Where Are the Preanalytical Stability Standards?

Corinne R. Fantz[1] and Dina N. Greene[2]*

Minimizing systematic and random errors within the testing process enables the reporting of quality results. Laboratories have developed multiple strategies to control the variables within the analytical testing phase, but often have little authority over the variables imposed during the pre- and postanalytical phases.

Unlike the postanalytical processes, which mainly rely on correct result transmission and subsequent interpretation, preanalytical processes can directly influence the analytical value reported by the laboratory (*1*). This is a conundrum that requires laboratory employees to define acceptability criteria for parameters such as proper mixing of samples with any required collection tube additives such as heparin or EDTA, the number of hours the sample can remain unprocessed (for serum/plasma tests), transport temperature, and the number of days following collection that additional testing can be added on, which is often storage temperature-dependent. All these parameters (and more!) can be generally classified under "stability."

During the assay development process, reagent stability receives significant attention. Manufacturers are required to ensure reagents are shipped at validated temperatures, and laboratories, in turn, document and maintain the required temperature of the reagents throughout storage and utilization. However, patient samples often do not receive the same level of scrutiny, either during the assay development process or during transport from collection site to receipt in the testing laboratory. In some cases, samples may require transport on ice, but rarely is a temperature log from collection to receipt in laboratory available for those specimens.

Even with measurands for which specimen transport on ice is not indicated, how do temperature fluctuations influence results? Many laboratories receive samples collected at outside clinics, where samples may remain in locked courier boxes for many hours, exposed to seasonal variations in temperature. Wiencek and Nichols observed seasonal variation owing to samples being stored in an outdoor lockbox before a courier delivered them to the laboratory. In their case, it was discovered that samples were subjected to temperatures ranging from −3.1 °C to 29.8 °C up to 72 h after collection (*2*). This report sheds light on the extreme temperature exposure that samples may experience in "ambient temperature," outdoor lockboxes. Although this study was limited to outdoor lockboxes and only tested a comprehensive metabolic panel collected in lithium heparin BD PST tubes, there are a number of other tests and transport variables that should be investigated. Other variables might include the impact of temperature on transporting whole blood vs serum or plasma in a broader sampling of measurands. In another study reported this month, Pedersen et al. examined preanalytical factors of calprotectin measurements in blood (*3*). These investigators demonstrated that while calprotectin samples were stable when collected in EDTA plasma,

[1]Roche Diagnostics; [2]Department of Laboratory Medicine, University of Washington, Seattle, WA.
***Address correspondence to this author at:** Department of Laboratory Medicine, NW120 Box 357110, 1959 Pacific Street, Seattle, WA 98195. Fax 206-598-6189; e-mail dngreene@uw.edu.
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measurements were susceptible to increased temperatures when the testing matrix was serum or lithium heparin.

Laboratories will often define their validation and verification protocols on the basis of national or international guidelines, which can be further exploited to prescribe regulatory standards. There are few recommendations for laboratories to develop their stability procedures. For example, the Clinical Laboratory Standards Institute's document GP-44-A4 (formerly H18-A4) has undergone several revisions (*4*). This document is helpful in outlining the publications that have determined the stability of measurands in unseparated serum and other matrices. However, GP-44-A4 is limited because it does not address any study caveats, distinguish between the used study designs, suggest appropriate sample sizes, or define the storage conditions that a laboratory should assess. Nevertheless, the document does include strong language that suggests mandating laboratories to determine adequate storage conditions.

Similarly, the Code of Federal Regulations standard CFR 493.1242 on specimen submission, handling, and referral outlines that laboratories must establish and follow written policies and procedures for patient preparation, specimen collection, specimen labeling, specimen storage/preservation, conditions for specimen transportation, specimen processing, specimen acceptability/rejection, and specimen referral (*5*). This standard provides tremendous flexibility for individual laboratories to accept or reject samples on the basis of their own local procedures. Stability studies need to consider environmental influences, and laboratories must implement temperature-control solutions if there are recognized risks in transporting specimens from distant locations.

The Code of Federal Regulations, 21 CFR 809.10 specifies the labeling requirements for manufacturers (*6*). It states that manufacturers need to include sample collection and preparation for analysis information, including preservatives, storage, handling, etc. Therefore, manufacturers perform stability studies to generate evidence supporting routine specimen-handling conditions in clinical use and to validate the use of stored samples during longer periods of storage, such as during clinical trials. Manufacturers include this information in their method sheets and make "claims" that are validated with data in their 510(k) submissions. Laboratories should be aware that deviations from these claims may not produce the same results. Typically, claims for sample stability are performed at room temperature, 4 °C, −20 °C, and −70 °C. Also, samples are frequently tested under repeat freeze–thaw conditions. These stability studies are specific to their supplies, reagents, and sample matrices and may not be representative of how samples are transported under routine conditions. Imeri et al. concluded that measurand stability of hematological parameters varied according to the test parameter, storage temperature, and measurement system (*7*). For example, platelet stability decreased when stored at colder temperatures (4–8 °C) when tested by one manufacturer, but this same temperature increased the stability of the platelet count when measured by a different manufacturer. Careful attention is required to understand the details of how the manufacturer conducted their stability study and how this might differ from routine practice.

In the spirit of Peter Drucker's quote that "you can't manage what you [don't] measure," quality indicators of specimen integrity are essential for reducing the likelihood of reporting inaccurate results. For example, deemed-status organizations could consider implementing checklist items to address sample integrity concerns when samples are transported over long distances or are exposed to extreme temperatures. Similarly, criteria should be outlined for evaluating the stability of analytes in biobanking, particularly when these samples will be used for improved measurand analysis or novel testing. Guidelines for laboratories and

manufacturers from the federal registry are not prescriptive and allow flexibility to suit the individual needs of the laboratories and manufacturers. The literature is lacking quality data on how best to perform these analyses. As laboratories continue to consolidate, there is a growing need to understand and control for preanalytical variables that affect stability during the transport of specimen.

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