CASE STUDY

USE OF GENERIC PHARMACOKINETIC ASSAYS TO SUPPORT TOXICOLOGY STUDIES FOR NEW BIOLOGIC ENTITIES

INTRODUCTION

What is a generic PK assay and when to use it?

A generic pharmacokinetic (PK) assay, also known as a universal PK assay for New Biologic entities (NBE), is used to measure circulating human biotherapeutics, mostly antibody-based, post-administration across multiple non-human matrices. This approach is particularly useful when drug-specific reagents, such as anti-idiotype antibodies, are not available. These assays provide critical data for ranking multiple drug candidates, determining systemic drug exposure in animals, and adjusting dose range for GLP toxicology studies.

One size does not fit all

When selecting the Ligand-Binding Assay (LBA) method for generic PK, the characteristics of the candidate drug and the performance must be considered. Capture and detection reagents can be chosen based on the antibody scaffold, isotype, any specific engineering features such as humanization or glycoengineering, and the antibody format (monoclonal or multi-specific Ab, or an antibody-drug conjugate (ADC)). These reagents can be selected among either the antibody target, anti-species Ab targeting light chain, Fc fragment, or a specific isotype, that are commercially available. In addition, when measuring humanized antibodies in non-human primates (NHP), particular attention should be paid to cross-reactivity that may impact the specificity and sensitivity of the method.

The technological platform must be selected based on the expected dynamic range, sensitivity, sample volume, and matrix interference[2,3].

“Off-the-shelf” does not mean “Ready-to-use”

If an off-the-shelf generic PK assay can be used, it is crucial to ensure the suitability of the assay for the drug being studied. A few days of method development may be needed for adapting the method to the expected working range, sensitivity, and matrix effect before non-clinical sample analysis.

CASE STUDY 1 : SELECTION OF A LEAD MONOCLONAL ANTIBODY

In the context of the selection of a **lead monoclonal antibody (mAb) candidate**, the sponsor has requested KCAS Bio to qualify a generic method for ranking **3 candidates** (mAb 1, 2 and 3) in non-human primates (NHP). Preclinical serum samples were collected for PK analysis at different time points from 3 groups of animals intravenously injected with each of the three mAbs.

Objective	Selection of a lead mAb candidate (3 candidates)
Preclinical stage	Pre-toxicology, non-GLP
Species	Cynomolgus monkey
Matrix	Serum
Immunoassay	Customized ELISA
Drug	Humanized mAb, IgG1

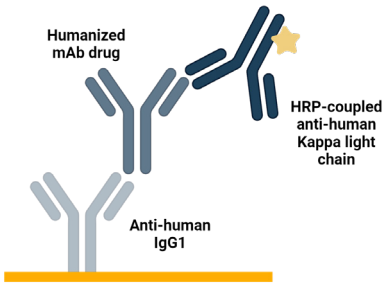

Figure 1 - **Assay format:** Sandwich ELISA

During the early stages of generic PK assay development, mAb1 exhibited slightly different behavior compared to mAbs 2 and 3. Therefore, two distinct methods were developed, based on the same format and working dilution but using two distinct calibration curves, one with mAb1 for quantifying mAb1, and another with mAb3 for quantifying mAbs 2 and 3. The lower and upper limits of quantification (LLOQ and ULOQ) were established. These methods were qualified for precision and accuracy, selectivity, and target interference prior to non-clinical sample analysis.

Results shown in **Figure 2** illustrate the PK profile of the three candidate mAbs over an 8-week period post-administration.

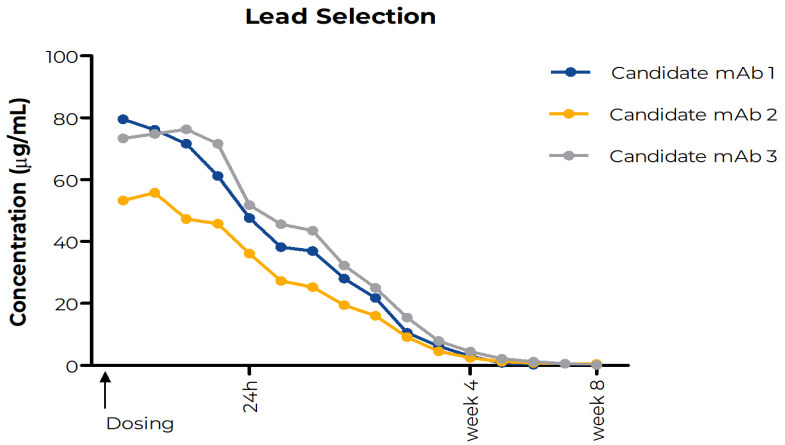

Figure 2 - **Serum concentration over time of 3 mAb candidates measured with generic ELISA based on mAb1 or mAb3 calibration curves.**

CASE STUDY 2 : DOSE RANGE FINDING OF A MONOCLONAL ANTIBODY

In the context of a **Dose Range Finding (DRF) study for a humanized agonist IgG1 mAb**, the sponsor has requested KCAS Bio to develop and qualify a method for measuring drug concentrations in a non-GLP toxicokinetic study in NHP. Preclinical samples were collected at different time points for PK analysis from 4 groups of animals intravenously injected or not with increasing doses of the mAb.

Objective	Dose Range Finding (1 candidate – 3 doses)
Preclinical stage	Toxicology, non-GLP
Species	Cynomolgus monkey
Matrix	Serum
Immunoassay	Gyrolab
Drug	Humanized mAb, IgG1

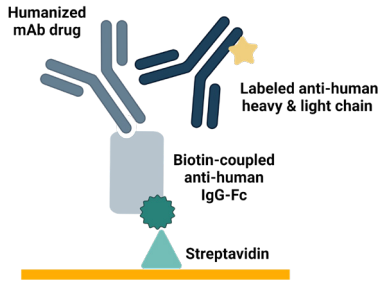

Figure 3 - Assay format: Gyrolab Generic PK Immunoassay

In addition to precision, accuracy and, selectivity, dilution linearity was assessed during qualification to ensure that the method can measure drug levels in the range of micrograms per mL in the NHP serum.

Results shown in **Figure 4** correspond to the quantification of the therapeutic mAb upon the first and third administration of increasing doses of the mAb drug at different time points. They show that measured drug levels are consistent with the injected doses.

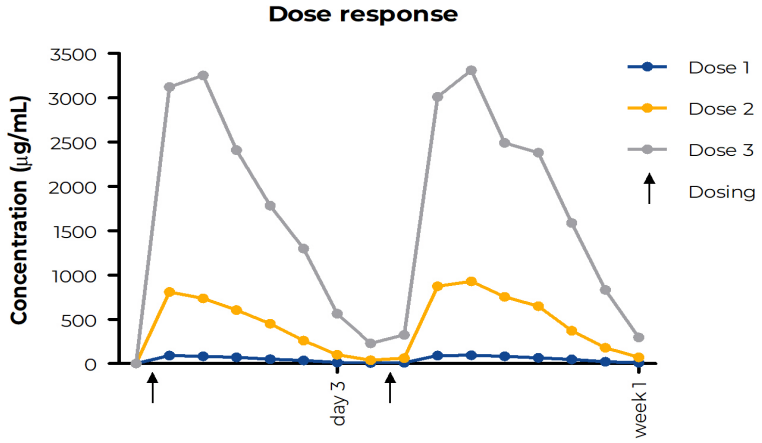

Figure 4 - Serum concentration over time for one mAb candidate administered at 3 doses, measured with Gyrolab generic PK immunoassay

CONCLUSION

KCAS Bio has leveraged its expertise to develop and troubleshoot multiple LBA-based generic PK methods over the past several years. The versatility and flexibility of generic PK assays not only accelerate the preclinical drug development process, but also offer significant cost savings. By quickly providing accurate data, these assays expedite the transition to late GLP toxicology and ultimately First-In-Human clinical phase.

REFERENCES

(1) https://kcasbio.com/blogs/biopharma-lba-generic-pk-assays-reduces-development-time-and-cost/

(2) Leary BA, et al., Bioanalytical platform comparison using a generic human IgG PK assay format. J Immunol Methods. 2013 Nov 29;397(1-2):28-36. doi: 10.1016/j.jim.2013.08.009. Epub 2013 Aug 29. PMID: 23994108.

(3) Dudal S, etal., Assay formats: Recommendation for best practices and harmonization from the global bioanalysis consortium harmonization team. AAPS J. 2014 Mar;16(2):194-205. doi: 10.1208/s12248-013-9552-9. Epub 2013 Dec 17. PMID: 24343771; PMCID: PMC3933581.