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PT, INR, and APTT Testing

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Illions of Americans take oral anticoagulant (e.g., Coumadin) therapy. For each of these patients, the Prothrombin Time (PT) and/or International Normalized Ratio (INR) are the laboratory tests(s) performed to monitor the medication dosage. The PT/INR and other coagulation tests are also used to assess unexplained bleeding or clotting in patients.

For waived single-use devices (e.g. Roche Diagnostics CoaguChek, ITC ProTime Microcoagulation System, etc.), the laboratory should refer to the manufacturer's instructions for specimen requirements and quality control requirements.

PT and APTT: The PT, INR, and Activated Partial Thromboplastin Time (APTT) tests are common coagulation laboratory tests used to assess the clotting ability of blood. The PT/INR test evaluates the extrinsic and common pathways of the coagulation cascade, while the APTT test evaluates the intrinsic and common pathways. Using both tests examines the integrated function of the coagulation factors.

INR: Differences in thromboplastin reagents have caused problems when comparing PT results across laboratories due to varied sensitivities of 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.

Inside This Issue

- 2-3 PT, INR, and APP Testing, cont'd
- 4 MTS/CLIA Licenses Expire June 30, 2013
- 4 Calendar of Events

The World Health Organization (WHO) recommends standardization of oral anticoagulant monitoring based on expressing PT results in terms of an INR. INR calculations are intended to yield identical INR results when a specimen is tested by two different 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).

To calculate the INR, one must use the appropriate International Sensitivity Index (ISI) value for the lot of reagent being used, and determine the normal range of patient testing.

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 WHO reference plasma, and then assigning an ISI value to the lot of reagent.

continued on page 2

Practice Guidelines

The following practice guidelines have been developed by the Clinical Laboratory Advisory Council. They can be accessed at the <u>LQA website</u>.

Acute Diarrhea Lipid Screening
Anemia PAP Smear Referral
ANA Point-of-Care Testing

Bioterrorism Event Mgmt PSA
Bleeding Disorders Rash Illness
Chlomydia Ped Call Tran

Chlamydia Red Cell Transfusion Diabetes Renal Disease

Group A Strep Pharyngitis
Group B Streptococcus
Hepatitis
HIV
Urinalysis
Infectious Diarrhea

Kellal Disease
STD
Thyroid
Tuberculosis
Urinalysis
Wellness

Intestinal Parasites

PT, INR, and APTT Testing, cont'd from page 1

Normal Patient Mean: Each laboratory must determine its own normal patient mean in order to calculate an accurate INR. Test a minimum of 20 un-anticoagulated healthy patients evenly distributed between males and females to establish the normal patient mean.

Calculating the INR: 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 different than the 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:

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

When performing the calculation, the values for the patient PT and the PT normal range mean contain one decimal place (e.g. 12.0) and ISI includes two decimal places

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(e.g.1.05). The INR should be rounded and reported to one decimal place (e.g. 2.5; two significant digits). Specimen Collection variables that affect coagulation testing:

Specimen Labeling: The Clinical Laboratory Standards Institute (CLSI) recommends that specimens be collected, labeled, and stored in a manner that respects patient privacy in accordance with HIPAA. Positively identify the patient at the time of collection. Label the specimens in the patient's presence after the blood is drawn. Include on the label 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 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: Although discard tubes are no longer required for PT and APTT, 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.

Reject Specimens for the following reasons:

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

Specimen Handling/Centrifugation: Check the blood specimen for gross clot formation prior to centrifugation.

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

continued on page 3

PT, INR, and APTT Testing, cont'd from page 2

The CLSI recommends that the capped specimen tube be centrifuged for sufficient time and speed (10 minutes at 1500g at room temperature) to consistently create platelet-poor plasma, since 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.

CLSI defines platelet poor plasma as plasma with a platelet count of less than $10,000/\mu L$. This is crucial for specimens that will be frozen. However, for fresh plasma samples tested within 24 hours for PT or 4 hours for APTT, the samples are not affected by platelet counts as high as $200,000/\mu L$.

In order to determine if the centrifuge time and speed can attain platelet poor plasma, 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 $10,000/\mu L$, the sample should be centrifuged for a longer period of time. Once the laboratory establishes the optimum time and speed to process the specimen, a periodic check should be performed to ensure that the platelet count is still acceptable.

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 period, the platelet-poor plasma should be frozen.

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.

New lot comparison studies: 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. With each new lot number of PT reagent, there are certain CLIA requirements that must be met including:

- establish a new normal patient mean;
- program the correct ISI (International Sensitivity Index) into the coagulation analyzer;
- comparison between the new and old lot numbers of PT reagent; and
- document the manual check of the INR calculation

Policies and Procedures: Review your laboratory's policies and procedures for performing coagulation testing and 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 signed and approved 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 their procedures.

Quality Control: The MTS/CLIA regulations state, "For all non-manual coagulation test systems, the lab must include two levels of control material each eight hours of operation and each time a reagent is changed."

Test Requests-Standing Orders: Many patients on oral anticoagulant therapy have standing orders from their physicians for PT/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.

Special thanks to Nancy Grove, BS, MT(ASCP) & Kristine Rotzoll, BS, MT(ASCP), Iowa CLIA Laboratory Program

References:

- 1. Clinical Laboratory Standards Institute (CLSI); H21-A5, Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline-Fifth Edition.
- 2. Clinical Laboratory Standards Institute (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; http://www.uhl.uiowa.edu/publications/cliacorner/20063/2006q3.pdf

MTS/CLIA Licenses Expire June 30, 2013

Medical Test Site (MTS) final notice license renewal fee cards were mailed on May 20, 2013. Contact the LQA Office at 253-395-6746 if you have not received your renewal notice.

The renewal fee payment for MTS licenses was due by June 1, 2013. MTS and CLIA licenses expire on June 30, 2013.

You will no longer be able to receive reimbursement for laboratory testing from Medicare and Medicaid and other third party payors if you do not renew your licene by June 30, 2013.

Visit the <u>LOA website</u> to obtain additional information about the MTS/CLIA license renewal process.

Calendar of Events

Training Classes:

2013 ASCLS-WA Spring Meeting

April 25-27

Lynnwood

2013 Northwest Medical Laboratory Symposium

October 16-19

Lynnwood

20th Annual Clinical Laboratory Conference

November 6

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.

For persons with disabilities, this document is available upon request in other formats. To submit a request, please call 1-800-525-0127 (TTY/TDD 1-800-833-6388).



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