BOX SCIENTIFIC

Data Quality Quick Reference

PROCESS VARIABILITY IN CLINICAL ASSAYS

As the complexity and sensitivity of Clinical Assays are pushed to the limits of our process capabilities, the opportunities and sources for process variability increase accordingly. Human specimens like biopsies, DNA & RNA are sensitive to environmental conditions even in-process. Other components like enzymes and oligonucleotides have temperature dependent functionality that is often specific and targeted. And even the best buffering systems can be challenged by rigorous or repeated freeze thaw. These factors, left uncontrolled, can introduce variability into an otherwise well controlled process. And can often show up in your output data. However with some basic best-practices these factors can be minimized or eliminated quite easily.

POTENTIAL PITFALLS – AN OVERVIEW

Much published study is available detailing any manner of process variability, their sources and remedies. There is no such thing as knowing or understanding your process too well. The wise scientist should embrace this wealth of information when embarking on any process improvement effort.

BOX Scientific's core focus is on process variability that occurs during preclinical sample preparation, between the freezer and process input. This has long been known to be the primary source of clinical error. And it is also the most frustrating as it has the ability to bring external variability into an otherwise tightly controlled process. The good news is that most preclinical errors spring from common causes that can be easily remedied. In our experience, nearly any clinical process can be benefitted by controlling a handful of omnipresent risk factors.

Of primary concern are:

-pH shifts

-crystallization

-stratification / dissociation

-thermal variability

-heterogeneity

This guide's intention is to illuminate these factors one by one, understanding where they arise and how to remedy them.

VARIABILITY – CAUSES AND REMEDIES

pH shifts- buffering is a skill we've mostly mastered in the clinical domain. Its easy to take for granted just how well our buffer systems work and the protection they provide where needed. However the freeze/ thaw process can prove to be a challenge for buffered solutions carrying components with disparate melt temperatures and solubility profiles. As components phase transition separately, the opportunity for localized pH spikes increases. This can be further influenced by the container geometry, rate of freeze/thaw, or other environmental factors. Denaturation, and precipitation are just some of the potential pitfalls.

Rapid Freeze/Thaw at a sustained rate has long been understood to offer remedial benefits to this phenomenon. Thankfully a broad array of freezer and sample storage systems are readily available. Some even offer optimum freezing parameters for a variety of sample types.

However while rapid freezing is easy to achieve, rapid thawing comes with a host of additional considerations. 'Unassisted thawing' has long been the standard for delicate specimens and reagents. The primary aim being to avoid compromising sample integrity via external influences employed to speed thawing. Excesses of heat or radiation can compromise sample integrity, and introduce thermal variability if not applied properly. BOX Scientific bridges the gap by employing convection to facilitate rapid heat exchange at ambient conditions. This enables thawing up to 75% faster than traditional 'unassisted thawing'. And drives rapid phase transitions that minimize localized pH spikes. It is safe for samples and delivers high uniformity.

Crystallization- parallel to the risk of pH shifts, are the effects of crystallization in aqueous solutions. They stem from the same cause but have differing effects. At sub-optimal freeze/thaw rates the aqueous phase transition can yield alternating intervals of decrystallization and recrystallization, often in localized regions. This can affect localized solubilities causing pH spikes. It can interfere with molecular folding/unfolding. And it can disrupt the overall solubility of the solution. This can introduce a host of unwanted effects, including precipitation, molecular cleavage and denaturation.

Rapid Freeze/Thaw again is the primary countermeasure to these effects. A sustained freeze/thaw gradient to drive steady and rapid phase transition should be the central focus of any efforts to this end.

Stratification- Stratification can occur in complex solutions often without any visual cues. It can result from of disparities in solubility, melt temperature and specific gravity being amplified by substandard handling protocols. Common assay components like Oligonucleotides and DNA and RNA standards are particularly subject to stratification during the thawing process. Especially where DMSO is employed. These compounds will have long unassisted thaw times, often lacking sufficient heat exchange to phase transition multiple components simultaneously. This will result in individual components phase transitioning one at a time. During phase transition, solubility and specific gravity are dynamic. As such localized disparities like immiscibility, hydrophobia, and pH spikes can manifest easily and repeatedly.

In the worst case, this can result in a tiered stratification with the least dense components atop stratified layers of increasing density and solubility as you move downward through the sample. Left unremedied, any subsequent attempts to aliquot the solution will result in broad heterogeneity both from aliquot to aliquot and between individual aliquots and the original source. And in process these disparities will cause variation, compromised functionality, and sub-optimal data quality.

Thaw/Mix Protocol- the simplest remedy to these effects is a comprehensive thaw/mix protocol. A thawing methodology that allows continuous rapid heat exchange will allow the entire solution to phase transition cohesively, minimizing the risk of phase separation. Furthermore, by introducing interval mixing to the thawing protocol, small separations that may still occur can be re-integrated into solution and their net effects minimized. BOX Scientific has an application note available on our website describing a basic thaw/mix protocol featuring a representative case study.

Thermal Variability- As many of our clinical processes are temperature dependent, thermal variability between process inputs will logically result in variability in in-process performance and outcomes. From common tasks like gene amplification and enzyme activity, to complex protocols like IVF, where temperature dependency exists, it is crucial to assure thermal uniformity across process inputs. In the case of established, commercialized assays, this uniformity must be maintained across facilities and long timescales.

To the extent that this fact is acknowledged and tools exist to remedy it, there is no substitute for quantitative verification that the intended effect is actually being achieved. For example, most modern PCR sequencers offer a preheat cycle aimed at minimizing thermal variability between sample wells. However applying a fixed quantity of heat to a set of samples that enters the process with random thermal disparities, may not be enough to normalize those differences. Likewise applying a uniform temperature ramp for a fixed time interval is not sufficient to assure that thermally disparate samples will reach the same temperature target at the same time. This can potentially have implications on the quality of the data reported. It is also important to note that many processes are *solely* dependent on fundamental physical attributes like enzyme activity, molecular folding, Tg, all of which are in turn directly dependent on the thermal state of sample. So its logical to assume that thermal variability between process inputs, will return similar variability in output data.

Equilibrium Thawing- Nature itself has provided us the perfect countermeasure to all of this. Equilibrium. Frozen specimens thawed to equilibrium with the ambient environment will naturally achieve high thermal uniformity across all samples. Furthermore, when held at equilibrium right up to the point of process input, they will logically return the most consistent data outputs. This is perhaps the primary factor that makes BOX Scientific's thawing platforms superior to conventional 'unassisted thawing'. Samples can be thawed to equilibrium with the ambient environment quickly, then held in that state indefinitely. And the continuous application of an ambient air convection current creates an 'active state' of equilibrium immune to minor external influences or variations in localized temperatures .

Heterogeneity- As a composite the preceding four sources of variability can certainly be lumped under the heading 'heterogeneity'. Each has unique underlying factors, but in each case the common result is some manner of heterogeneity. In day to day operations, many additional unexpected, unprecedented and totally random sources of heterogeneity can seemingly manifest out of nowhere. Lot to lot differences in reagents, changes in the weather, personnel changes, supplier changes, etc. can all disrupt the continuity of an otherwise stable process. Your process may be adequately robust to any single factor, but still susceptible to disruption by cumulative small variations happening in concert. Randomness happens. There will inevitably be things that are impossible to anticipate or predict. But it doesn't have to keep you up at night.

Procedure- In the end the best remedy to these and all sources of preclinical variability is procedure. Just as variation is the enemy of procedure, so too is procedure the antidote to variability. While some may be more challenging than others, no step in your process is more important than another. No step in your process requires less control than any other. It all works together to deliver one outcome, and thus every step, no matter how small, is crucial. Understanding the underlying factors of every step of your process and anticipating potential pitfalls is always a worthwhile effort. And applying effective procedures to remedy them is an invaluable pre-emptive step towards eliminating problems before you have them.

SO YOU KNOW

What do you really know? This is our credo here at BOX Scientific. Time and experience have taught us much, but we make a point not to assume we know things we haven't taken the time to know. Or that we understand things we haven't taken the time to understand. However you *will really know* the things you do take the time to learn and understand.

Observation, hypothesis, procedure, experimentation, quantitation, evaluation, conclusion. This is how we do science. And even the most insignificant step in your scientific process is still science. This sequence of steps, properly applied, will always yield new understanding. So in your quest to root out and remedy any and all potential sources of variability in your process, leave no stone unturned. Or untested.

We sincerely hope this guide has provided you some greater insight into the potential sources of variation in preclinical processes. BOX Scientific also offers many tangible resources to help you in your efforts. From our incomparable lineup of rapid thawing platforms, to the many application notes and case studies available on our website, to the ability to create unique custom solutions to meet your handling needs, we're here to help you achieve the new standard of the world. Visit us at our website: www.boxscientific.com

BOX Scientific – Thaw Right!

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