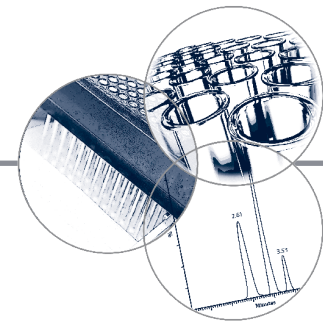

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Incurred sample reanalysis: it is just a matter of good scientific practice

"Integral to the successful development of drugs are accurate and precise data. Reliable and robust analytical methods remain the basis for the reproducible quantification of therapeutic drugs in biological matrices…"

Keywords: documentation ■ event investigation ■ good scientific practice ■ incurred sample reanalysis ■ method development ■ reliability ■ reproducibility ■ robustness

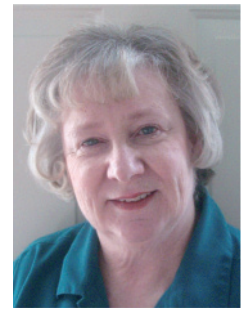
Is it not interesting that when an unforeseen event turns disastrous, or at least damaging, it has the ability to focus our attention on exactly those key issues that demand resolution? In fact, even the path to resolution becomes amazingly clear.

For example, throughout the 1990s, the bioanalytical community understood that it had to be aware and test for immune responses to protein drugs. Yet the process was vague and deciding which tests to perform was characterized by individual interpretation. When, after years on the market with no unexpected clinical sequelae, an erythropoiesis-stimulating protein formulation suddenly induced an immunogenic response, it did not take long before the tiered approach to testing for immunogenicity was described. Tied to that directive was the further elucidation of what could cause an immune response, whether patient, formulation or study related [1–3]. The requirement for incurred sample reanalysis (ISR) falls into this same unforeseen but impactful category as well.

Integral to the successful development of drugs are accurate and precise data. Reliable and robust analytical methods remain the basis for the reproducible quantification of therapeutic drugs in biological matrices to assist in the decision making. The data are used early in the drug-development process for the interpretation of pharmacokinetics and selecting effective dosing regimens in nonclinical species, and to project the starting dose in the first clinical trial. Later in the development process, the data are used to characterize the pharmacokinetic/pharmacodynamic relationship in order to develop models to enter clinical trials with some understanding of the drug's behavior. Thus, considering that the intended use of the data is to establish human safety and efficacy, ensuring accurate, precise and reproducible data is paramount [4,5].

The LC–MS/MS methods, using LC separation coupled with MS ionization/analysis and employing individualized high-quality internal standards, are inherently specific and robust, ensuring the accurate and precise measurement of drug concentration. Ligand-binding assays (LBA), on the other hand, demonstrate more analytical variability. Whether it is due to the absence of an internal standard, lack of extraction from the biological matrix, lack of a heterogeneous reference standard, necessity to produce new lots of unique critical reagents or any other of the challenges of developing a robust LBA, nonetheless, the greater imprecision persists. To compensate for this variability, LBA measurements are typically performed in replicate, either duplicates or triplicates, and acceptance criteria are placed on the replicate values to maintain some control over the variability. It may be argued that for LBAs the use of replicates with related acceptance criteria (%CV [coefficient of variation]) is, in fact, an example of an internal ISR. However, the use of replicates in this instance would not meet the implied appropriate execution of an ISR since the former process uses a single pipetting and is performed within the same 'plate' or analytical batch. Nonetheless, duplicate testing was always intended to provide bioanalysts with control over intra-assay variability and some reassurance of the reproducibility of their methods. It could be expected that for LBAs, in the absence of multiple repeats for failed replicate CV during sample analysis, ISR results should prove reproducibly acceptable.

Knowing the method controls that bioanalysts put in place to ensure reliable data seems to contradict the need for ISR. Nonetheless, when at a large CRO, technical mis-steps while performing an LC–MS/MS extraction process, compounded by the lack of a thorough investigation into the apparent spurious results, came to light, attention



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was quickly focused on the issue and its resolution. Again, it did not take long for the regulatory authorities to see a clear path forward and to require some assurance of data reproducibility, namely ISR [6,7].

In reviewing the series of analytical events that culminated in the US FDA's ISR requirement, it seems clear that the issues at the CRO were strictly operational in nature, including a lack of good process management and an inadequate or unproductive event investigation. Not surprisingly, the majority of the methods investigated during the eventual review were found to be valid, indicating poor execution rather than poor method development, a concept discussed in the recent European Bioanalysis Forum White Paper [8].

While the impetus to require ISR was not triggered by a method reliability issue, it has now become the umbrella under which aspects of method reproducibility and robustness are monitored. Robustness, the measure of the capacity of the assay to remain unaffected by small, but deliberate changes in method parameters, provides an indication of its reliability during normal run conditions. But experiments to monitor and quantitate robustness do not usually include those nondeliberate operational challenges (i.e., mistakes) that we encounter while attempting to validate a method under tight timelines and with less than optimal reagents – something that happens with regularity for the LBA scientist when supporting early nonclinical protocols.

So what lessons have been learnt? One can argue ISR is not simply addressing a robustness issue. Both platforms discussed above provide some internal assurance of reproducibility when the method is sufficiently validated. Of course, it is understood that emphasis should be placed on developing a valid method as opposed to simply validating a developed method. When developing a new procedure in the name of efficiency, it is as important to not only execute a series of experiments but to understand the possible impacts, both positive and negative, of operational steps on the whole process. In another words, it is imperative that bioanalysts look not only at the individual steps that go into building a method but keep an eagle-eyed view of the whole process and how the elements of the process relate to each other. In addition, it is mandatory that we conduct a rigorous review of the raw dataset we employ prior to the release of the summary data to our client, typically the pharmacokineticist. It behoves each of us to apply stringent criteria right from the start, and when data appear contradictory in any study, not just the study employing ISR, to conduct an event investigation. In such cases, the findings, the resolution and the evaluation of the impact on the method validation and study protocol should be reported in a timely manner. In the end, small, unexplained discrepancies could be an indicator of an impactful issue.

Employing good scientific practices is at the heart of the matter. They should be incorporated into our daily routine. The safety of patients and the credibility of our data are at stake.

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Bibliography

1 Rosenberg AS, Worobec AA. Risk-based approach to immunogenicity concerns of therapeutic protein products, part 1: considering consequences of the immune response to a protein. *Biopharm. Int.* 17, 22–26 (2004).

2 Rosenberg AS, Worobec AA. Risk-based approach to immunogenicity concerns of therapeutic protein products, part 2: considering host-specific and product-specific factors impacting immunogenicity. *Biopharm. Int.* 17, 34–42 (2004).

3 Rosenberg AS, Worobec AA. Risk-based approach to immunogenicity concerns of therapeutic protein products, part 3: effects of manufacturing changes in immunogenicity and the utility of animal immunogenicity studies. *Biopharm. Int.* 18, 32–36 (2005).

4 Kuang B, King L, Wang F *et al.* Therapeutic monoclonal antibody concentration monitoring: free or total? *Bioanalysis* 2(6), 1125–1140 (2010).

5 Lee J, Kelley M King L *et al.* Bioanalytical approached to quantify 'total' and 'free' therapeutic antibodies and their targets: technical challenges and PK/PD application over the course of drug development. *The AAPS J.* 13(1), 00–110 (2011).

6 Viswanathan CT, Bansal S, Booth B *et al.* Quantitative bioanalysis methods validation and implementation: best practices for chromatographic and ligand-binding assays. *AAPS J.* 9(1), E30–E42 (2007).

7 Fast D, Kelley M, Viswanthan C *et al.* Workshop report and follow-up-AAPS workshop on current topics in GLP bioanalysis: assay reproducibility for incurred samples – implications of Crystal City recommendations. *AAPS J.* 11(2), 238–241 (2009).

8 Timmerman P, Lowes S, Fast DM, Garofolo F. Request for global harmonization of the guidance for bioanalytical method validation and sample analysis. *Bioanalysis* 2(4), 683 (2010).