Understanding and Reducing Analytical Error—Why Good Science Requires Operational Excellence

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“Analysis and Control of Variation” is dedicated to revealing weaknesses in existing approaches to understanding, reducing, and controlling variation and to recommend alternatives that are not only based on sound science but also that demonstrably work. Case studies will be used to illustrate both problems and successful methodologies. The objective of the column is to combine sound science with proven practical advice.

Reader comments, questions, and suggestions will help us fulfill our objective for this column. Case studies illustrating the successful reduction or control of variation submitted by readers are most welcome. We need your help to make “Analysis and Control of Variation” a useful resource. Please send your comments and suggestions to column coordinator John McConnell at john@wysowl.com.au or journal coordinating editor Susan Haigney at shaigney@advanstar.com.

KEY POINTS DISCUSSED
The following key points are discussed:

• Good science in discovery, development, production, and in laboratories requires stable operations with low variation.
• Unstable analytical systems signals from the analytical process add variation to production data.
• Actual examples of variable processes are presented.
• “Stabilize first” is the first principle. Stable processes are predictable.
• Variation in laboratory operations may mask causal relationships in other areas.
• Compliance to procedures is not acceptable rationale for a variable process.
• Senior management should remove obstacles to conquering variation by making it a strategic imperative.
• In environments where high degrees of variation are possible (e.g., in biologics), the need for very low levels of variation in operations is greatest.
• Reduced variation means fewer deviations, fewer resources tied up conducting investigations and reports, more resources dedicated to doing the core work, and increased security from robust processes with known capabilities.
• Operating in a low-variation environment results in easier detection of causal relationships and fewer errors in interpreting data.
• The US Food and Drug Administration’s process validation guidance recommends statistical process control techniques to measure and evaluate process stability and process capability.

INTRODUCTION
This article continues discussion initiated in “Blame the Laboratory—Understanding Analytical Error” (1).
That article generated more comment and discussion than any other article published in this column, and it soon became clear that readers required more detail and guidance. As this article was being written, one of the authors visited a large pharmaceuticals site producing biological products. Earlier in the year, a slow and long-term upward drift in the level of analytical error had been demonstrated. In addition, it was noted that a significant drop in the average of the production data was matched with a similar drop in the average for laboratory reference standards. Further studies revealed that analytical error was likely increasing variation in the formulation of the final product.

It was clear that analytical error was excessive and that it needed to be reduced. The cell count for reference standards met the desired minimum level only about 40% of the time. A project to reduce analytical error was initiated. Six weeks after this project was introduced, remarkable results had been achieved. Cell count met the standard 90% of the time, and the standard deviation for this cell count was less than half of that which existed before the project commenced. A quiet revolution is taking place in this analytical system. Analytical error has been slashed, and the project is far from over; in truth, it has barely begun. Interestingly, nearly all the improvement work has been done by the technicians. In this example, the science remains unchanged. It is the conduct of operations that has improved. Central to this article is the notion that if we are to do good science, we are well served to start by conquering variation in operations.

GOOD SCIENCE REQUIRES GOOD OPERATIONS
Pharmaceuticals companies are designed, built, and managed by scientists. This is only as one might expect. Nearly always, one of the most important criteria for promotion will be technical skills and ability. This results in pharmaceuticals businesses having a culture strongly biased towards technical excellence both at a business unit and at an individual level. Technical excellence is a very good objective. However, when such companies encounter a problem, the nature of the business and the people who staff them is to address the problem from a scientific or technical perspective. This can be a terrible mistake, especially if the process under examination is not statistically stable.

Some Actual Examples
Before we ask the scientists in the discovery, development, production, or analytical areas to do good science, we ought to create stable operations. Unfortunately, much of the industry has yet to discover this truth, let alone use it to their advantage. To illustrate the situation, two control charts are shown in Figure 1 (2). They show the results of a plant trial whose objective was to drive variation to minimum levels in everything. The same people using the same technology made the same product for the period of the chart. There is no change in the science involved. What changed was operational rather than chemical or biological. What changed was that everyone involved, from the plant manager down, became intolerant of variation in any form. Training was conducted, operational definitions were created, and method masters were appointed to ensure almost exact performance between shift and between operator and analyst repeatability. Instruments were tested and calibrated to ensure excellent replication across instruments. Bacteria from only one working cell bank were used in fermentation. The aim was never concerned with accuracy for any characteristic. The aim was always to create maximum precision, to conquer variation, and to create repeatability. Nowhere was this done better than in the laboratory.

There are two elements that ought to be kept in mind when examining Figure 1. First, it should be clear that not only was the factory (in this case the fermentation step for a biologic) successful in conquering variation, but also so too was the analytical laboratory involved. The laboratory manager and the technicians involved reduced assay variation by just as significant a proportion as did the production people. This must be true; otherwise, the change in factory performance would not have been so obvious. Secondly, if the instrument failure noted in the pH chart had occurred before the trial, there is every chance that it would have gone unnoticed. It is axiomatic that as we reduce variation in any process, ever smaller signals can be detected through the reduced background random “noise.”

This is a critical understanding if we are to do good science. Nowhere is this truer than in the analytical world. The lower the variation in assays, the easier it is to detect disturbances in the analytical process and to correct them before they cause deviations or other trouble. The customer of the laboratory also benefits. The lower the variation is in the assay, the easier it is for production people to detect signals in the production data. Figure 2 shows a chart of laboratory controls (reference material) in another
company. The production people believed the assay to be inaccurate and were demanding more replicates in an attempt to improve assay accuracy. The analytical laboratory manager disagreed, suggesting the problem was in assay variation rather than in accuracy. He assigned a statistician to drive variation in the conduct of operations to a minimum. Again, a dramatic decrease in analytical error is observed. As before, the improvement is entirely operational, and nearly all the improvement work was done by the analysts. The entire project lasted for a week. No change was made to the science.

Good science requires good knowledge and a good understanding of that which is being investigated. This requires understanding of causal relationships. The charts in Figure 3 come from the same trial as those results shown in Figure 1. The two variables should have shown a strong correlation based on the science, but until the operations were stabilized with minimum variation, the scientists could not understand the process well enough to do good science. In Figure 3, not only do we note a much reduced scatter and a vastly increased $R^2$ factor, but also that the angle of slope of the regression line is fundamentally altered (the shallow slope in the left chart of Figure 3 is caused by instability). From a scientist’s perspective, both are critical understandings. After the trial, the data made sense and the correlation that always existed was clearly demonstrable. This was not possible with variation at the level prior to the steady-state trial.

**WHAT SHOULD BE THE INITIAL AIM?**

Stabilizing the process and reducing variation should be the initial aim for every analytical process. In particular, variation in laboratory operations, which masks the causal relationships from the scientists, ought to be an early target.

Have any of us ever met anybody working in pharmaceuticals or biologics who is not interested in variation, and if possible, reducing it? Every chemist, biologist, virologist, analyst, manager, or operator with whom we have discussed this subject has been in agreement that reducing variation is a good thing to do. Some might claim that it is sometimes not possible in certain circumstances—that we have hit the limits of our technology. Others might be adamant

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**Figure 1:** Results of a plant trial to reduce variation.
that whilst it is necessary to reduce variation, their hands are tied because the real causes of variation lie in a different department, and so on. Nevertheless, it seems we are all in agreement that reducing variation is a good thing to do. The reasons that understanding and reducing variation is always a good thing to do are many.

**Polio Vaccine Clinical Supplies**

From a quality perspective, lower variation means more predictable and better quality product. Jonas Salk understood well the need for repeatability and predictable outcomes as a key quality indicator. In 1954, the first batches of polio vaccines were manufactured for the massive clinical trial. Over 400,000 doses were administered without any serious incidents or negative effects. The National Foundation for Infantile Paralysis had demanded that to have their vaccine accepted for the trial, manufacturers were required to make 11 successive batches, all of which demonstrated that the live virus was completely inactivated. Only two manufacturers met this criterion and only these two provided vaccine for the clinical trial. After the successful trial, the federal government assumed oversight of manufacturing and large-scale vaccination. The requirement to make 11 successive inactivated batches was dropped. Soon afterwards, a man-made polio epidemic followed that was created almost exclusively by a single manufacturer who was not part of the initial trial and who had never made more than four batches in a row without detecting live polio virus in finished batches. Live virus was, in some batches, being missed during testing and these batches were paralyzing and killing children. Other issues did exist. However, the subsequent investigation showed that if the requirement for repeatability and predictability had been maintained, the man-made epidemic would never have occurred because the problematic vaccine would never have been released for use (3).

**Shewhart and Deming**

Eighty years ago, Dr. Deming edited Dr. Shewhart’s seminal work, *Economic Control of Quality of Manufactured Product* (4). Until his death in 1993, Deming pleaded with western business to work at understanding and reducing variation in everything they do. Deming stated “It is good management to reduce the variation of any quality characteristic ... whether this characteristic be in a state of control or not, and even when no or few defectives are produced. Reduction in variation means greater uniformity and dependability of product, greater output per hour, greater output per unit of raw material, and better competitive position (5).”

Unfortunately, 80 years later we are still learning that Shewhart and Deming were correct. To this day when analytical managers in the pharmaceuticals industry are made aware that their processes contain unnecessary variation many respond with, “but I am compliant ...what is the problem?”

**Why No Progress?**

Some are trying to convince the industry that the approaches developed by Shewhart, Deming, Smith, Juran, Harry, and others holds the promise of improved quality and productivity as well as fewer deviations and regulatory issues (2). Unfortunately, change is occurring slowly. In the case study depicted in Figure 2, the laboratory manager and statistician who led this analytical revolution presented the results of their project to colleagues and peers. They intended to explain the methodology and demonstrate its benefits.
For the most part, their audience was unresponsive. Generally speaking, they were meeting the required standards. Even if a similar project in their laboratories might yield similar results, why should they bother to drive analytical error to even lower levels? No argument moved the detractors. Neither improved service to customer departments nor the potential to reduce regulatory deviations impressed them; nor did the opportunity to provide a better platform for scientific work, now and in the future.

Until senior management removes options to conquering variation by making it a strategic imperative, we ought not to be surprised if some refuse to switch their focus from technical to operational issues. When trouble occurs in the process, there is a strong tendency for scientists to search for the “smoking gun.” Sometimes it exists, and sometimes it does not. Where it does exist, it will be much easier to find in a low variation environment. In many cases, however, what exists is not so much a smoking gun as a plethora of operational issues that combine to produce a noisy environment with high variation in which it is very difficult to do good science.

From a compliance perspective, reduced variation means fewer deviations, fewer resources tied up conducting investigations and preparing reports, more resources dedicated to doing the core work. This enables security for all (i.e., the company, the US Food and Drug Administration, and the consumer) that springs from a predictable, repeatable, and precise analytical process with a known capability.

From an operational perspective, Little’s Law explains why Deming was right when he claimed that reducing variation increased output. Increased output from the same resources (people and equipment) means lower costs.

Finally, from a scientific perspective, operating in a low-variation environment results in easier detection of causal relationships and fewer errors in interpreting data. Consider pre-clinical trials. If the scientists are operating in a low-variation environment, there will be fewer type I and type II errors (2). A type I error occurs if we conclude that two candidate molecules produced different effects when in fact there was no difference between them. A type II error occurs if it is concluded that the two candidate molecules produced the same effect, when in fact there exists a real difference in performance. One is inclined to wonder how often high levels of analytical error have sent the wrong candidate molecule to the clinic and what the associated costs might be. We can never know the answer to such musings. What we can do is to work now and forever to minimize the variation in operations to give the scientists the best chance at doing good science.

**THE FIRST PRINCIPLE**

“Stabilize first” is the first principle (2). Figure 4 shows a stable and an unstable process, side-by-side, as a series of distributions (2). What are the implications of instability? First, by definition, an unstable process is not predictable. A modern Jonas Salk would rightly exclude the unstable (unpredictable) supplier of product or of analytical services. In addition, until it is stable, a process has no known capability (4). One can do the calculations, but the resultants of these
calculations mean nothing if the data are unstable. What does this imply when the laboratory controls investigated by the authors have never once been stable at the commencement of investigations? First and foremost, instability makes a mockery of the estimates provided for analytical error. If the laboratory controls are unstable, no degree of confidence can be applied to the degree of likely analytical error in the future, which is what process capability measures in a laboratory. Because process capability implies prediction (what will be the likely analytical error next month?), a glance at Figure 4 soon reveals that any measure of process capability only has meaning if the data are reasonably stable (4). Finally, how do scientists establish causal relationships when the data are unstable? The Winter 2010 issue of the Journal of Validation Technology (6) illustrates this issue. In one example, it resulted in a potential root cause being moved from the bottom of the list to the top. Too often, significant errors in interpreting the science are made. It is not possible to do good science when the data are so unstable.

However, if the data exhibit stability, they are predictable. This makes the analytical process trustworthy and easier to manage. It greatly simplifies scheduling and allows us to provide analytical capability and service guarantees that actually mean something. Stable data reveal causal relationships much more readily. Stabilizing a process is akin to lifting a fog that hitherto had concealed the truth from all. This allows the scientists to do good science far more often. Fewer type I and type II errors are made. In the laboratory, analytical error can be even further reduced. In production, yields rise and costs fall. In discovery and development, scientists are able to detect smaller changes in the performance of a molecule or cell and to do a better job of selecting the most promising candidate to send to the clinic.

**BIOLOGICAL ASSAYS**

By their nature, biological assays are usually more variable than their chemical counterparts. It is too easy to shelter behind what seems to be unavoidable variation, and to claim that the level of variation observed is inherit in the biology and largely unavoidable. In an attempt to overcome this high level of variation, a common reaction is to add more replicates and more cost. However, if a control chart made with laboratory reference standards shows instability, the inevitable conclusion is that the same people and instruments can produce results with reduced variation if only they could stabilize the process. Figure 5 shows two recent examples of reference standard performance in biological assays. Both are unstable. This means that stabilizing the process will significantly reduce analytical error. Regardless of whether the assay under examination is chemical or biological in nature, stability is more often an operational issue than it is a technical issue. When it is a technical issue, causes for the trouble can be found much more rapidly and with more certainty when the assay is stable with minimum variation. Consider the charts at Figure 4. Once the correct control band has been calculated, often only one to three points reveal a change in the system, triggering a search for root causes while whatever changed is still there to be found. Alternatively, if a deliberate change has been made, often very few points are needed to demonstrate an improvement to the process. A well-constructed control chart leads to faster, more effective interpretation of time series data. Laboratory controls are a good place to start.
Every example in this article came from biological processes. Some were vaccines; others were biological therapeutics; but all were biological. Operational excellence (i.e., reduced operational variation) is most important when the potential for variability in the science is higher and when data are scarce or expensive. Therefore, in biological analytical processes the need to achieve operational excellence is greater than might usually be the case. The same can be said of development areas where data are much more scarce. If we combine these two considerations, it is difficult to avoid the conclusion that assay development for biologics is one key area where the requirement to design for operational excellence and robustness is at its greatest.

CONCLUSION

In part, the FDA Guidance for Industry-Process Validation: General Principles and Practices (7) states:

“We recommend that a statistician or person with adequate training in statistical process control techniques develop the data collection plan and statistical methods and procedures used in measuring and evaluating process stability and process capability. Procedures should describe how trending and calculations are to be performed and should guard against overreaction to individual events as well as against failure to detect unintended process variability. Production data should be collected to evaluate process stability and capability. The quality unit should review this information. If properly carried out, these efforts can identify variability in the process and/or signal potential process improvements.”

REFERENCES

4. W. A. Shewhart, Economic Control of Quality of Manufactured Product, Van Nostrand, 1931.