

Pre-Analytical Factors that influence INR system Comparisons

The INR (International Normalized Ratio) is PT (Prothrombin Time) and MNPT (Mean Normal PT) ratio raised to the power of the ISI (International Sensitivity Index) according to the following equation:

$$\text{INR} = (\text{PT}/\text{MNPT})^{\text{ISI}}$$

The (ISI) is a measure of the sensitivity of the PT reagent. The ISI for a PT reagent is determined by comparing that reagent to the WHO, (World Health Organization) standard. Using the INR scale theoretically eliminates differences between PT reagents, and allows results to be compared between labs regardless of the type of thromboplastin.^{1,2} When the INR system was introduced, it was believed that it would eliminate the differences seen between PT reagents. However, there were a number of variables that were not quantifiable at the time that continue to cause differences when comparing systems.³

Technical Bulletin 105 broadly explains the various factors that influence INR comparisons. Let us discuss in detail one of those factors, the Pre-Analytical Variables. It is important to maintain the integrity of the sample as local instrument variability in the INR system still remains a problem.⁴

The following common Pre- Analytical errors contribute to inaccurate INR results.

Pre-Analytical errors in Finger Stick^{5,6:}

1. Trying to get a sample from a cold hand or a hand with poor circulation (peripheral vascular disease).
2. Using a finger that has not been cleaned (visible contamination).
3. Using sites with callous and wounds.
4. Using a site that is bruised, swollen or cyanotic.
5. Not drying the site before performing the finger stick. (Residual alcohol on site causing blood to smear on the finger).
6. Improper depth of lancet penetration.
7. Re-sticking the same site.
8. Double dropping (adding two drops to one strip)
9. Finger painting (smearing blood on the strip sample well).
10. Milking the finger causing contamination with interstitial fluids.
11. Introducing bubbles to the strip when using a micro capillary tube.
12. Scraping the micro capillary tube against the puncture site.

Pre-Analytical errors in Venipuncture.^{7,8}

1. Using vein from above an IV site.
2. Using a sclerosed vein.
3. Using a vein around a hematoma.
4. Using a vein from an arm with a fistula/shunt.
5. Using specimens from more than one venipuncture or venipuncture with tissue trauma.
6. Having the tourniquet tied for more than 1 min can cause hemoconcentration and petechiae.
7. Drawing the blood too slowly.
8. Using an inappropriate needle gauge causing clotting or hemolysis.
9. Drawing from an indwelling catheter is not recommended due to the possibility of heparin contamination and/or dilution of the specimen.
10. Drawing into a syringe is discouraged because of the increased risk of hemolysis and clotting.
11. Not following the order of draw for multiple specimen collection.
12. Using the incorrect top tubes (blue top).
13. Using siliconised or plastic tubes instead of glass.
14. Shaking or mixing the tube, causing hemolysis (gentle inversion only).
15. Using 3.8% citrate tube instead of 3.2%.⁹
16. Under-filling of the collection tube decreasing the blood:anticoagulant ratio (9:1), leading to falsely prolonged results.
17. Overfilling causing an increased blood:anticoagulant ratio, which may result in erroneous results or specimen clotting.
18. Using inappropriate tubes for certain patients e.g. patients with elevated hematocrit (polycythemia $\geq 55\%$) may require special Coagulation tubes.
19. Using hemolyzed specimens may lead to falsely decreased PT
20. Using icteric or lipemic samples depending on the instrument used.

Not following stringent timing, processing and storage guidelines can alter the PT results of venous samples. The following conditions are recommended:

1. If batching is done for delivery then the plasma must be separated from the red cells and frozen at -20° C within one hour of collection.
2. Centrifuging at 1,500 g for no less than 15 min at RT is recommended.
3. Whole blood tubes to be kept at 18 to 24° C and tested within 24 hrs.
4. Storage at 2 to 4° C may result in cold activation of Factor VII, altering the PT result.
5. Transport temperature should follow the storage temperature guidelines.

When comparing a point of care device to a reference lab it is important to understand that all these above mentioned pre-analytical variables can affect the INR results and cause potential discrepancy. All these variables require standardization at the local level. Strict control over these variables is required to correctly determine the relationship (correlation) between plasma based lab systems and whole blood point of care devices finger stick devices.

References

1. Bailey EL, Harper TA, Pinkerton PG. The "therapeutic range" of one-stage prothrombin time in control of anticoagulant therapy: the effect of different thromboplastin preparations. *Can Med Assoc J.* 1971;105:1041-3.
2. Nichols, W. L. & Bowie, E. J. W. (1993). Standardization of the prothrombin time for monitoring orally administered anticoagulant therapy with use of the international normalized ration system. *Mayo Clinical Procedure*, 68, 897-898.
3. Hirsh, J. & Poller, L. (1994). International normalized ratio: A guide to understanding and correcting its problems. *Archives of Internal Medicine*, 154, 282-288.
4. [Journal of Clinical Laboratory Analysis](#), Volume 14, Issue 3 , Pages 101 – 114, Published Online: 19 Apr 2000 Copyright © 2000 Wiley-Liss, Inc.
5. Thomson JM. Specimen collection for blood coagulation testing. In: Koepke JA ed. *Practical Laboratory hematology*. New York: Chrchill Livingstone, 1991; 313-28.
6. Poller L. The Prothrombin Time test. In: Jespersen J, Bertina RM, Haverkate F eds. *ECAT assay procedures*. Utrecht: Kluwer, 1992; 41-5.
7. Clinical and Laboratory Standards Institute. Document H12-A4. *Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Gudeline- Fourth Edition*.
8. British Society for Hematology, Guidelines on oral anticoagulation, 2nd edn, rev Poller L. *J Clin Pathol* 1990; 43: 177-83.
9. Ingram GIC, Hills M. The prothrombin time test: Effect of varying citrate concentration. *Thromb Haemost* 1976; 36: 230-6.