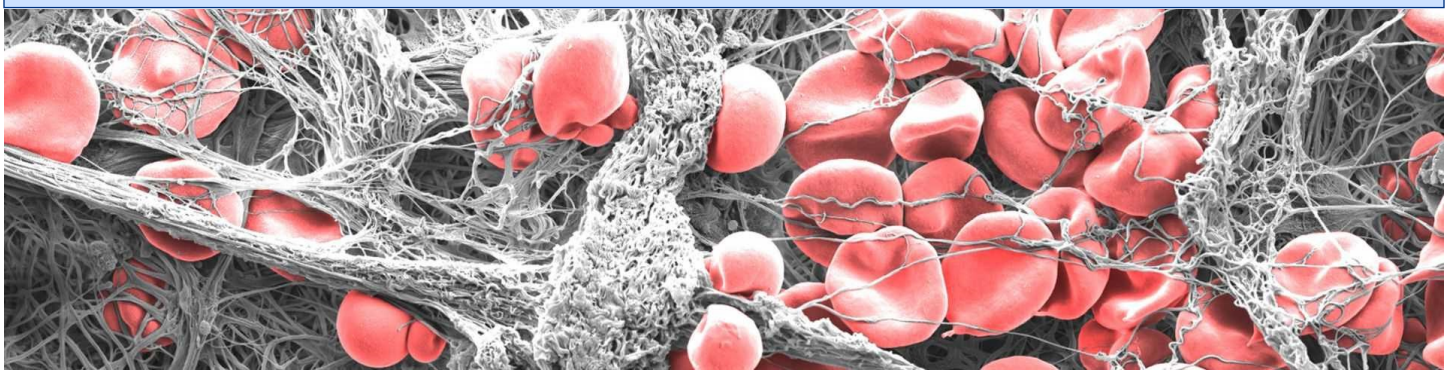
The University of Iowa | State Hygienic Laboratory

CLIA CORNER

State Hygienic Laboratory at The University of Iowa Second Quarter 2023

In This Issue…

- **Requirements for non-waived, non-manual coagulation test systems**



Prothrombin Time (PT) testing is performed to measure how long it takes for a patient's blood specimen to clot. Automated coagulation test systems sample the plasma, combine the plasma with the reagents, detect an end point or clot formation, and display the test results without operator intervention. The International Sensitivity Index (ISI) is the correction factor for variable sensitivities of thromboplastins (PT reagent).

Most laboratories report PT test results as the International Normalized Ratio or INR. The INR is a calculation primarily used for monitoring a patient's oral anticoagulant therapy. The INR corrects for the variability in PT results attributable to the ISI. This allows all PT's to be corrected to an international standard.

When performing coagulation testing, the laboratory must consider multiple factors.

Test Requests: Standing Orders

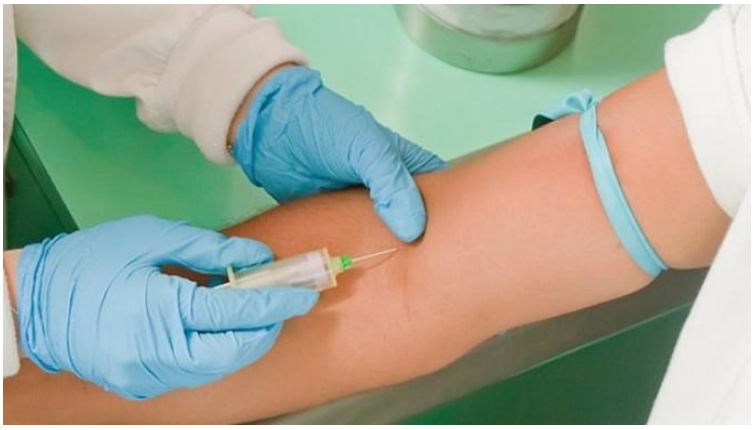
Many patients on oral anticoagulant therapy have standing orders for PT/INR testing from their physician. The laboratory must have a written policy for standing orders. It must define the frequency with which these orders need to be reviewed and renewed by the ordering physician.

Procedures

The laboratory must have procedures for performing coagulation testing, including specimen collection, labeling, storage, preservation, handling, and rejection criteria. If the laboratory uses the operator's manual and/or package insert as its procedure, anything specific to the laboratory that is not included in the operator's manual (i.e. specific control and calibration testing, reporting patient results, corrective action for unacceptable controls or calibrations, etc.) must also be included in a separate, written procedure. All procedures, including operator's manuals and package inserts, must be approved, signed, and dated by the current laboratory director.

Specimen Collection and Processing

For instruments that use a plasma sample, the laboratory must review the operator's manual and package inserts to determine the correct procedures for specimen collection and processing. In general, coagulation testing is done on platelet-poor plasma specimens, which is plasma with a platelet count of less than 10,000/µL. If the manufacturer specifies that platelet-poor plasma must be used for testing, then checks must be performed to ensure a platelet count of less than 10,000/µL can be achieved. The laboratory must establish the optimal speed and time for each centrifuge used to process coagulation specimens. Once the speed and time is determined, the laboratory must perform a periodic platelet poor plasma check to ensure an acceptable specimen is achieved. If the manufacturer defines a frequency for the check, the laboratory must follow it. If it is not specified, the laboratory must define it in a policy and/or procedure, perform it with the established frequency, and document it.



Equipment Maintenance and Function Checks

The laboratory must follow and document all manufacturer requirements for equipment maintenance and function checks. For maintenance and function checks not defined by the manufacturer, the laboratory must establish protocols and the frequency of the maintenance and/or function checks, including centrifuge timer and speed as well as other timers. The laboratory must also monitor and document temperatures where necessary: equipment, reagent refrigerators and/or freezers, room temperature, humidity, etc.

Quality Control

For all *non-waived, non-manual* coagulation test systems, the laboratory must include two levels of control materials each eight hours of operation and each time a reagent is changed. **This also applies to coagulation testing performed on chemistry instrumentation.**

For test systems for which the manufacturer recommends quality control be done at an interval less than every 8 hours of operation, the laboratory may develop an Individualized Quality Control Plan (IQCP). Refer to the following issues of the CLIA Corner: First Quarter 2014, Third Quarter 2014, Fourth Quarter 2014, and First Quarter 2015.

Calibration and Calibration Verification

The laboratory must follow the manufacturer's calibration guidelines for its test system. If calibration is required, the laboratory may also be required to perform calibration verification procedures. *CLIA does not require calibration verification for test systems which include instruments that cannot be adjusted or calibrated because they are factory or manufacturer calibrated (e.g. unit use devices). Calibration verification is also not required for non-quantitative tests, (e.g. PT and PTT tests, which are measured in time.)*

Analytes that require calibration must be calibrated following the manufacturer's test system instructions, using calibration materials provided or specified, and with at least the frequency recommended by the manufacturer. Calibration verification must include three levels of verifiers (a minimal or zero value, a mid-point value, and a maximum value) and must be performed at least once every six months. This means that if the laboratory has the same lot of reagent for longer than six months and is not required to calibrate at a lesser frequency, the laboratory must perform calibration verification procedures. To satisfy the calibration verification requirement, the laboratory may calibrate the analyte again if the calibration materials include a minimal or zero value, a mid-point value, and a maximum value, or it may use other calibration verification materials purchased separately.

PT Testing and INR Calculations

The INR is equal to the ratio of the patient's PT (in seconds) to the laboratory's established normal mean PT (in seconds), then raised to the power of the ISI.

$$INR = \left\{ \frac{PT\ (pat)}{Pt\ (n)} \right\}^{ISI}$$

PT (pat)= Patient's prothrombin time
PT (n) = Normal reference range
ISI = International sensitivity index
(the optimal ISI is 1.3 to 1.5)

labpedia.net

NOTE: A scientific calculator is needed to calculate the INR.

Example:
Patient PT (in seconds) =18.5
Normal mean PT (in seconds) =12.9
ISI value (obtain from the package insert of the laboratory's current lot of thromboplastin reagent) =2.002

18.5 ÷ 12.9 = 1.434 (Patient Ratio)
$1.434^{2.002}$ = 2.056 (INR Result)
Report the INR as: INR = 2.1

Specific guidelines for PT/INR testing can be found in the CLIA Regulations and Interpretive Guidelines at:

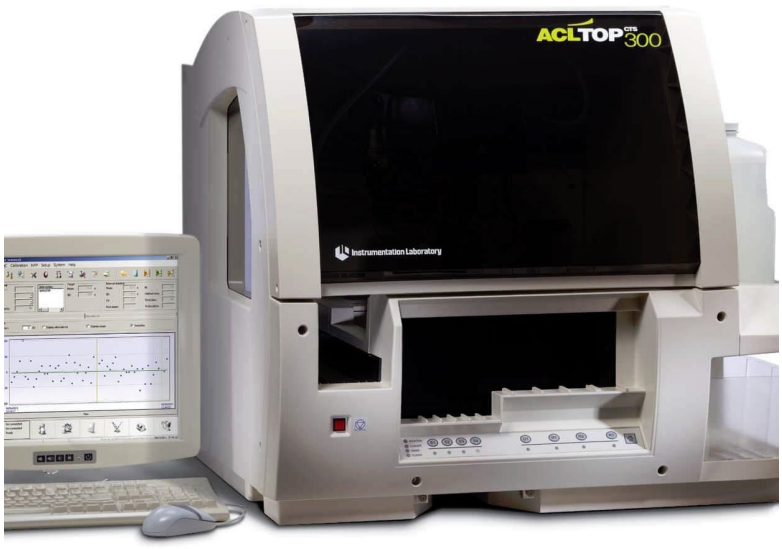
D5411 - §493.1252 *Standard: Test systems, equipment, instruments, reagents, materials, and supplies;*
(a) Test systems must be selected by the laboratory. The testing must be performed following the manufacturer's instructions and in a manner that provides test results within the laboratory's stated performance specifications for each test system as determined under §493.1253.

For ***International Normalized Ratio (INR) calculations***, the laboratory must perform the following:

→ **Establish a normal patient PT mean with each new thromboplastin lot number**.
Establishing a new normal patient PT mean is important because the value is used to calculate the INR results. The laboratory should collect specimens from a minimum of 20 normal individuals (check the manufacturer's guidelines for the specific criteria and exclusions for the "normal" pool of individuals) and perform a PT using the new lot of thromboplastin reagent. Using these results, the laboratory then calculates an average PT and establishes a new normal patient PT mean. Check the manufacturer's guidelines for exceptions to this. Each laboratory must establish its own normal patient mean with each lot of reagent even if the same equipment and lot numbers of reagent are used in multiple laboratory locations. It must also establish a separate normal patient mean for each instrument used in the same laboratory.

→ **Verify that the normal patient PT mean study has been performed according to the manufacturer's instructions.**

→ **Incorporate the current and pertinent normal patient PT mean and ISI value for each lot of thromboplastin (manual, instrument, or LIS)**.
When the new lot of thromboplastin is put into use, the newly established normal patient PT mean and ISI value for the lot number are programmed into the coagulation instrument or laboratory information system (LIS), whichever is used to calculate the INR result. Each new lot number of thromboplastin reagent is assigned a specific ISI value based on the manufacturer and model of coagulation instrument. It is the laboratory's responsibility to check the manufacturer's package insert for each specific lot number of thromboplastin reagent and use the correct value.



PT Testing and INR Calculations, continued...

→ **Document a manual check of the INR calculation for each new lot number.**

A manual check of the INR calculation can be accomplished by using a scientific calculator or the chart provided with the thromboplastin reagent package insert. If the manual calculation does not match the one from the instrument or LIS, then double check to make sure the correct normal patient mean and ISI value are programmed into the analyzer or LIS system. *Select an abnormal low and abnormal high PT result and verify the calculations.*

→ **Document each thromboplastin lot number, with the normal patient PT mean and the ISI value provided by the manufacturer (manual or instrument)**.

Check out our sample worksheet – this could be your laboratory's means of completing this requirement. (This is NOT a CMS/CLIA sanctioned form.)

→ **Periodically verify, for each thromboplastin lot number in use, the correct normal patient PT mean and the International Sensitivity Index (ISI) value are being used for calculating the INR value.**

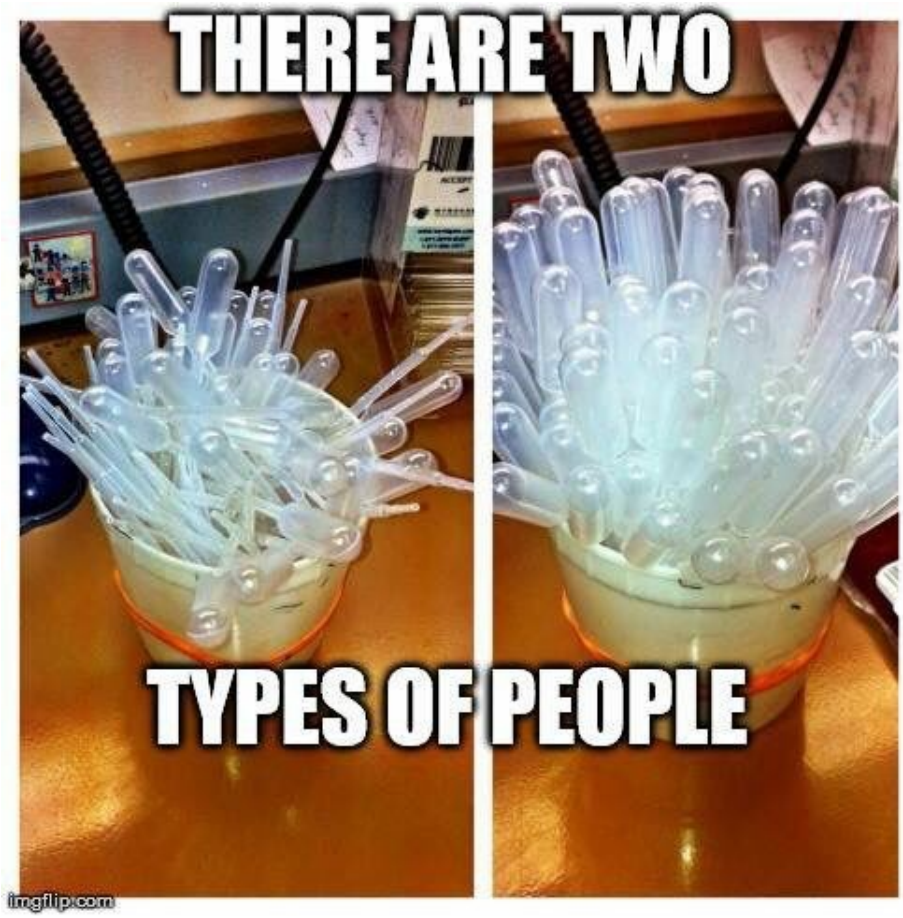
Verify that the ISI used in the calculation correlates with the ISI specified in the reagent package insert. *It is up to the laboratory to determine the frequency for performing these periodic checks.*

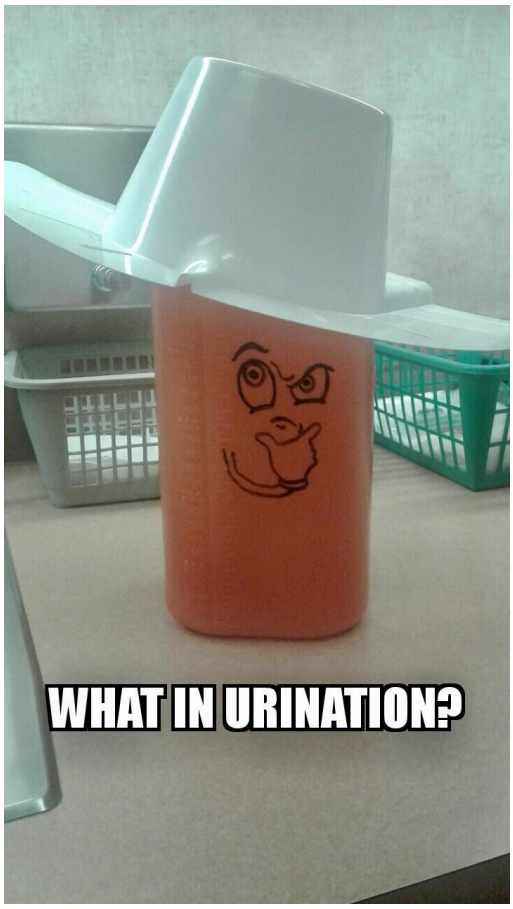
→ **Periodically verify the accuracy of the INR calculation (manual, instrument or LIS).**

Again, it is up to the laboratory to determine the frequency for performing these periodic checks.

If you would like your name added to our CLIA Corner google group, send an email to:

Kristine-Rotzoll@uiowa.edu or Melinda-Bochmann@uiowa.edu





New Lot of PT Reagent Worksheet

Date: _____

Reagent: _____ Lot #: _____ Exp Date: _____

Establishing Normal Patient Mean

Review manufacturer's instructions for normal patient selection.

Normal Patient Mean PT results					New Patient Mean Value
1	5	9	13	17	
2	6	10	14	18	
3	7	11	15	19	
4	8	12	16	20	

New ISI value: _____

Date normal patient mean and ISI programmed into analyzer/LIS: _____

***Manual INR Check:**

Select normal and abnormal (high and low) patient

Date	Patient's Name or Accession Number	Automated INR Result	Manual INR Result

Platelet Poor Plasma Check

Spin down 5 Sodium Citrate (blue top) tubes and verify platelet count <10,000/µL

Date	Platelet Count				
	1	2	3	4	5

Periodic Checks – Verifying PT Reagents

To be performed every six months until reagent expiration or new reagent is implemented.

Date	Verified correct Normal PT mean and ISI	Verification of INR value Patient's Name or Accession Number	Automat-ed INR Result	Manual INR Result

Date reviewed: _____

Laboratory director or designee signature: _____

*INR = (Patient PT ÷ Normal Patient Mean PT) ^{ISI}