Practical limitations of using a 1.0% failure rate of low positive control in ADA assays

The use of antibody-based therapies and biopharmaceuticals is becoming more common in the treatment of various diseases. However, these therapies can provoke immune responses that may reduce their efficacy and lead to unwanted side effects. Detection and characterisation of anti-drug antibodies (ADAs) is essential in developing and testing new biopharmaceuticals. One major practical challenge for validating and using ADA assays is setting and maintaining appropriate low positive control samples (LPCs) to ensure assay sensitivity at a 1.0% rejection rate. Biological variability and changing assay conditions can lead to inconsistencies and increased rejection rates of runs based on LPC responses. The diverse portfolio of ICON's clients provides the opportunity to apply a variety of methodologies, continuously improving the reliability and accuracy of our ADA assays and delivering reliable outcomes.

Introduction

Anti-drug antibodies (ADAs) can impact the therapeutic efficacy of biopharmaceuticals in several ways. They can neutralize therapeutic proteins by binding them, potentially preventing their interaction with the target antigen and thus mediate loss of drug efficacy by blocking the biological activity of the drug. Furthermore, ADAs can accelerate the clearance of therapeutic antibodies from the body, which reduces their half-life and consequently their therapeutic efficacy. In some cases, ADAs can form immune complexes which could trigger autoimmune reactions and further complicate the patient's condition. The impact of these immune responses varies widely, ranging from negligible effects to severe and life-threatening outcomes. This highlights the importance of accurate immunogenicity testing to detect and evaluate ADAs, ensuring both the safety of patients and the effectiveness of treatments.

Setting up a bioanalytical assay for the measurement of ADAs presents significant challenges, particularly in the selection of an appropriate positive control (PC). Unlike pharmacokinetic assays, where quality control samples are available, ADA assays rely on surrogate PCs that are typically generated by immunising animals (e.g. rabbits) with the therapeutic protein and purifying the antibodies formed. Traditionally, these surrogate polyclonal antibodies have been used as PCs due to their broad epitope recognition and cost-effectiveness. However, surrogate antibodies often come with challenges, including variability and lower specificity, which can lead to cross-reactivity and inconsistent results. Monoclonal surrogate PCs, on the other hand, offer high specificity and reproducibility, but may not reflect the heterogeneity of antibody responses in study subjects. Despite its benefits, monoclonal antibody development is still complicated and expensive. The choice between using surrogate polyclonal or monoclonal antibodies depends on various factors, including the stage of drug development, assay requirements, and resource availability.

In an ADA assay, three levels of PCs are typically used: low PC (LPC), mid-PC (MPC), and high PC (HPC). Each is spiked with surrogate PCs in a pooled blank matrix to cover a wide range of ADA concentrations. LPC is used to monitor the assay's sensitivity at low levels of ADAs, ensuring that the assay can detect even minimal immune responses. MPC evaluates the assay's accuracy at moderate levels of ADAs, providing a benchmark for expected ADA concentrations in patient samples. HPC is used to verify the assay's performance at high ADA concentrations, ensuring that the assay remains accurate and reliable across a wide range of ADA levels.

The 1.0% failure rate approach to low PCs

Due to the generally low and variable levels of ADAs in patient samples, sensitive assays are often required to ensure accurate detection and monitoring. High sensitivity ensures that even low levels of ADAs that may be clinically significant in terms of drug efficacy or safety are identified. In practice, many assays are more sensitive than the 100 ng/mL as expected by the FDA.

In accordance with FDA guidelines, a robust statistical method is used to determine the sensitivity of the assay. To achieve this and to establish the LPC concentration, ICON routinely performs a minimum of nine independent runs with sensitivity curves, performed by at least two analysts on different days. These sensitivity curves are prepared using a PC that is serially diluted two-fold above the screening cut point until they no longer yield a positive result, providing a robust dataset for reliable statistical analysis.

Assay sensitivity is then calculated using either linear regression based on the concentrations and signal responses directly above and below the plate-specific cut point or using non-linear regression (4-PL/5-PL). The sensitivity results from each curve are used to calculate the overall average and standard deviation. To ensure assay reliability, it is recommended by the FDA that the LPC concentration be set to achieve an approximate 1.0% expected failure rate, meaning that one LPC out of every 100 is expected to fail and score negative instead of positive. This dataset is then utilised to determine the screening LPC (sLPC) and confirmatory LPC (cLPC) concentrations using the following formula:

Mean screening assay sensitivity + (t0.005df * standard deviation screening assay sensitivity)

Mean confirmatory assay sensitivity + (t0.01df * standard deviation confirmatory assay sensitivity)

If the two concentrations are similar (e.g., both are approximately 10.0 ng/mL), or if the anticipated screening/non-drug spiked signal responses are within ±10% of each other, a single concentration may be selected for use as both the screening and confirmatory LPC concentration. However, if there is a significant difference between the two calculated concentrations (e.g. a difference greater than 10.0 ng/mL), separate sLPC and cLPC concentrations will be established and qualified for each respective assay tier.

If the calculated screening and/or confirmatory LPC concentrations fail to produce a positive response in all evaluations, a fresh preparation of the affected LPC concentration(s) is made and analysed in the respective assay tier. Simultaneously, an additional set of screening and/or confirmatory controls will be prepared at approximately 20% higher concentration to ensure reliable assay performance.

Applying the LPC based on a 1.0% failure rate in sample analysis: theory versus practice

Although theoretically, the statistically calculated LPC concentration should lead to the rejection of an individual LPC 1.0% of the time, this is not always the case in practice. While validation typically takes a few weeks, clinical study sample analysis can span several years and multiple lots of critical assay reagents, further contributing to assay variability. At ICON, numerous ADA studies are conducted and in several of those sample analysis studies, particularly those for multiple-year ADA studies, the calculated LPC fails at a specific point in time, resulting in a rejection rate exceeding 1.0%. This, in turn, increases sample analysis costs due to unnecessary run rejection.

While the statistical approach described above establishes a useful LPC level, it may not always offer enough data points to fully capture all variations and factors affecting the assay. During validation, the LPC is established in a two-day time period with a specific batch of materials, but there are multiple other factors that can impact the reproducibility of the LPC. In the long run new batches of blocking, assay and read buffers, new labeled batches of coating and detection, a new matrix pool, new assay plates and even new qualified lab analysts will be introduced. All these modifications can significantly impact assay sensitivity and performance, potentially increasing variability and leading to failure rates beyond the acceptable 1.0%. There are also occasions where the actual LPC spike does not reflect the anticipated 1.0% failure rate. This can occur because the LPC is established by diluting the HPC to below the cut point, and the actual LPC is volumetrically spiked with multiple pre-dilutions (figure 1). These examples highlight the importance of ongoing evaluation and adjustments throughout the ADA assay lifecycle. These adjustments can include additional LPC determinations upon consistent LPC failure in consecutive bioanalysis runs and following introduction of the new set of materials and analysts. Adjustments during the lifecycle of the ADA assay should not be unusual as long as they are within the recommendations and acceptance ranges. If properly supported an increase in the LPC should be possible for an assay that demonstrates a sensitivity well below 100 ng/mL, as requested by the FDA in their most current guidelines.

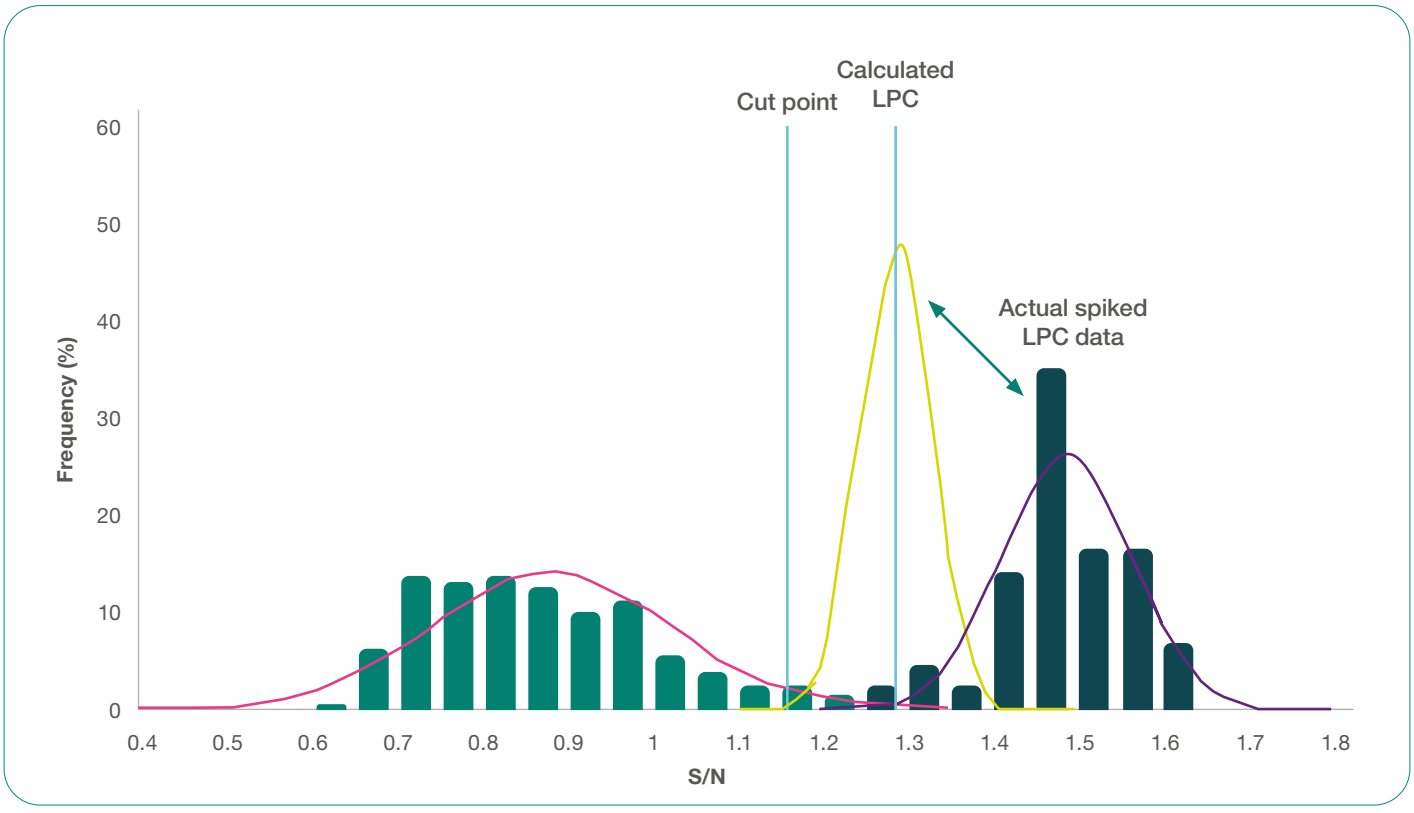

Figure 1: An illustration of how a volumetrically spiked LPC may not always consistently reflect the expected theoretical 1.0% failure rate. Here, the signal over noise ratio (S/N) of the actual spiked LPC is about 20.0% higher compared to the established LPC. Even though, in this example the LPC will consistently score positive with a failure rate well below 1.0%, a different spike might also end up at the left end of the calculated LPC curve and exceed the expected 1.0% failure rate.

Mitigation strategies when the 1.0% failure rate is exceeded

When the expected 1.0% LPC failure rate is exceeded during sample analysis, it is advisable to avoid unnecessary run rejection by raising the LPC concentration. Here, several options for increasing the LPC level are proposed.

Performing a log transformation on the sensitivity results from each curve during validation and in the end back-transform the calculated LPC to the original scale, can increase the LPC concentration. Through logarithmic transformation the impact of outliers is reduced, skewed data will approximate normality and variance in heteroscedastic datasets is stabilised.

If, during sample analysis the LPC does not align with the anticipated 1.0% failure rate, an extended LPC qualification may be performed following the actual bridging of the new spike. This qualification involves analysing 40 newly spiked LPC samples on a single plate, with 39 of 40 samples required to meet the criteria. If qualification fails, increasing the LPC may be considered based on a scientific evaluation (e.g. the LPC level can be increased by 20.0% and re-evaluated).

Moreover, for phase three trials an in-study cut point is calculated when a sufficient number of pre-doses of the diseased population are available. When the in-study cut point and the validation cut point differ significantly, it can be decided to use a separate QC-based cut point and a sample-based cut point, however, it is advisable to recalculate the LPC with the in-study cut point factor to align with the newly established assay sensitivity.

Finally, duplicate analysis leaves less sample space on the plate. Therefore, when possible, validate the ADA assay in singlicate, as this reduces the need to switch to new batches of critical material, new matrix pools and labeled batches of coating and detection. Thus, minimising the variability that may cause the failure rate to exceed 1.0%.

In conclusion, numerous variables may contribute to assay variability. Therefore, continuous monitoring of the LPC scoring and failure rate is crucial to maintain accuracy and consistency over time. If necessary, appropriate steps can be taken to mitigate a potential increased failure rate. These should be in line with the current guidelines and supported by scientific judgement.

References

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