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 antibodies eliminates the need for multiple rounds of assay/reagent development and validation to support experimental activities using limited 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 analyze concentration. Early in development, sample volumes may 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 (Uygun AE et al. Pharm. Res. 2014; Jul;31(7):1825-33).

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

Immunassay

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 CD 1000 HD CD or Gyrolab Bioaffy 200 HD CD was used on a Gyrolab xP or Gyrolab xP® system throughout the evaluation.

Gyrolab Bioaffy CD

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

About Gyros technology: Gyrolab® xP workstation and Gyrolab® xP® perform automated immunassays 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® xP runs single CD, Gyrolab® xP® workstation can run up to five CDs unattended.

Consumption of sample and reagents is dramatically reduced compared with plate-based ELISA. 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 GAMP and GLP environments.

Results

IgG subclass reactivity and compatibility with different species

Monoclonal IgG subclasses IgG1, IgG2 and IgG4 were spiked 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).

Figure 1: Overlay of labeled standard curves for an IgG1 analyte diluted in 10% mouse, rat and cynomolgus serum. Figure 2: Standard curves of an IgG1 analyte diluted in 10% cynomolgus serum (left) and 10% mouse serum (right).

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.

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Figure 3: Working range for PK and TK samples. Figure 4: Working range for PK samples. Figure 5: Working range for TK samples.

Conclusions

Using Gyros 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 immunassay that supports:

- Several 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 0.1 ng/mL

Table 2: QC samples quantified in six separate runs for 10% cynomolgus serum using the Gyrolab Generic PK kit and 50% cynomolgus serum using the Gyrolab Generic TK kit. The concentrations are back calculated to neat serum.

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Acknowledgements: CV, Coefficient of Variation; SD, Standard Deviation; RE, Relative Error; TE, Total Error; MRD, Minimum Required Dilution; LQC, Lower Limit of Quantitation (LOQ); HQC, Upper Limit of Quantitation.