Current industry practices for in-study cut point setting for clinical immunogenicity assays

For biotherapeutics, the development of custom anti-drug antibody (ADA) assays to monitor unwanted immunogenicity is an integral part of clinical development programs. ADA analysis is commonly performed following a tiered approach. Samples that potentially contain ADAs are initially identified in the screening tier. Positively screened samples are subsequently analysed in a confirmatory assay to verify whether the initial positive screening results are true positives or false positives. Confirmed (i.e. true-) positive samples can be further characterised with titer-, neutralising- and/or binding affinity- assays. The distinction between positive and negative samples in each tier is made using statistically derived response thresholds (cut points) that are set during method validation.

Assay responses used to set these cut points (CPs) can vary substantially between subject populations due to factors such as disease state and/or demographic background. This can lead to a mismatch between the established validation CP and the clinical study population, which may have several unwanted consequences. On one hand, underestimation of the CP will lead to increased reporting of false positive results that do not have any clinical relevance. A high incidence of false positive samples will also result in larger sample numbers requiring confirmatory analysis, causing unnecessary resource allocation and increased costs. On the other hand, overestimation of the CP can cause false negative results and thus

increase the risk of obscuring potentially relevant immunogenicity. CPs that are established during method validation therefore need to be verified in each clinical study and adjusted when applicable.

Recent white papers have addressed industry best practices for in-study CP verification and calculation. Nevertheless, in the immunogenicity field, there is ongoing discussion on this topic and many different approaches are applied due to differences in interpretation, sponsor specific practices and context of use. This commentary describes ICON's observations of current practices for in-study CP verification and calculation.
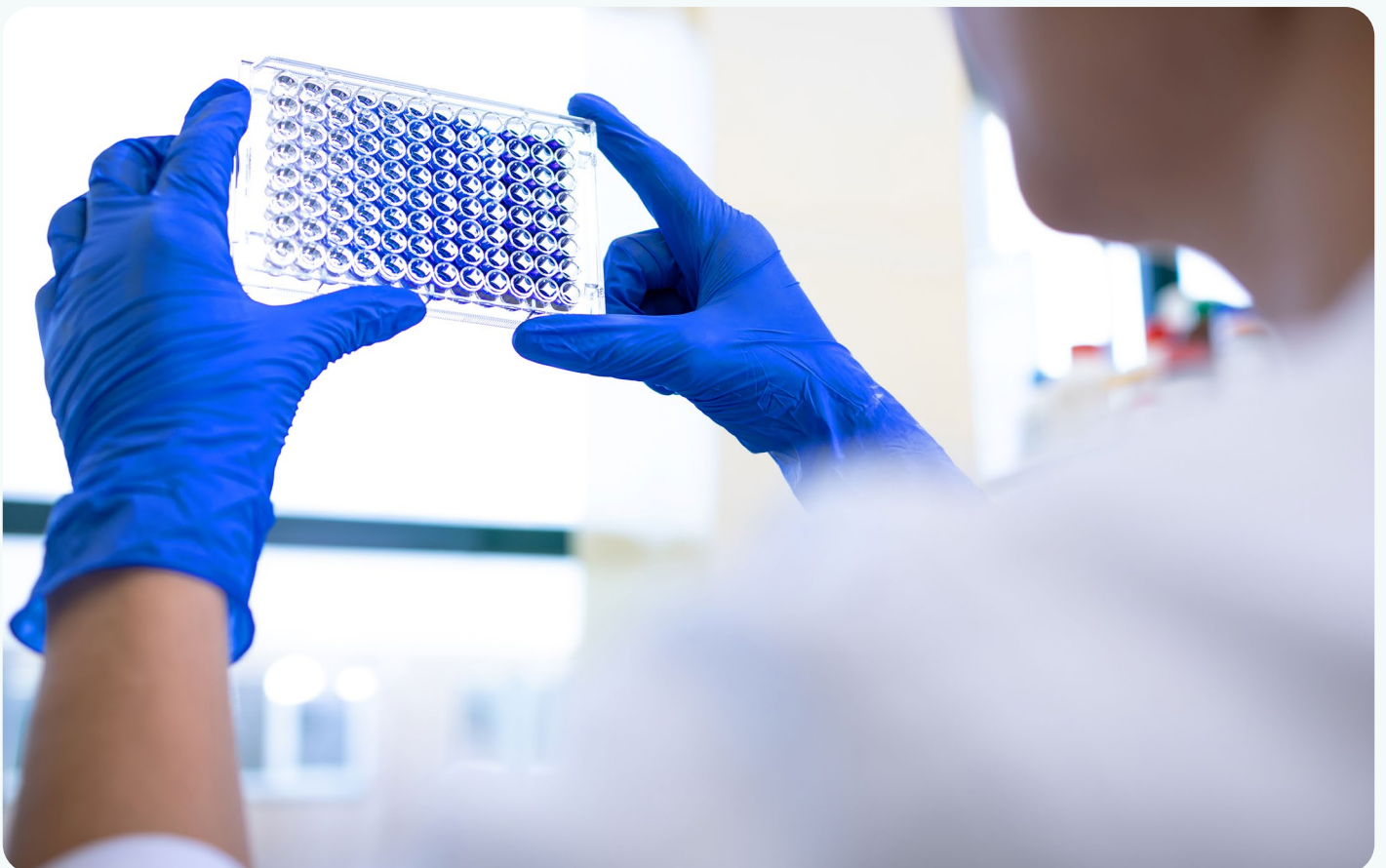
When to use an in-study cut point

Regulatory agencies have stipulated that where feasible, the CP should be statistically determined using samples from treatment-naive subjects. Pre-dose study samples could be used for CP determination; however, study samples are often not readily available during method validation. In addition, late-stage clinical development programs may encompass a variety of different diseases, which could make it even more challenging to set an appropriate population-specific CP for each disease state. In practice, it is therefore accepted that validation CPs are mostly determined using matrix samples from commercially sourced healthy subjects. In those cases, the applicability of the validation CP should be verified at a later stage, once treatment-naïve (pre-dose) samples from the clinical study population become available. Based on this verification, it can then be decided whether the validation CP may be applied in sample analysis or whether an in-study CP should be considered.

To assess the need for an in-study CP, pre-dose clinical study samples are commonly evaluated using the validation CP. If, after the removal of positively confirmed samples the observed false positive rate (FPR) is within the expected 2 – 11% range, the validation CP can be used. Otherwise, an in-study CP is considered. It should be noted that this does not always result in applying an in-study CP. For instance, for certain (e.g. early stage) clinical trials, the sample number may be too low to reliably calculate the FPR. Consequently, the calculated FPR percentage should be interpreted with caution. In such cases, an FPR exceeding the anticipated 11% will have relatively little impact and is often considered acceptable because, as with relatively low sample numbers, the increased workload is limited. Conversely, an FPR < 2% is more likely to trigger an in-study CP due to the potential risk associated with missing immunogenicity.

In the past, the requirement for an in-study CP was often solely based on the FPR. However we observe that it is becoming more common to combine FPR calculations with additional statistical evaluations. In particular, when an in-study CP is indicated based on the FPR, the study pre-dose and validation CP samples can be compared in terms of means (Levene's test), and/or variances (ANOVA) and then visualised using boxplots. If this comparison confirms significant differences in means and/or variances between the validation and pre-dose samples, this provides additional supporting evidence that the use of an in-study CP would be appropriate. Another approach that we have applied in some rare cases, based on specific sponsor requests, is the pre-emptive calculation of an in-study CP for each disease state population. This mitigates the need for complex statistical evaluation but can also lead to unnecessary changes in CPs between studies. Nevertheless, for methods where responses are known upfront to differ substantially between populations, this can be a reasonable approach.

How to set an in-study cut point

The approach for setting a validation CP is extensively documented in white papers and regulatory guidance and therefore well standardised. This is in contrast to setting in-study CPs, which is less straight forward and involves various experimental approaches.

The validation CP is typically defined by analysing 6 individual measurements of at least 50 drug-naïve representative samples. These measurements should be conducted on at least 3 different days by at least 2 laboratory technicians. Usually, a balanced design is used for CP runs, which will provide higher statistical power and a better understanding of the variation observed in the tested population. With this approach, analytical and biological outliers can be determined and excluded from further CP analysis.

In-study CPs are set on naïve pre-dose study samples but use various experimental approaches. Firstly, the in-study CP can be determined and evaluated during an ongoing sample analysis study. It can be determined as soon as a sufficient number of pre-dose samples have been analysed, e.g. >50 pre-dose samples. In cases where less pre-dose samples are available, using a minimum of 20-30 pre-dose samples is advised. As a consequence of this approach, pre-dose samples used for setting an in-study CP will be analysed only once, in contrast to the repeated measurement set-up of the validation CP. Here it is assumed that the pre-dose samples are distributed across multiple bioanalytical runs, which ensures that the relevant biological and analytical variability is captured.

Alternatively, pre-dose samples can be analysed separately from the bioanalytical study in a similar fashion to setting a validation CP. The number of replicates or repeat assessments of a set of pre-dose samples can be identical to validation but are usually less (e.g. two repeats). Hybrid approaches are also sometimes followed, where the initial analysis data from pre-dose samples are supplemented with dedicated runs to increase the statistical power of the assessment. For this approach the available volume of pre-dose samples and the number of freeze/thaw cycles should be taken into account. Also, informed consent from the study subjects should allow for these additional assessments, which is not always the case.

Consequences of using an in-study cut point

When an in-study CP is applied, the impact of this change in CP on the assay reproducibility and data interpretation needs to be considered. A significant shift in CP, could theoretically lead to altered assay characteristics, such as sensitivity and drug tolerance. In practice, most validation parameters are not re-evaluated when the CP is changed and only occasionally a selected number of validation experiments (primarily sensitivity and drug tolerance) are repeated. However, re-evaluation is important in case of a change in CP and the need to repeat the sensitivity or drug tolerance assessments should be considered.

If the in-study CP is higher than the validation CP, this can also affect the reproducibility of the low positive control (LPC) that was statistically set close to the detection limit of the method during validation. In those cases, it is advisable to re-establish the LPC concentration, based on existing data using the in-study CP to avoid unnecessary run failures. If the in-study CP is significantly different from the validation CP, this can also affect the scoring of bioanalysis samples. Therefore, an in-study CP should be established as soon as possible, to prevent re-evaluation of study samples, which were initially evaluated with the validation CP.

In conclusion, continuous monitoring of CPs is a critical process to ensure reliable immunogenicity assessment throughout different trial phases and between populations. These CPs may need adjustment based on statistically evaluating clinical study populations, to avoid false positives or negatives. Contrary to the CP setting during method validation, which over the years has evolved into a well-documented and standardized process, in-study CP setting is less straightforward and more context dependent. Hence, it is essential to thoroughly evaluate the experimental and statistic methodology on a case-by-case basis.

References

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