Reducing regulatory risks in PCR assays for bioanalytical validation

Polymerase chain reaction (PCR) based bioanalytical testing is critical for the proper delivery, safety, and efficacy of cell and gene therapies (CGTs)

PCR assays resemble immunoassay based pharmacokinetic (PK) assays in overall concept and desired outcome

Using a similar assay framework can be beneficial; however, the technologies differ in sample preparation requirements and detection methods, which can affect the validation parameters of the PCR assay

When developing a quantitative nucleic acid method for PK analysis, key differences from standard ELISA assays must be considered

Current guidance for validation testing parameters of immunoassay PKs is clear. However, there is no regulatory guidance for validation of nucleic acid PK assays.

Key differences between PCR and ELISA

Sensitivity

Limits of detection (LOD) can be variable and dependent on sample-processing (extraction and purification) and quantification cycle (Cq) calculation.

Risk Mitigation

Use lower limit of quantitation (LLOQ), as it is less affected by variability in the assay set-up.

Accuracy

Accuracy is reported in copies per reaction. For qPCR, use a standard curve, as for ELISA. For ddPCR, there is no standard curve due to the nature of the technology.

Risk Mitigation

For ddPCR, calculate quantity based on partitioning and poisson distribution of the positive fraction of target DNA.

Precision

Across runs, quantification cycles (Cqs) are subject to inherent variation and are not an appropriate measurement of assay reproducibility.

Risk Mitigation

Use absolute values (ddPCR Poisson-corrected results or interpolated qPCR results) rather than raw Cq values.

Recovery

Naked DNA or RNA should never be added directly to a matrix due to rapid degradation; therefore, if numerous matrices are required to be tested, the true analyte is not available for LOD and/or LLOQ.

Risk Mitigation

Controls should be generated in a matrix-free diluent complemented by a recovery experiment.

The validation parameters outlined reflect the need to understand current regulatory recommendations in the context of use and applicability for PCR-based PK assays, to best mitigate risks resulting from lack of clear regulatory guidelines.

Working with a partner that continually evaluates trends, guidance, and industry recommendations on building a model-validated PCR assay for PK can enable you to navigate adoption of new methods, understand regulatory considerations, and provide an agile response to your study's needs.

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