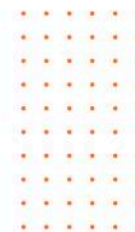


ECL-BASED ADA ASSAYS:

Choosing the Right QC
Strategy for Your
Programme





→ Introduction

Immunogenicity and Clinical Risk

Immunogenicity testing is a regulatory requirement for most CGT programmes. Whether you are screening patients for pre-existing immunity to AAV serotypes or monitoring immune response following administration, the data your assay generates has direct consequences for what comes next. Patient inclusion and exclusion decisions, safety monitoring, programme timelines, and regulatory submissions all depend on the quality and reliability of that data. Getting the assay right from the outset is not optional.

•

Regulatory tension

Regulatory agencies recommend that the low positive control (LQC) concentration is set so that no more than 1% of values fail during normal assay use. In practice, for an assay operating with a floating cut-point, the threshold of positivity shifts from plate to plate, making it structurally difficult to set an LQC concentration that reliably meets that expectation. This is a known tension between regulatory guidance written for quantitative assays and the biological reality of ADA testing.



As a CRO, you have to be clear towards your sponsors, the regulators, the scientists or medical doctors who have to take decisions based on your results. Expertise is the key to confidence.



Sabrina Triffault, Analytical Director, GenoSafe

Why Assay Decisions Matter

ADA assays are mandatory for most innovative biologic therapies. Whether you are seeking a specific immune response, as in cancer vaccination, or screening to exclude patients with pre-existing immunity, as in gene therapy, the quality of your immunogenicity data shapes the clinical decisions that follow, from patient inclusion and exclusion to safety monitoring throughout the programme.

72%

of healthy adults carry pre-existing antibodies to AAV2, the most prevalent serotype, making patient screening a prerequisite for treatment eligibility.

These assays are custom-built for each therapy, validated from scratch, and developed in an environment where no universal standard exists.

For example, two CROs given the same brief will approach it differently. This should not be assumed to be a quality failure, but rather a structural feature of the field, and understanding it is the starting point for being able to closely manage it.

The decisions made at validation, about which acceptance approach to use and why, have consequences that run for the lifetime of a study. Getting those decisions right requires experience with how these assays behave in practice.



59%

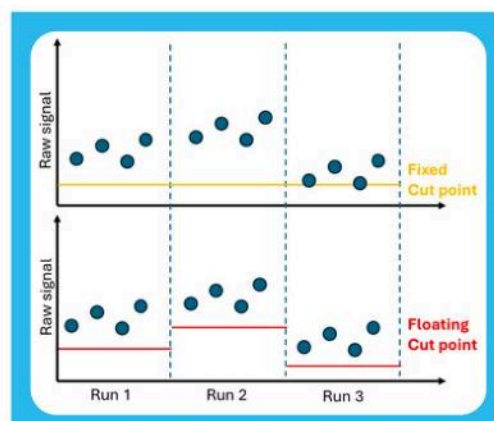
of healthy adults carry neutralising antibodies to AAV2, the serotype most commonly used in gene therapy, capable of blocking vector transduction entirely.

→ The QC Challenge



ECL-based platforms have become the preferred technology for ADA assays in many programmes, offering better sensitivity, dynamic range, and lower background signal than earlier methods such as ELISA and radioimmunoassay. This improved sensitivity, however, introduces a problem that is easy to overlook during assay development and difficult to manage once you are into a long-running clinical study.

Most ADA assays use a floating cut-point, where the threshold between a positive and negative signal is recalculated for each analytical plate based on the negative control signal on that plate. This accounts for normal day-to-day variation in background signal and is scientifically sound. The complication arises when you combine a floating cut-point with fixed QC acceptance ranges.



Fixed QC ranges are set during validation to detect drift, reagent decay, and procedural variability. The difficulty with ECL platforms is that background signal drift occurs over time, a natural feature of the technology, causing QC values to shift even when the assay is performing correctly, and samples are being classified accurately. The practical consequence is plate rejection without a scientific basis, with associated delays and cost implications for a programme that may already be under time and budget pressure.



“Even with a validated assay, you do observe significant differences in raw signal from one experiment to another, **even if performed with the same reagents or by the same technician.** It does not usually impact the result associated with a given sample. The bad news is that you sometimes must reject the results which otherwise look good **because QC acceptance ranges are no more aligned**”



***Sabrina Triffault**
Analytical Director
GenoSafe*

→ Understanding ECL Signal Drift

Several factors specific to ECL platforms contribute to this drift over time:

- **Background signal variation between runs**
- **Differences between MSD plate lots**
- **Instability of biotin and SULFO-TAG reagents**
- **Edge effects on multi-well plates**
- **Timing-sensitive signal variation**

Most of these can be managed through stability studies, precise incubation timings, or reagent controls. Background signal drift is different. It can occur with no change in reagents, no evident explanation, and no predictable timeline for resolution. It cannot simply be engineered away.

In a regulated environment, the practical response is not to chase a universal fix but to choose a QC acceptance strategy

appropriate to your programme's specific context from the outset.

The purpose of the assay matters: a short preclinical study where all samples are analysed within weeks has different requirements from a long-running clinical programme where reagent lot changes are inevitable. The four approaches below reflect that reality, each with different trade-offs between simplicity, sensitivity to drift, and regulatory auditability.

Approach	Handles signal drift	Suitable for long studies	GCP regulatory simplicity	Patient inclusion decisions
• Raw values only	X	X	✓	✓
• Hierarchical ranges	~	~	~	X
• Ratio of signals	✓	✓	~	✓
• Normalisation over a 100% reference	✓	✓	✓	✓



Four Approaches to QC Acceptance Ranges

01

RAW VALUES ONLY

QC acceptance ranges are defined as fixed raw signal values during validation. Each plate is accepted or rejected based on whether the QC signals fall within the predefined values, independent of the negative control level on that plate.

WHEN TO USE

Short-duration studies in which reagent lots are unlikely to change and signal variation is expected to be minimal. A reasonable starting point for assays in early development, where long-term robustness has not yet been established.

LIMITATIONS

Signal drift over time causes increasing plate rejection rates even when sample classification remains accurate. Not recommended for assays running across multiple months or reagent lot changes without reassessment. The simplest approach, and the least adaptable.

02

HIERARCHICAL RANGES

Acceptance is based on maintaining the correct hierarchy of QC levels: the high positive control must exceed the low positive, the low positive must exceed the negative, and variability between QC sets must remain within a defined %CV threshold. Raw values are not monitored directly.

WHEN TO USE

Preclinical GLP studies where all samples will be analysed within a short window and no patient inclusion or exclusion decision depends on the assay result. Well-suited to programmes where the relationship between QC levels matters more than absolute signal stability.

LIMITATIONS

Signal drift may go undetected in the absence of raw value monitoring. Requires careful scientific justification in regulated clinical settings where auditors may challenge the absence of fixed reference values.

03

RATIO OF SIGNALS

Acceptance is based on maintaining a minimum ratio between QC levels across the plate, for example the mean HQC divided by the mean LQC. The assay retains its ability to discriminate between signal levels regardless of overall background drift.

WHEN TO USE

Assays with known or anticipated background signal drift where the critical performance indicator is discrimination between positive and negative signals rather than absolute value. Particularly useful in long-running clinical studies where reagent lot consistency cannot be guaranteed.

LIMITATIONS

Multiple ratio combinations are possible, and the calculation method must be precisely defined before validation. Regulators will expect a clear rationale for the chosen ratio and pre-defined thresholds documented in the validation plan.

04

NORMALISATION OVER 100% REFERENCE

All signal values are normalised against a high-signal reference control, with each QC expressed as a percentage of that reference rather than as a raw value. This provides inherent correction for plate-to-plate signal variation and mirrors the methodology used in cell-based neutralisation assays.

WHEN TO USE

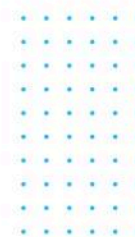
Assays requiring tight cross-run comparability, particularly where reagent lot changes are anticipated across the lifetime of a long study. A strong choice for programmes nearing commercialisation where batch-to-batch consistency of the assay carries regulatory weight.

LIMITATIONS

The reference control must be carefully chosen, stable and reproducible, and produce a signal reliably within the linear range of the assay. If the reference control drifts, the normalisation fails. Also adds a data processing step that must be clearly documented and auditable.



Choosing the Right QC Strategy



Signal variation is an inherent feature of ECL-based ADA assays, and in a well-validated assay managed by an experienced team, plate failure is a predictable consequence of the technology rather than an indicator that something has gone wrong. It can be anticipated and designed around from the outset.

The four approaches described here reflect decisions that analytical teams make in practice, often under time pressure and regulatory scrutiny. Knowing when to apply each one, and how to justify that choice to a sponsor or an agency, is where expertise has the most practical value.

Immunogenicity testing in CGT is one of the most technically demanding areas of clinical bioanalysis.

The gap between a well-chosen CRO partner and a poorly-chosen one has direct consequences for programme timelines, regulatory outcomes, and ultimately patient access.

Choosing between approaches for a specific programme, justifying that choice under regulatory scrutiny, and knowing when to revisit it as a study evolves, is where analytical experience has the most direct commercial and clinical value. That judgement accumulates over years of working with these assays across different programmes, platforms, and regulatory environments. While guidelines can document the options, they cannot replicate the experience of having navigated them in practice.



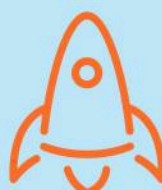
Read the full scientific paper, including full methodology, figures, and insights



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