Challenges in transferring bioanalytical methods

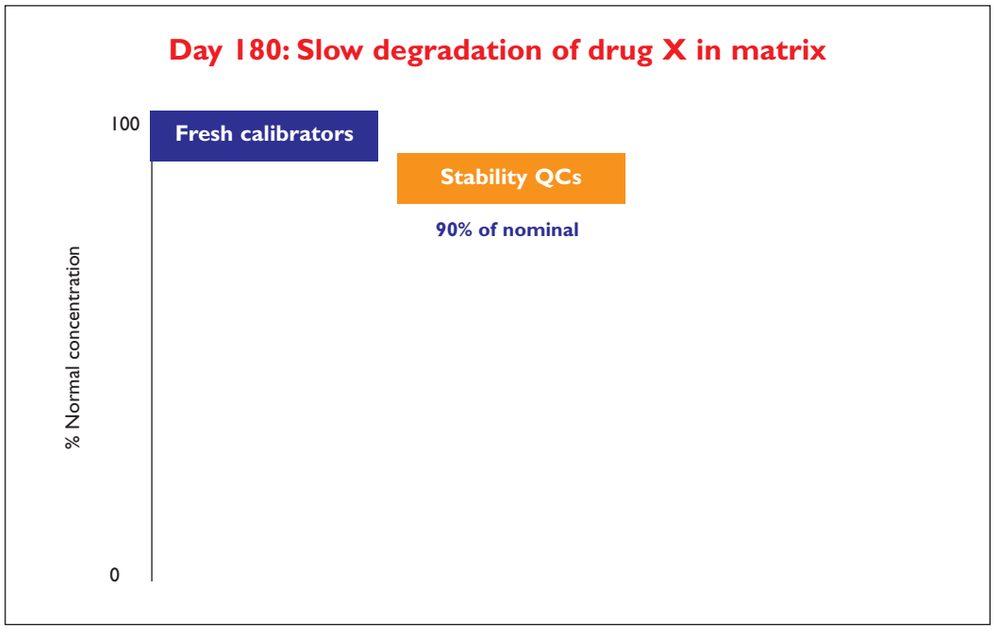
Drug development is arduous and expensive with any delay to market resulting in significant financial impact. Delays at any stage of the process cause challenges to programmes, however, some are easier to predict and plan for than others. Development of bioanalytical methods – essential for any drug development programme – is one area where delays often occur, but with proper planning, could be avoided. It is often necessary to move bioanalytical methods from one laboratory to another over the course of a drug development programme. It is therefore critical to understand the challenges of method transfers and plan for this eventuality to ensure rapid transition between the different stages of drug development and to constrain one's predictable cost.

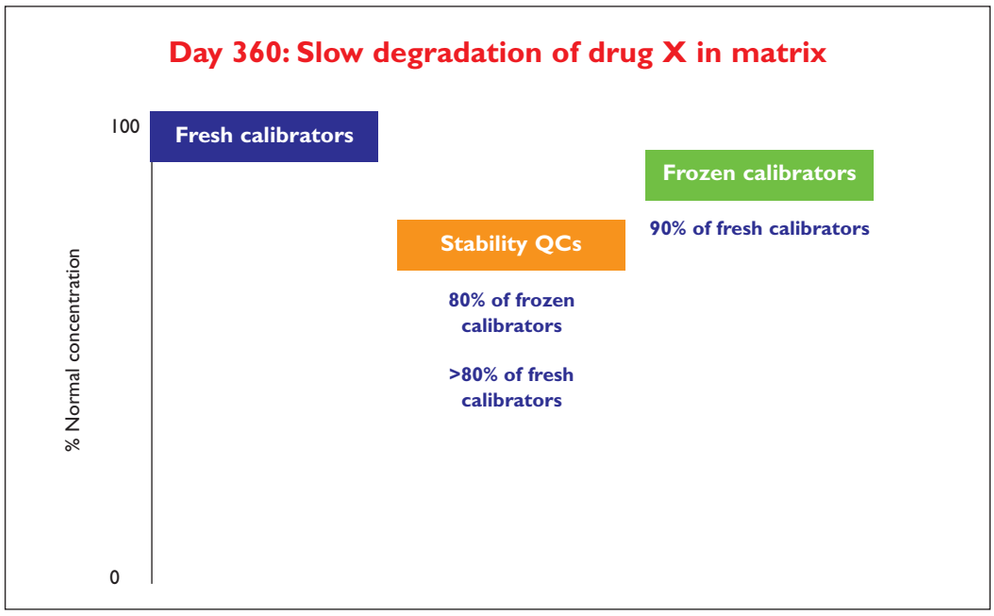
By Dr Chris Beaver

Method transfers occur for a number of reasons. Typically a sponsor has a method available in-house and requires a contract research organisation (CRO) to increase throughput for the assay. In other instances, a sponsor must transfer assays to a different laboratory because of financial opportunity, logistical requirements or dissatisfaction with services offered from internal or external suppliers. Method transfers frequently occur between preclinical and Phase I clinical studies, or Phase I and Phase II clinical studies. If in-house methods have been developed and analytical resources exist, a sponsor will often elect to analyse the preclinical and early clinical samples in order to maintain control over studies that support an IND submission or early clinical studies. Some of these studies involve smaller sample numbers and have fewer logistical issues than later-phase studies. If methods are transferred between Phases I and II, the majority of these will have already been validated – either by the sponsor or contracted laboratories – according to existing regulations and guidelines for validation of pharmacokinetic, immunogenicity or cell-based neutralising antibody assays, and in line with the needs of the programme[1-5]. If assays are transferred between preclinical and clinical phases, it is often due to a sponsor's internal resources being limited or redirected to support other programmes; consequently it may not be possible to manage the sample analysis. In some instances, transferred methods are in a partially evaluated or pre-validated state, as they have only been used in an unregulated capacity where such a method may be sufficient for providing exploratory data to support pharmacology, toxicology, drug metabolism or formulation studies.

The challenges of method transfer apply to both small- and large-molecule programmes. Large-molecule therapeutics tend to encounter these challenges more frequently, partly because compounds are often unique, having been modified in some manner to improve or extend their pharmacological activity or bioavailability. Companies with large-molecule candidates will have more developed analytical methods to detect their product utilising specific reagents (eg antibody pairs), which will more frequently necessitate a method transfer. In addition, due to the immunogenic nature of biological therapeutics, bioanalytical programmes for large molecules have additional

Figure 1





complexity. Sponsors will require validated methods for both pharmacokinetic measurements (ie, quantity of analyte in matrix) and immunogenicity measurements (ie, the immune response to the drug product).

Generally, issues that occur during bioanalytical method transfers can be grouped into the following categories: method development issues, performance expectations and information transfer issues.

Method development

The success of the method transfer will depend to a great extent on the method development scientists' experience and knowledge of applicable regulatory guidelines. Laboratories that have limited experience in developing assays for bioanalysis in preclinical or clinical studies may not appreciate or be completely aware of the requirements of a fully validated method, or even have

relevant experience to resolve common issues that may arise.

An example of this problem can be seen in the process by which analyte stability is established. A method might be transferred under the assumption that long-term stability of the molecule in relevant biological matrices has been established at either -20°C or -80°C. When tested at the laboratory after being transferred it fails to meet stability acceptance criteria. Upon further investigation, it has often been discovered that the calibrator curve was not prepared immediately before analysis, or that stability had not been established for drug stock solutions used to prepare the calibrators. Industry bioanalytical method validation consensus requirements are clear that stability must be established against calibrators that have been freshly prepared on the day of the evaluation. The reason for the discrepancy can be illustrated schematically (**Figure 1**). The first graph demonstrates an evaluation of stability quality control samples (QCs) that have been stored for 180 days. When tested against freshly prepared calibrators (blue bar) the stability QCs (orange bar) have degraded but are still within ±20% of the nominal concentration, acceptable by consensus criteria. In the second graph, stability QCs have now been stored for a further 180 days to bring the total storage time to 360 days. In this example, the stability QCs are being compared against calibrators stored for 180 days, shown in the green bar, rather than against fresh calibrators. When compared with the stored calibrators, the recovery of the QCs is approximately 20% below the nominal concentration. However, if the stability QCs were compared to freshly prepared calibrators (shown in the green bar) the stability evaluations would have failed. The degradation of the QCs below an acceptable limit would be masked by the use of frozen calibrators. This situation has been likened to 'the shrinking yardstick' where your ruler is changing to the same extent as the item it is meant to measure. This results in a method with inadequate stability for the intended purpose and will require more method development before it can be used in a regulated environment.

For large-molecule assays, selectivity testing is one aspect of method development that is often overlooked. Selectivity is defined as the ability of the method to detect analyte in the biological matrix of interest. It is negatively affected by matrix components that obstruct the assay's ability to detect the analyte of interest, either through binding directly with the analyte or through indirect effects on the assay system. Selectivity is evaluated by fortifying individual lots of matrix with the analyte at or near the lower limit of quantitation of the assay, and at a higher concentration (typically the high-quality control) and assessing the accuracy of recovery. Ideally, it should be possible to accurately measure analyte concentration in the individual lots. Conversely, a method for which many lots show poor recovery requires further development. For some methods, selectivity testing may have only been performed in a small number of lots or even a single pool of matrix before transfer. Testing in a pool of matrix obscures lot-to-lot differences and commonly results in further method development, sometimes after validation attempts fail. CROs consider this a critical question to resolve before method transfer can be considered advisable.

CROs develop and validate numerous methods per year and possess standard operating procedures (SOPs) that govern how a method should be validated. A best practice before transferring a method from sponsor to CRO is to ensure that both parties' scientific teams communicate early and frequently about method characteristics. A sponsor should request a review of the CRO's method validation SOP or a summary of the evaluations performed. If possible, a face-to-face review should take place to discuss the way evaluations are performed with the CRO's team.

Performance expectations

Another aspect of a drug development programme that can lead to an unsuccessful method transfer is failure to recognise the stresses to which methods are subjected in a high-throughput laboratory. A method that functions well when run infrequently by a single individual in a pharmaceutical or academic research lab may not demonstrate robust performance in a high-throughput environment where hundreds or thousands of samples are analysed each week by multiple analysts. It is incumbent on the sponsor and CRO to outline their throughput expectations and explain how the method will be used. Thoroughly testing the robustness of the method prior to method transfer is required. Testing by multiple individuals over many days ascertains whether methods are able to withstand the rigors of the bioanalytical testing environment. CROs often use this approach to evaluate a method before formally validating the method.

Transfer of information

Less-than-optimal method performance will be compounded by a limited transfer of technical information between sponsor and the CRO performing the validation. Although it may seem

obvious, thorough sharing of information during transfer is critical to success. Both individuals and organisations develop technical habits that differ slightly from other laboratories (eg, methods of pipetting, storage of reagents, analyte stock solution formulation, use of specialised equipment, plate washers, etc). Differences between the laboratories may exaggerate inherent method variability and cause a validation failure due to unacceptable accuracy and precision. In addition, laboratories source common reagents from several suppliers. Differences in the purity and preparation of chemicals between suppliers are common. This can affect the performance of both large- and small-molecule methodologies, often in different and unpredictable ways. Furthermore, difficulties with reagent bridging and qualification are not restricted to the project validation phase and may be encountered throughout validation and sample analysis. Securing the availability of antibodies, antibody conjugates and other detection reagents for the foreseeable duration of the project is critical for immunochemical methods. For projects with timelines spanning several years, it is strongly recommended that a strategy for sourcing these reagents be adopted early. Plans ensuring suitability of new reagent lots and/or manufacturers will be critical. However, it must be stressed that even slight changes in common buffers, kit solutions, columns and assay plates have been known to produce aberrant results. Even in evolving laboratory environments that adhere to significant Good Manufacturing Practices – like attention to event investigations, corrective action/preventative action and change control – a failure in a critical reagent can lead to delays both in validation and sample analysis. It is the responsibility of the CRO to ensure reagents are received and stored appropriately.

Immunogenicity assays are equally influenced by the factors described above. In addition, project-specific information for the validation is very important. Immunogenicity screening assays are statistically-based. Samples are deemed to be positive or negative for antibodies to the drug product on the basis of their response in the assay system relative to a threshold set during validation. The threshold is calculated on the performance of matrices (typically around 50 lots) that have not been fortified with the surrogate antibody. The lots are chosen to mimic a study population unexposed to the drug and the threshold is usually set as the mean of the negative control run in the assays, plus a factor derived from the standard deviation of the lots tested during validation. (Mire-Sluis et al, 2004). Setting the threshold accurately for sample analysis relies upon selecting the correct lots of matrix for the validation. A best practice is to select lots of matrix that are from individuals as close to the study population as possible (eg, age, weight, gender, diet, ethnicity, etc). In some circumstances this is difficult, if not impossible, to achieve due to the availability of certain patient-specific matrices. The clinical study protocol should be supplied to the CRO at the time of method transfer, along with the biological matrix specifications.

It is important the sponsor and CRO collaborate to ensure that the pertinent details of the sponsor's method are clearly communicated at the time of transfer. As much detail as possible should be provided in the method SOP or procedure document. Seemingly irrelevant information can be useful in clarifying procedures. Identifying key reagents for the method and testing different lots/suppliers ahead of time will ensure that the method will function with reagents from variable sources. When possible, assay performance should be tested with multiple individuals to ensure method transferability. Ideally, individuals from both the sponsor and CRO should run the method side by side at the laboratory from which the method originated or, preferably, at the CRO or laboratory where the transfer is taking place. The geographical location of the two laboratories can in some instances make this an expensive proposition. However, this short-term investment is insignificant when compared to overall drug development costs. When the cost of travel is weighed against that of expending additional drug development time or resources, which typically run up to the hundred thousands per diem[6], it is a very worthwhile investment.

Incurred samples in method transfers

Given the significant financial pressures on drug development for meeting analytical milestones, a best practice for project managers is to ensure methods are validated at more than one site. This ensures an alternate or back-up laboratory is available should an issue arise with the capacity at a single bioanalytical site. Once a method is transferred into multiple sites the question of the comparability of results commonly arises. A great deal of attention has been focused recently on the use of incurred (in-study) samples for the assessment of bioanalytical method performance. The robustness and reliability of a method is assessed through examining the reproducibility of results for a portion of the study samples. While no official guidance has been delivered, the general consensus

derived from meetings between industry representatives and the FDA held over the past few years is that small molecule/chromatographic methods are considered to have acceptable reproducibility if the repeat result of a minimum of two-thirds of tested samples is within ±20% of the original result (http://www.aapspharmaceutica.com/meetings/files/112/Closingremarkssummary.pdf). For macromolecule ligand binding assays this criterion may be widened to ±30% due to the larger variability associated with these types of assays[2]. While primarily aimed at determining the reproducibility of the method itself, this type of analysis can reveal non-methodological issues such as technical errors, differences in matrices, changes in reagents, etc.

In some cases incurred sample analysis has been used as a means of evaluating the acceptability of a method transfer. At method validation completion, once the precision and accuracy has been established, incurred or spiked samples that have previously been analysed at another location are analysed at the validation site and evaluated for concordance. In a manner similar to incurred reanalysis, the results and the methods are deemed to be equivalent if they meet predetermined acceptance criteria (eg, 2/3 of results must be within 20% of the original value). Delivering results from methods that meet acceptance criteria instills confidence that samples could be analysed at either site with an equivalent outcome. However, in contrast to the situation described for in-study incurred sample analysis where the evaluation is for a method at a single site, an additional set of questions may arise from a method when data from two different sites fail acceptance criteria. Since both methods met acceptance for precision and accuracy and would be considered validated at each site, judging which of the two methods requires investigation is very difficult. Objective evaluation of cross-site data remains a topic of spirited discussion. The potential for divergent data may call into question the strategy of placing a single assay at multiple laboratories.

The comparability of the qualification samples at different sites may not be an issue, depending upon type of analyte under investigation. While for quantitative pharmacokinetic assays inter-lab accuracy is an absolute requirement, for some pharmacodynamic assays this stringency may be less critical and, in some cases, impossible to obtain. For example, with some biomarker assays sponsors may be less interested in knowing the absolute value of the measurements than the relative change in biomarker levels. The criteria for establishing concordance between laboratories under these circumstances would require a fit-for-purpose approach.

Summary and conclusions

The average pre-approval costs of drug development run at just above $800 million[6] or roughly $183,000 per day over a 12-year timeframe. The loss of a day of patent life of a therapeutic has a considerable impact. It is therefore essential to adopt strategies for successful and efficient transfers of bioanalytical methods, which occur frequently in the drug development process. Good communication, close collaboration and planning between sponsor and CRO are the keys to their success. Likewise, the use of incurred samples offers a powerful tool for assessing the suitability of the transfer, but one whose outcomes must be carefully discussed and planned prior to use in order to avoid any delays resulting from unexpected or inconclusive results. Strategic relationships between sponsors and CRO partners facilitate these processes through the sharing of common processes and expectations. DDW

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References

1 Guidance for Industry – Bioanalytical Method Validation (US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research, May 2001).

2 Viswanathan, CT, Bansal, S, Booth, B, DeStefano, AJ, Rose, MJ, Sailstad, J, Shah, VP, Skelly, JP, Swann, PG, Weiner, R. Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. Pharmaceutical Research. 2007, 24:1962-73.

3 DeSilva, BS, Smith, WC, Weiner, R, Kelley, M, Smolec, J, Lee, B, Khan, M, Tacey, R, Hill, H, Celniker, A. Recommendations for the Bioanalytical Method Validation of Ligand-Binding Assays to Support Pharmacokinetic Assessments of Macromolecules, Pharmaceutical Research, 2003, 20(11): 1885 – 1900.

4 Mire-Sluis, AR, Barrett, YC, Devanarayan, V, Koren, E, Liu, H, Maia, M, Parish, T, Scott, G, Shankar, G, Shores, E, Swanson, SJ, Taniguchi, G, Wierda, D, Zuckerman, LA. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. Journal of Immunological Methods, 2004 289:1-16.

5 Gupta, SR, Indelicato, V, Jethwa, T, Kawabata, M, Kelley, AR, Mire-Sluis, SM, Richards, B, Rup, E, Shores, SJ, Swanson, E, Wakshull. Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics. Journal of Immunological Methods. 2007, 321:1-18.

6 DiMasi, JA, Hansen, RW, Grabowski, HG. The price of innovation: new estimates of drug development costs. Journal of Health Economics, 2003, 22:151-85.