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PT, INR, and APTT Testing

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Millions of Americans take oral anticoagulant therapy (e.g., Coumadin). For each of these patients, the Prothrombin Time (PT), International Normalized Ration (INR), and Activated Partial Thromboplastin Time (APTT) are the laboratory tests performed to achieve and monitor the appropriate medication dosage. These tests are also 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 samples, and
5) miscellaneous information.

Please 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 also be ordered for patients suspected of 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 due to 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

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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
Anemia
ANA
Bioterrorism Event Mgmt
Bleeding Disorders
Chlamydia
Diabetes
Group A Strep Pharyngitis
Group B Streptococcus
Hepatitis
HIV
Infectious Diarrhea
Intestinal Parasites
Lipid Screening
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Point-of-Care Testing
PSA
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Renal Disease
STD
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Tuberculosis
Urinalysis
Wellness

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identical INR results when a single specimen is tested by two laboratories; one using a more sensitive thromboplastin (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 the 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 the geometric mean.

The INR is calculated from the following formula:

$$INR = (PT_{patient} / PT_{normal})^{ISI}$$

$PT_{patient}$ 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).

ISI: The International Sensitivity Index (ISI) reflects the sensitivity of the reagent 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.

Activated Partial Thromboplastin Time (PTT): The PTT test is used to reach and maintain therapeutic heparin levels and assists in determining bleeding disorders. This test may be ordered for unexplained bleeding or bruising, DIC, chronic diseases, blood clots, and pre-surgical workups.

This test evaluates the intrinsic "surface contact" and common pathways. The PTT is used to evaluate the coagulation factors XII, XI, IX, VIII, X, V, II (prothrombin), and I (fibrinogen) as well as prekallikrein (PK) and high molecular weight kininogen (HK). The PTT test is reported in seconds.

2) Changing Lot Numbers
Requirements for New Lot comparison studies: In addition to manufacturer's requirements, laboratories must do comparison studies before switching to a new lot of PT reagent or changing methodology to confirm accuracy of the assigned ISI value.

These requirements include:
- establishing a new normal patient PT mean value,
- programming the correct ISI (International Sensitivity Index) into the coagulation analyzer,
- comparisons 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 PT mean for the

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patient population it serves in order to calculate an accurate INR. To establish the normal patient PT mean, 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/medications are not included in the study for the normal patient PT mean.

Emergency department or pre-op patients should not be used for the normal patient pool because their blood may contain acute phase reactants that are elevated in times of stress, and inflammation can shorten the normal range. Additionally, testing should be completed over a period of several days to include intra-lab variability.

3) Interfering Substances for Prothrombin Time
Please note that certain substances are known to interfere with Prothrombin Time and INR results. Do not select patients taking these substances to establish the new normal patient PT mean value.

Substances that may increase Prothrombin Time and INR:
Antiiotics
• Penicillins (except Dicloxacillin, Nafcillin), Doxycycline, Cephalosporins, Fluoroquinolines, Macrolides, Metronidazole, Timethoprim-sulfamethoxazole
Medications
• Acetaminophen, Amidorone, Allopurinol, Amiodarone, Androgens (e.g., testosterone, methyltestosterone, oxandrolone), Azole antifungals and cancer therapies
Alcohol
• in larger amounts (three drinks per day)
Vitamin K
• Decreases in vitamin K consumption increases the effect of Coumadin (a significant change in vitamin K consumption may result in a significant change in your Prothrombin Time/INR)

Substances that may decrease Prothrombin Time and INR:
Vitamin K
• Increases in vitamin K consumption decreases the effect of Coumadin (a significant change in vitamin K consumption may result in a significant change in your Prothrombin Time/INR)
Antibiotics
• Dicloxacillin, Nafcillin, Griseofulvin, Rifampin
Medications
• Azathioprine, Cholestryramine, Ritonavir, Sucalfate

Note: Herbs and supplements may affect the Prothrombin Time; consult a doctor or pharmacist.

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.

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 9 parts blood to one part citrate (9:1). Under-filled tubes will contain an excess of anticoagulant, causing erroneous testing results.

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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 due to 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 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: The CLSI defines platelet poor plasma as plasma with a platelet count of less than 10,000/µL. 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 /µL.

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 if 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/µL, the sample should be centrifuged for a longer period of time.

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

Specimen Storage: 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, 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 4 hours. If testing cannot be performed within this time period, the platelet-poor plasma should be frozen. Do not refrigerate APTT samples.

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5) Miscellaneous Information:
Policies and Procedures: Review your laboratory's policies and procedures for performing coagulation testing. Verify that the specimen collection policy is up to date including specimen labeling, storage, preservation, processing, and rejection criteria.

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 guide must include specific quality control policies, calibration policies, and the laboratory's system for entering patient results. If the operator's guide does not contain all of the necessary information, it is the laboratory's responsibility to include this information in its 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 Partial Thromboplastin Time (APTT) Test; Approved Edition.
3. Hygienic Laboratory at the University of Iowa and the Iowa CLIA Laboratory Program; CLIA Corner, 3rd quarter 2006.

Washington's Laboratory Complaint Process

Reporting Device-Related Adverse Events to the FDA

Please see the January/ February issue of the Elaborations Newsletter

Calendar of Events

Training Classes:

2018 ASCLS-WA Spring Meeting
April 26-27 Renton

2018 Northwest Medical Laboratory Symposium
October 24-27 Portland, OR

25th Annual Clinical Laboratory Conference
November 2018 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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