The data are expressed as observed.

The method has been validated fit.

Validation samples were prepared at 5 levels

The assay system is designed to facilitate transition of PK analyses from one preclinical species to the next.

Linearity of dilution passed acceptance criteria for

as a dilution factor.

standard curve.  All QC samples and study samples were back

A standard curve was generated for each run using purified human IgG.  MSD Discovery

unspiked plasma samples for any of the 3 species.

95% matrix to spike proportion, and final human IgG concentrations at the HQC and LLOQ levels,

Selectivity was assessed by spiking 10 individual plasma samples from the 3 species, equally

Validation samples (VS/QC) were prepared at concentrations of 7.5, 20, 150, 4,500, and 5,500

range, with a 1:100 MRD factored in the calculation, was 7.5 to 5,500 ng/mL human IgG.

QC and standards were prepared in ≥95% of the respective species pool.  The standard curve

Assessments

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

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 into standard 4-well MSD plates for capture, 1 µg/well, in pH 7.0 phosphate-buffered saline (PBS). 50 µL/well with incubation overnight at 4°C. The plate was washed with PBS containing 0.05% Tween-20 (PBS-T) and blocked with 150 µL/mL of PBS containing 3% bovine serum albumin (BSA). Standards, quality control (QC) samples, and test samples were diluted to the MD of 1:100 with PBS containing 1% BSA/PBS/TBSA. After washing 3x with PBS/TBSA, the diluted samples were added into the designated wells. 50 µL/well, followed by 1 hour incubation at room temperature. After another wash step, well plate of the were incubated with 50 µL/PBS/PBSA containing 200 µg/mL of blocking solution, 1:100 dilution of blocking solution, human IgG. After incubation (1 hr., room temperature), the wells were washed again, followed by addition of strepavidin SULFO-TAG. The data are expressed as reciprocal of dilution, wash steps, the plate was developed with biotinylated-gold anti-human IgG. followed by strepavidin

Accordingly, we propose to use the present method of analyzing for the presence of human IgG, and evaluate the performance of the LBA under the following conditions: 

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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).