M1020

A Generic Pharmacokinetic (PK)/ Toxicokinetic (TK) Assay Kit for Analysis of Biotherapeutic Antibodies in Sera from Several Species for Early-stage Development using Gyrolab™ Platforms

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Purpose

The development of recombinant therapeutic IgG is often challenged with multiple candidates per program, reagent selection and limited sample volume. In addition, lead candidates have to be evaluated in two pre-clinical species, to assess both efficacy and safety parameters which involve the use of a variety of experimental animals such as mouse, rat and cynomolgus monkey. A high performance generic PK assay recognizing human monoclonal antibody eliminates the need for multiple rounds of assay/reagent development and validation to support experimental activities using less sample volumes for a variety of studies early in development.

Ideally a generic assay should be compatible with the most common species used in pre-clinical phase and for drug candidates belonging to different IgG subclasses. Furthermore, given the wide analyte concentration range that may occur in samples from pre-clinical studies, the assay should be easily adaptable to a wide variation in sample analyte concentration. Early in development, sample volumes may also be extremely limited, e. g. when using transgenic mouse models, and dramatically reduced biological variability for PK can be achieved using the one mouse – one PK profile approach (Joyce AP et. al. Pharm. Res. 2014 Jul; 31(7):1823-33).

The intention is to provide a generic PK assay kit, compatible with Gyrolab platforms that can be used in studies throughout early-stage and pre-clinical development of recombinant human intact antibodies of different IgG subclasses in different species.

Methods

Immunoassay

The generic nature of the PK assay was achieved by using epitopes present on all relevant IgG subclasses (IgG1, IgG2, IgG4) for assay design. The assay was also designed to generate signal from human IgG only. Immune reagents were therefore selected that would not cross-react with IgG in species employed for *in vivo* experiments (primarily cynomolgus monkey, mouse and rat).

Sample preparation

Therapeutic antibodies of different human IgG subclasses (IgG1, IgG2 and IgG4) were spiked in 100% of mouse, rat or cynomolgus serum. The samples were then diluted 1:10 in sample dilution buffer (Gyros Protein Technologies) and quantified using the corresponding standard and matrix in Gyrolab.

CDs and Instruments

Gyrolab™ Bioaffy 1000 HC CD or Gyrolab™ Bioaffy 20 HC CD was used on a Gyrolab™ xP or Gyrolab xPlore™ system throughout the evaluation.

Gyrolab Bioaffy CD

Sandwich assay on streptavidin bead | *Affinity-capture column (15 nL)* | *Bioaffy CD microstructure* | *Gyrolab Bioaffy CD* | *Typical analyte concentration and dynamic range for Gyrolab Bioaffy CDs.*

Gyrolab system streamlines the immunoassay workflow by automating sample addition, washing and detection using nanoliter volumes in the microfluidic format of Gyrolab Bioaffy CD.

About Gyros technology: Gyrolab xP workstation and Gyrolab xPlore perform automated immunoassays within nanoliter-scale microfluidic structures in a Compact Disk (CD) format. Each structure on the CD comprises a 15-nanoliter affinity column pre-packed with streptavidin-coated particles, supporting a variety of assay types including sandwich and indirect antibody assays. While Gyrolab xPlore runs single CDs, Gyrolab xP workstation can run up to five CDs unattended.

Consumption of sample and reagents is dramatically reduced compared with plate-based immunoassays. Microfluidic control ensures that all samples on a CD are processed in parallel, giving consistent results. Each microstructure equates to one data point, eliminating cross talk. The control and analysis software is 21 CFR part 11 compliant, ensuring that assays can be developed and transferred through to GMP and GLP environments.

Results

IgG subclass reactivity and compatibility with different species

Monoclonal IgG of subclasses IgG1, IgG2 and IgG4 were serially diluted in 100% of cynomolgus, rat or mouse serum followed by dilution in sample buffer as described in methods to a final concentration of 10% serum. The resulting nine standard curves were run using the Generic PK kit and the overlay is shown in figure 1.

The assay performance was comparable between IgG subtype and species, confirming that the assay can be used for a wide range of applications.

Assay performance

A monoclonal antibody of IgG1 subtype was selected to evaluate assay performance using a Minimum Required Dilution (MRD) of 1:10.

To evaluate precision, standard curves were diluted in 10% cynomolgus serum and analyzed in six separate runs. The assay range of the standard curve was 0.2 – 3000 ng/mL, corresponding to 2 – 30000 ng/mL back calculated to neat serum.

The six standard curves superimpose with intra-run % CV typically below 10% and inter-run % CV below 20% (Fig 2 and Table 1).

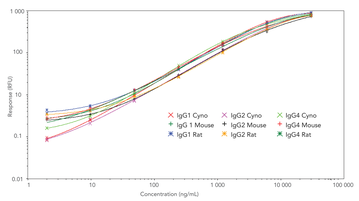

Figure1: Standard curves of IgG subclasses IgG1, IgG2, and IgG4 diluted in 10% of cynomolgus, mouse or rat serum. The concentrations are back calculated to neat serum.

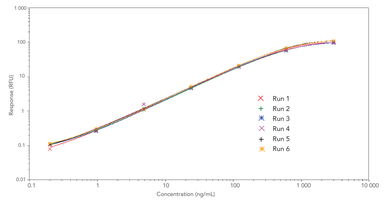

Figure 2: Six standard curves of IgG1 analyte diluted in 10% cynomolgus serum. The concentrations are back calculated to neat serum.

Table 1: Precision of six standard curves of IgG1 analyte diluted in 10% cynomolgus serum analyzed in duplicates. The concentrations are back calculated to neat serum.

	Conc. ng/mL	Avg response	RE%	Intra run %CV (n=2)	Inter run %CV (n=12)
Standard 1	2	0.1	1.3	2.0	10.9
Standard 2	9.6	0.3	-1.6	9.9	7.0
Standard 3	48	1.1	1.9	4.3	13.8
Standard 4	240	4.6	0.8	0.7	3.9
Standard 5	1200	19	-1.5	0.6	1.5
Standard 6	6000	59	0,9	2.8	4.8
Standard 7	30000	99	-0,5	0.7	18.1

Working range

The antibody was spiked in 100% cynomolgus or mouse serum at different concentrations between the anticipated LLOQ and ULOQ followed by dilution to 10% serum. Corresponding standard curves in 10% cynomolgus or mouse serum were prepared. The QC samples were quantified in duplicate in three separate runs (Table 2). All QC samples were quantifiable with a total error of less than 30%, which suggested a working range in of 10 – 14000 ng/mL, back calculated to neat serum for cynomolgus or mouse serum.

Shifting the working range for TK samples

To eliminate the need for excessive dilutions in the case of TK studies, the working range of the assay was shifted to a higher range of quantification by using the Gyrolab™ Bioaffy 20 HC CD. The working range of the assay shifted from approximately 10 – 10,000 ng/mL to 500 – 1,000,000 ng/mL.

Abbreviations: CV, Coefficient of Variation; SD, Standard Deviation; RE, Relative Error; TE, Total Error; MRD, Minimum Required Dilution; LLOQ, Lower Limit of Quantitation; ULOQ, Upper Limit of Quantitation.

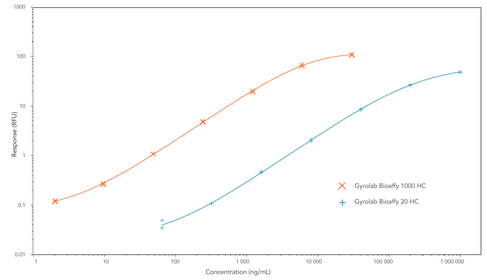

Figure 3: Standard curves of IgG1 analyte diluted in 10% cynomolgus serum using ethier Gyrolab™ Bioaffy 1000 HC CD or Gyrolab™ Bioaffy 20 HC CD. The concentrations are back calculated to neat serum.

Table 2: LLOQ and ULOQ samples quantified in duplicate in 10% serum in three separate runs. The concentrations are back calculated to neat serum.

	Sample Id	Exp Conc ng/mL	n	Mean ng/mL	SD	%CV	%RE	%TE
Cyno	LLOQ 1	10	4	1.08	0.03	2.8	8.0	10.8
	LLOQ 2	30	6	3.43	0.20	5.8	14.3	20.1
	LLOQ 3	50	6	5.77	0.80	13.9	15.4	29.3
	ULOQ 1	9000	6	727	35.6	4.9	-19.2	24.1
	ULOQ 2	12000	6	1068	126	11.8	-11	22.8
	ULOQ 3	14000	6	1215	48.1	4.0	-13.2	17.2
Mouse	LLOQ 1	10	6	0.99	0.10	10.5	-1.0	11.5
	LLOQ 2	30	6	2.66	0.16	6.1	-11.3	17.4
	LLOQ 3	50	6	4.87	0.25	5.1	-2.6	7.7
	ULOQ 1	9000	6	793	68.7	8.7	-11.9	20.6
	ULOQ 2	12000	6	1168	84.5	7.2	-2.7	9.9
	ULOQ 3	14000	6	1180	71.3	6.0	-15.7	21.7

Accuracy

QC samples covering the working range of the assay were spiked in 100% cyno serum and diluted to 10% serum with sample dilution buffer. The samples were quantified in six separate runs using a standard curve in 10% cynomolgus serum (Table 3). The accuracy was excellent, with total error less than 20% in the linear range and less than 30% at LLOQ and ULOQ.

Table 3: QC samples in 10% cynomolgus serum quantified in six separate runs. The concentrations are back calculated to neat serum.

Species	Sample Id	Exp Conc ng/mL	n	Mean ng/mL	SD	%CV	%RE	%TE
Cyno	ULOQ	12000	12	1326.29	247.34	19	10.5	29.5
	HQC	8000	12	763.42	85.67	11.4	-4.6	16
	MQC	500	12	47.45	1.73	3.6	-5.1	8.7
	LQC	100	12	9.85	0.53	5.6	-1.5	7.1
	LLOQ	30	12	3.10	0.30	10	3.3	13.3

Conclusions

Using Gyrolab Generic PK/TK kit, human therapeutic antibodies belonging to different IgG subclasses and presented in serum from multiple species, were quantified with excellent analytical performance. The generic character of the assay provides a single immunoassay that supports:

- Several different animal species commonly used in PK experiments
- Different IgG subclasses (IgG1, IgG2 and IgG4)
- Quantification of ≤ 10 ng/mL IgG analyte back calculated to neat serum with absolute sensitivity being dependent on drug molecule and matrix
- The Gyrolab Generic PK/TK kits combined provide an assay with 5 logs of analytical range

Ready to use Gyrolab Generic PK/TK kits streamlines evaluation of drug candidates for multiple programs using only nanoliter volumes and eliminates the time and resources needed for assay development and reagent screening. This provides a valuable tool to be used throughout early-stage and pre-clinical development of intact human therapeutic antibodies.