Wellness
Intestinal Parasites	

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tin (yielding a higher PT result) and the other using a less sensitive thromboplastin (yielding a lower PT result).

The INR result is the patient's PT result in seconds divided by the geometric mean of PT result of the laboratory's normal patients, as calculated by each laboratory.

The geometric mean is an average that is different from the simple arithmetic average. It is calculated by multiplying all the PT results together (in this case, the 20 normal PT results) raised to the reciprocal of the number of results (in this case, 1/20). The geometric mean is used to avoid bias that may be caused by the inclusion of extremely high or low values. A calculator or software program is necessary to calculate geometric mean.

The INR is calculated from the following formula:

- $INR = (PT \text{ patient} / PT \text{ normal}) \text{ ISI}$
- PT patient is the patient's PT result expressed in seconds.
- PT normal is the laboratory's geometric mean value for normal patients expressed in seconds.

When performing the calculation, the values for the patient PT and the PT normal range mean value contain one decimal place (e.g., 12.0) and ISI includes two decimal places (e.g., 1.05). The INR should be rounded and reported to one decimal place (e.g., 2.5).

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ISI: The international sensitivity index (ISI) reflects the sensitivity of the reagent to decreased levels of vitamin K dependent coagulation factors as compared to an international standard. The manufacturer of the thromboplastin reagent determines the ISI by comparing each batch of reagent to a World Health Organization reference plasma and then assigns an ISI value to that lot of reagent.

2) Requirements for New Lot comparison studies:

In addition to manufacturer's requirements, laboratories must do comparison studies before switching to a new lot of thromboplastin, quality control material or changing methodology to confirm accuracy of the testing.

These requirements include:

- reviewing the procedure
- ensure you have an up-to-date procedure for lot changes establishing a new normal patient PT mean value for new lots of thromboplastin,
- programming the correct ISI (international sensitivity index) into the coagulation analyzer,
- establishing the new ranges for quality control
- performing comparison studies between the new and old lot numbers of PT reagent, and
- documentation of the manual check of the INR calculation.

The laboratory must maintain the documentation of the studies, including the raw data, for at least two years.

Establishing the Normal Patient PT Mean Value: Each laboratory must determine the normal patient PT mean value for the population it serves in order to calculate an accurate INR. This is done when you change lot numbers because the ISI value is likely to change from lot to lot. The failure of re-establishing the normal patient PT mean value may result in inappropriate Coumadin dosage changes.

To establish the normal patient PT mean value, check the manufacturer's instructions. In general, a minimum of 20 un-anticoagulated healthy patients evenly distributed between males and females should be tested with the new lot of thromboplastin. Ensure that patients taking interfering substances and medications are not included in the study for the normal patient PT mean value by checking the manufacturer's guidelines, discussing interferences with your laboratory director, and adhering to your laboratory policies.

Emergency department or pre-op patients should not be

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used for the normal patient PT mean value because their blood may contain acute phase reactants that are elevated in times of stress, and inflammation can shorten the values. Additionally, testing should be completed over a period of several days to include intra-lab variabilities.

3) Interfering substances for prothrombin time / INR: Please note that certain substances are known to interfere with prothrombin time and INR results in normal patients being considered for establishment of the normal patient PT mean value. Additionally, certain substances affect the prothrombin time and INR when taking Coumadin/warfarin. Refer to a physician for more information and for decisions regarding acceptable patients for your normal patient mean studies.

Substances that may affect the normal prothrombin time and INR in patients being considered for the establishment of the normal patient PT mean value:

Substance	Potential effect	Reference
Antibiotics	May increase or decrease the INR	Direct Link: Merck Manuals Direct Link: Impaired hemostasis caused by beta-lactam antibiotics. Direct Link: The effects of aminoglycoside antibiotics on platelet aggregation and blood coagulation. Direct Link: Antibiotic treatment and associated prolonged prothrombin time.
Vitamin K deficiency Protein deficiency	May increase INR	Direct Link: Mayo Clinic: Prothrombin time test Direct Link: What Causes High Prothrombin Time?
Blood-thinning medications	May increase INR	Direct Link: Mayo Clinic: Prothrombin time test
Estrogen/progestogen oral contraception medications	May decrease INR	Direct Link: Mayo Clinic: Prothrombin time test Direct Link: Coagulation effects of oral contraception.
High intake of foods that contain vitamin K, such as liver, broccoli, chickpeas, green tea, kale, turnip greens and products that contain soybeans	May decrease INR	Direct Link: Mayo Clinic: Prothrombin time test
Supplements that contain vitamin K	May decrease INR	Direct Link: Mayo Clinic: Prothrombin time test

Note: Herbs and supplements may affect the prothrombin time and INR; consult a doctor or pharmacist.
 Note: Warfarin/Coumadin: Warfarin prescriptions are dispensed annually with widespread use. Patients on Coumadin therapy should not be used for the establishment of the normal patient PT mean.

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4) Specimen collection and variables that affect coagulation testing

Pre-analytic procedure failures are the source of many problems in coagulation testing. The testing site must adhere to manufacturer instructions, package inserts and operator's manuals to ensure adequate specimen collection and processing.

Preanalytical Variable	Cause of False Elevation of PT and or aPTT Test Result	Corrective Action
Blood collection tube is inadequately filled.	Improper ratio of blood to anticoagulant. Excess anticoagulant causes prolonged PT or aPTT result.	Recollect specimen ensuring proper fill to achieve a blood to anticoagulant ratio of 9:1.
Patient has a hematocrit level above 55 percent.	Improper ratio of blood to anticoagulant. Excess anticoagulant causes prolonged PT or aPTT result.	Prepare a specimen collection tube that contains less anticoagulant. Refer to your laboratory's procedure for the proper amount of anticoagulant.
Specimen is clotted.	Coagulation factors have been activated; insufficient levels left in the plasma. PT and aPTT results will be affected.	Recollect the specimen.
Specimen collected from an arm with a heparin lock or from a heparinized vascular access device (VAD).	Heparin contamination will prolong the aPTT.	Collect the blood from a vein rather than a VAD. If blood must be drawn from the VAD, flush it first with 5 mL of saline, and discard the first 5 mL of blood before collecting the specimen.
Patient is receiving heparin therapy.	Heparin will prolong the aPTT	If the patient is being evaluated for possible factor deficiencies or coagulation inhibitors, use a heparin digesting enzyme as a pretreatment before testing the PT or aPTT.

Source: [LabCE Direct Link](#)

Specimen Labeling: The CLSI recommends that specimens should be collected, labeled and stored in a manner that respects patient privacy in accordance with HIPAA. The patient should be positively identified at the time of collection, and specimens should be labeled in the patient's presence after the blood is drawn and that each label contains the patient's full name, a unique identifier, date and time of collection, and any other information required by your regulatory agency and your facility.

Specimen collection tubes and devices:

- Use and proper filling of tubes:
- It is critical that 3.2 percent citrated tubes are used and filled properly to maintain a ratio of nine parts blood to one part citrate (9:1). Under-filled tubes will contain an excess of anticoagulant, causing erroneous testing results.
- It is never acceptable to pour partially filled tubes together to make one full tube as this tube will contain too much anticoagulant.
- Discard tubes:
- Note: Although discard tubes are no longer required for prothrombin time and partial thromboplastin time, the practice is still recommended for other coagulation studies because of lack of sufficient evidence that discard tubes are not needed. Always check with the manufacturer of your instrument and/or your reference laboratory for the current recommendations.
- Butterflies and winged devices:

If using a butterfly or winged device to draw the sample, a non-additive discard tube should be used if the coagulation tube is

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the first tube to be drawn. This technique fills the tubing dead space and ensures a proper anticoagulant to blood ratio.

Specimen Rejection:

The laboratory should ensure it follows the procedures for specimen rejection for the following problems:

- Clotted specimens
- Specimens with the wrong anticoagulant
- Under-filled tubes
- Over-filled tubes
- Mislabeled or unlabeled specimens

Specimen Handling: Prior to centrifugation, the blood specimen should be checked for gross clot formation.

Centrifugation: The laboratory should review the operator's manual for the coagulation analyzer and the reagent package insert to determine the optimal speed and time to process specimens.

Platelet Poor Plasma for coagulation testing: The CLSI defines platelet poor plasma as plasma with a platelet count of less than 10,000/ μ L and this is crucial for specimens that will be frozen. However, for fresh plasma samples, the APTT, PT/INR and thrombin time are not affected by platelet counts as high as 200,000 / μ L.

The CLSI recommends that the capped specimen tube must be centrifuged for sufficient time and speed (10 minutes at 1500 g at room temperature) to consistently create platelet-poor plasma, because the presence of platelets in the specimen can shorten clotting times. Centrifuges such as "Stat-spin", which spin at higher rates and shorter duration, are acceptable.

In order to determine whether the centrifuge time and speed can attain platelet poor plasma, the laboratory should centrifuge the specimen for the determined amount of time and then run the plasma portion of the sample through the hematology analyzer to determine the platelet count. If the platelet count is higher than 10,000/ μ L, the sample should be centrifuged for a longer period.

Once the optimal time is determined, periodic checks (at minimum annually) should be performed to ensure the centrifuge continues to perform optimally.

Specimen Storage for coagulation testing: Specimens for PT testing may be stored at room temperature for up to 24 hours, provided that the collection tube remains unopened. If testing cannot be performed within this time period, the platelet-poor plasma should be frozen. Do not refrigerate PT/INR samples.

Specimens for APTT testing may be stored at room temperature for up to four hours. If testing cannot be performed within this time period, the platelet-poor plasma should be frozen. Do not refrigerate APTT samples.

5) **Miscellaneous information:**

Policies and Procedures: Review your laboratory's policies and procedures for performing coagulation testing and verify that the procedures and policies are up to date. The policies and procedures must be approved, signed and dated by the current medical director.

If the laboratory is using the coagulation analyzer operator's manual as the procedure it must be approved and signed by the laboratory director. The operator's manual must include specific quality control policies, calibration policies, and the laboratory's system for entering patient results. If the operator's manual does not contain all of the necessary information, it is the

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laboratory's responsibility to include this information in their procedures.

Quality Control: For non-waived automated coagulation test systems, the lab must perform two levels of quality control testing each eight hours of operation and each time a reagent is changed.

A note about IQCP: If the coagulation system qualifies for an individual quality control plan (IQCP), follow the control frequency that is established and approved by the laboratory director.

Test Requests-Standing Orders: Many patients who are on oral anticoagulant therapy have standing orders from their physicians for prothrombin time/INR testing. The laboratory should have a written policy clearly defining the use of standing orders, describing which tests may be covered by standing orders and at what intervals standing orders should be reconfirmed with the physician.

Periodic Checks: Periodically ensure the INR calculation, ISI and lot numbers are accurate in your LIS and/or analyzer. The laboratory is responsible for determining the frequency of these checks.

References:

1. Clinical Laboratory Institute Standards (CLSI); H21-A5, Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline-Fifth Edition.
2. Clinical Laboratory Institute Standards (CLSI); H47-A, One-Stage Prothrombin Time (PT) Test and Activated PartialThromboplastin Time (APTT) Test; Approved Edition.
3. [Direct Link: CLIA Corner: CLIA Updates for Prothrombin Time & INR Testing](#)

26th Annual Clinical Laboratory Conference

November 12, 2019

Mark Your Calendars
Now!!!!!!

Calendar of Events

Training Classes:

[2019 Northwest Medical Laboratory Symposium](#)

October 9-12 Lynnwood, WA

[26th Annual Clinical Laboratory Conference](#)

November 12 Tukwila

Contact information for the events listed above can be found on page 2. The Calendar of Events is a list of upcoming conferences, deadlines, and other dates of interest to the clinical laboratory community. If you have events that you would like to have included, please mail them to ELABORATIONS at the address on page 2. Information must be received at least one month before the scheduled event. The editor reserves the right to make final decisions on inclusion.



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