INR Verification Guidelines

The Prothrombin Time (PT) test is used globally to monitor antivitamin K (AVK) therapy such as Coumadin-based treatment for patients at risk for blood clots due to heart disease or other conditions. Historically, PT results vary significantly depending on combinations of coagulation instrument manufacturers, methodology and reagent in use. This makes it challenging to monitor oral anticoagulant testing in order to keep patients within the therapeutic range. As a result of these challenges with PT testing, the International Normalized Ratio (INR) system for reporting PT results was introduced by the world Health Organization to address this problem. The INR is a mathematical conversion of the PT result based on the responsiveness of the thromboplastin reagent used in testing.

To ensure accurate verification good laboratory practices should be used at all times. This includes proper operating and maintenance of the instrument; all reagents used within expiration date/time; use of one lot number of reagent; and correct storage of all reagents according to manufacturer's specifications and thawed or reconstituted as per the manufacturer's requirements. It also includes the use of the correct ISI and MNPT in the INR equation.

The following are required components of verification:

1. **Geometric Mean:**
   To report an accurate INR result the correct mean normal prothrombin time (MNPT) is required. The MNPT is defined as the geometric mean of the PT of healthy adult population. INR is calculated using the following equation:

   $$INR = (Plasma\ PR/MNPT)^{ISI}$$

   If the wrong MNPT is used it can have a clinically significant and systematic effect on the INR that can inappropriately increase or decrease the results.

   a. Sample Criteria
      - Use at least 20 fresh samples from healthy individuals, with an equal number of samples from both sexes.
      - The same samples used for the reference range can be used to calculate the geometric mean.
      - The blood should be collected, processed and tested in the same manner; within 5 hours of collection and stored at room temperature.
      - If using frozen samples, they must be tested within two hours of thawing. (Note: Only frozen samples that have been separated and aliquot using the two-spin technique can be used.)

   b. Testing and Results

- Equation to calculate geometric mean:

$$X = \text{Antilog} \sum \left(\frac{\text{Log(PT)}}{N} \right)$$

- The geometric means is calculated using computer-based statistical program such as an Excel formula. See *VAL 2010_INR Calculators Worksheet.*
- The MNPT should be determine using the laboratory's own thromboplastin-instrument combination and population.
- The Arithmetic mean should not be used. The use of arithmetic mean will be disproportionately higher than the true MNPT value.

2. INR Certified Verification:

The ISI is a mathematical index of the responsiveness of the PT reagent and instrument combination. It can be found in the package insert of the reagent used for testing. It is highly recommended that the ISI used is for the specific thromboplastin reagent and specific instrument system in use. It is recommended to use reagents with an ISI < 1.5 for their instrument. Reagent with an ISI > 1.5 are less sensitive to coagulation factors. Generic ISI values should not be used unless there is no other option.

Verification is performed with any change in reagent, reagent lot number, or instrument or following major instrument repair. If there is a major change in quality control or major discrepancies in EQA are evident that cannot be explained by standard troubleshooting a new verification should be performed. Verification of the new INR is recommended for instrument-specific ISIs. If the laboratory is using a generic ISIs then verification is mandatory.

a. Sample Criteria
   - Obtain the INR certified reagent from either a reagent supplier or manufacturer supplier. This process uses certified plasma with specific INR values to ensure the laboratory INR is calculated correctly.
   - The certified plasma should include at least three vials with an INR therapeutic range from 1.5 to 4.5.

b. Testing and Results
   - Run samples in duplicate and if possible over a two day period to allow for day-to day variation.
   - If unable to do multiple days then duplicate testing is required. External quality assurance (EQA) programs can also verify INR values.

c. Acceptability Criteria

- +/- 15% or as indicated in the package insert of the Certified Plasma. (Note acceptance criteria may differ depending on kit used. Use criteria for your kit.)

3. Method Approval

The final decision on INR verification and acceptance is made after a careful review of all the studies performed as part of the complete verification process. The Laboratory Director shall make the ultimate decision on INR verification. INR acceptance is based on the results from the above studies.

4. References

a. CLSI H54-A, Vol. 25 No. 23 Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline. 2005

b. CLSI H47-ED3, One-Stage Prothrombin Time (PT) Test and Activated Partial Thromboplastin Time (APTT) Test; Approved Guideline. 2023