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Efficient and Economical Preclinical Pharmacokinetic Analyses for Human Therapeutic Antibodies and Constructs, Using a Validated Human Immunoglobulin Immunoassay in Plasma of Mice, Rats, and Monkeys

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PURPOSE

This work was conducted to develop and validate (fit-for-purpose, FFP) an efficient means for pharmacokinetic (PK) analyses of large molecule drugs composed in part, or in whole, of human IgG. PK behavior of large molecule drug candidates in experimental animals is often a determination factor in the selection of which candidate(s) to pursue. Analysis of serum or plasma for the presence of such drugs can be expensive and time consuming, especially if an analytical method needs to be developed and validated for each candidate in a series of candidates. Accordingly, we propose to use the present method of analyzing for the presence of human IgG antigenic determinants in the plasma of mice, rats, or monkeys, subsequent to injection of human therapeutic antibodies, or constructs containing any part of the human IgG molecule.

A sandwich ligand-binding assay (LBA) was developed using Meso Scale Discovery (MSD®) electrochemiluminescence (ECL) technology. Affinity-purified, monkey IgG-adsorbed, goat anti-human IgG was used for capture. The same antibody, subsequent to biotinylation, was used for detection in conjunction with streptavidin-SULFO-TAG™ as the reporter. The method proved highly efficient and specific in its abilities to distinguish human IgG from other components in plasma of common preclinical animal species, i.e., mice, rats, and monkeys.

A FFP validation approach was used to include comparative analysis across the 3 species. Parameters assessed in the FFP validation included accuracy and precision (A&P), specificity, selectivity, linearity of dilution, and hook effect. Stability of human IgG in plasma of such experimental animals is well known and was not assessed, especially since stability would be a drug-specific characteristic. All testing of plasma was done with a minimum required dilution (MRD) of 1:100. The high sensitivity of ECL allows for this dilution factor, while maintaining sensitivity at <10 ng/mL after factoring in MRD. Consequently, as little as 5.0 microliters of sample is sufficient to conduct the analysis, a clear advantage when using small animals like mice.

METHOD(S)

Principal of the Human IgG LBA

Affinity-purified, monkey IgG-adsorbed, polyclonal goat anti-human IgG was used for capture and detect. The antibody was coated onto standard 96-well MSD plates for capture, 1 µg/mL In pH 7.0 phosphate buffered saline (PBS), 50 µL/well with incubation overnight at 2-8oC.

The plate was washed with PBS containing 0.05% tween-20 (PBS/T) and blocked with 150 µL/well of PBS containing 3% bovine serum albumin (BSA). Standards, quality control (QC) samples, and test samples were diluted to the MRD of 1:100 with PBS/T containing 1% BSA (PBS/T/BSA). After washing the plate with PBS/T, the diluted samples were added to the designated wells, 50 µL/well, followed by 1 hour incubation at room temperature. After another wash step, wells of the plate were inoculated with 50 µL/well of PBS/T/BSA containing 250 ng/mL of biotinylated-goat anti-human IgG. After incubation (1 hr., room temperature), the wells were again washed, followed by addition of streptavidin-SULFO-TAG with an incubation of 1 hr. at room temperature. Subsequently, following another wash step, 2x MSD read buffer T was added to the wells (150 µL/well). Assay signal (electrochemiluminescence units, ECLU) was measured using an MSD QuickPlex SQ 120 instrument.

Preparation of Standards, Validation Sample QC, and Spiked Recovery Selectivity Assessments

Standards and QC were prepared in pools of normal mouse, rat, and monkey K2EDTA plasma. All QC and standards were prepared in ≥95% of the respective species pool. The standard curve range, with a 1:100 MRD factored in the calculation, was 7.5 to 5,500 ng/mL human IgG. Validation samples (VS/QC) were prepared at concentrations of 7.5, 20, 150, 4,500, and 5,500 ng/mL for LLOQ, LQC, IQC, HQC, and ULOQ, respectively. Selectivity was assessed by spiking 10 individual plasma samples from the 3 species, equally divided between male and female. Spiking was conducted in neat plasma at a 1:20 ratio, yielding 95% matrix to spike proportion, and final human IgG concentrations at the HQC and LLOQ levels, 7.5 and 4,500 ng/mL, respectively. Endogenous human IgG reactivity was not observed in parallel unspiked plasma samples for any of the 3 species.

Calculations

A standard curve was generated for each run using purified human IgG. MSD Discovery Workbench software, v.4.0.12 (Meso Scale Discovery) was used to fit the data using a four-parameter logistic (4-PL) curve-fit of mean ECLU values versus nominal IgG standard values. Concentrations of human IgG in study and QC samples were calculated by interpolation along this standard curve. All QC samples and study samples were back-calculated using the MRD (1:100) as a dilution factor.

RESULT(S)

Figure 1.
Comparability of Human IgG Standard Curves in Assay Diluent and in 1% Mouse, Rat, and Monkey Plasma

Standard curves were prepared in assay diluent (PBS/T/BSA) and in plasma pools from mice, rats, and cynomolgus monkeys and diluted to the optimal MRD (1:100) with assay diluent before addition of the samples to wells of a standard MSD plate coated with goat anti-human IgG. After incubation and wash steps, the plate was developed with biotinylated-goat anti-human IgG, followed by streptavidin-SULFO-TAG. The data are expressed as means of duplicate wells/determination, except VS positions which are illustrative spot markers and not data points.

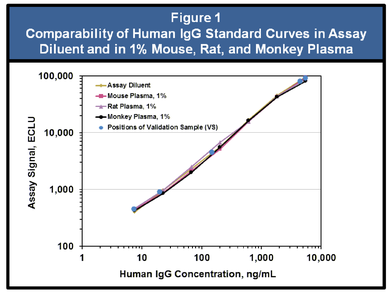


Figure 2.
PK Assay Specificity for Human IgG, and Little Reactivity with IgG, IgM, and Plasma of Mice, Rats, and Monkeys

Panel A illustrates the human IgG standard curve performance in assay diluent, as well as assay signals for mouse, rat, and monkey IgG and IgM. The human IgG curve exhibited a discernable response with as little as 10.5 pg/mL IgG. Purified IgG and IgM of mice, rats, and monkeys elicited minimal assay signals, less than the LLOQ. Panel B of Figure 2 demonstrates that the LBA for human IgG has little immunoreactivity with constituents of plasma from mice, rats, and monkeys. One percent of such plasma displayed the equivalent of <80.0 pg/mL human IgG.

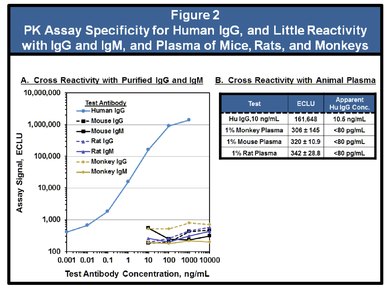
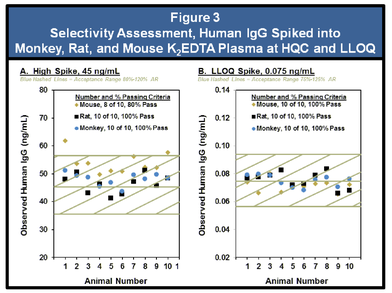


Figure 3.
Selectivity Assessment, Human IgG Spiked into Monkey, Rat, and Mouse K2EDTA Plasma at HQC and LLOQ

- Individual plasma specimens, equally divided between males and females, from mice, rats, and monkeys were spiked at the HQC (Panel A) and LLOQ (Panel B) levels with human IgG.
- The samples were analyzed against the corresponding standard curve (e.g., mouse samples vs. standards in 1% pooled mouse plasma).
- The data are expressed as observed concentrations in the spiked samples. The hashed bars denote the regions of acceptance for percent analytical recovery, 80%-120% for HQC spikes, and 75%-125% for LLOQ spikes.
- Most (≥80.0%) spiked samples met acceptance criteria at both the HQC and LLOQ levels for all species.



RESULT(S)(CONTINUED)

Table 1.
Inter-assay A&P for Analysis of Human IgG in Mouse, Rat, and Monkey EDTA Plasma

- Validation samples were prepared at 5 levels (LLOQ, LQC, IQC, HQC, ULOQ) in mouse, rat, and monkey K2EDTA plasma.
- The VS spanned the range of the calibrator curve (7.50 to 5,500 ng/mL).
- All VS, in all species, passed standard acceptance criteria, including Total Error (TE).
- No substantial difference were observed between species and, as shown by inter-species statistics, the cumulative results were largely the same as for the individual species.

Table 1
Inter-assay Accuracy & Precision for Analysis of Human IgG in Monkey, Rat, and Mouse EDTA Plasma

Individual Species	Inter-assay Statistics	Validation Sample, Nominal Concentration				
		LLOQ, VS1 7.50 ng/mL	LQC, VS2 20.0 ng/mL	IQC, VS4 150 ng/mL	HQC, VS4 4,500 ng/mL	ULOQ, VS5 5,500 ng/mL
Mouse	Mean Observed	7.36	19.2	120	4,037	5,292
	%CV, %DEV	11.9%, 4.4%	11.7%, 6.1%	7.4%, 18.5%	9.8%, 12.2%	11.4%, 8.1%
	%TE	16.9%	17.8%	26.9%	22.0%	16.6%
Rat	Mean Observed	7.34	18.8	151	4,182	5,020
	%CV, %DEV	11.2%, 2.1%	8.1%, 6.1%	5.9%, 0.9%	5.0%, 7.1%	4.2%, 8.7%
	%TE	13.3%	14.2%	6.8%	12.1%	13.9%
Monkey	Mean Observed	6.74	17.9	126.5	4,328	5,284
	%CV, %DEV	17.6%, 10.1%	7.7%, 10.5%	9.4%, 15.7%	5.9%, 3.8%	5.1%, 3.9%
	%TE	27.7%	18.2%	25.1%	9.7%	9.1%
All Species	Inter-species Statistics	LLOQ, VS1 7.50 ng/mL	LQC, VS2 20.0 ng/mL	IQC, VS4 150 ng/mL	HQC, VS4 4,500 ng/mL	ULOQ, VS5 5,500 ng/mL
Combined Species Data	Mean Observed	7.15	18.6	132	4,154	5,174
	%CV, %DEV	2.4%, 5.3%	2.8%, 7.0%	13.1%, 12.2%	4.6%, 7.7%	2.7%, 6.9%
	%TE	7.8%	9.8%	26.3%	12.3%	8.6%

Table II.
Summary of Validation Parameters for Quantitation of Human IgG in Mouse, Rat, and Monkey EDTA Plasma

- An assay range of 7.5 to 5,500 ng/mL human IgG was observed for plasma of all species.
- The LLOQ was established by A&P runs, as well as by spiked recovery in selectivity analyses at LLOQ (7.5 ng/mL) and HQC (4,500 ng/mL).
- Linearity of dilution passed acceptance criteria for all species.
- These results confirm that the assays will be able to quantitate human Ig therapeutic over a broad range preclinical dosages (e.g., 1-60 mg/kg).

Table II
Summary of Validation Parameters for Quantitation of Human IgG in Mouse, Rat, and Monkey K_2EDTA Plasma

Species	Assay Ranges, ng/mL		Accuracy and Precision (A&P) Inter-assay Ranges			Selectivity	Linearity on Dilution
	LLOQ	ULOQ	%CV	%AR	%TE	Pass/Fail % Pass	R^2, Nominal vs. Observed
Mouse	7.50	5,500	6%-12%	2%-20%	11%-26%	8/10, 80%	Pass, R^2 > 0.99
Rat	7.50	5,500	4%-11%	1%-%9	7%-14%	9/10, 90%	Pass, R^2 > 0.99
Monkey	7.50	5,500	5%-18%	4%-16%	9%-28%	10/10, 100%	Pass, R^2 > 0.99

CONCLUSION(S)

A. We describe an efficient and economical LBA analytical method for quantitating biologics with epitopes in common with human IgG, e.g., therapeutic antibodies and human Ig constructs.
B. The method has been validated fit-for-purpose, i.e., suitable for screening and identification of optimal drug candidates based on PK characteristics.
C. The assay system is designed to facilitate transition of PK analyses from one preclinical species to the next.
D. This LBA requires only a pilot run to verify suitability of the assay system to quantitate the biologic in question.
E. Client funded method development and validation are not required to use this PK analysis service, aside from the pilot run with the candidate drug(s).

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