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Application of a Novel Affinity Purified Chicken IgY in an Immunoaffinity LC-MS/MS Assay for Rapid Quantitation of Monoclonal Antibody Therapeutics and ADCs in Nonclinical Species

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PURPOSE

Today monoclonal antibody-based therapeutics (mAbs) serve as important targeted treatment modalities across numerous therapeutic areas. Thus, to facilitate development of new agents there is a need to increase the quality and throughput of bioanalysis to expedite assessments of nonclinical PK prior to initiating Phase I evaluation in humans. Accordingly, we have adapted an affinity purified chicken IgY with pan-specificity for human IgG subclasses which displays negligible cross-reactivity to IgG from common nonclinical species, e.g. cynomolgus monkey, for use in an immunoaffinity LC-MS/MS assay workflow to establish a bioanalytical method for rapid quantitation of monoclonal antibody-based therapeutics, including antibody drug conjugates (ADCs).

METHODS

Immunoaffinity Capture Beads

The immunoaffinity capture is performed by combining the following components in a protein LoBind Eppendorf tube and incubating for at least 2 hours at RT with gentle mixing: PBS pH 7.4 containing 0.2% BSA + 3% PEG (8 kDa mol. Wt.) binding buffer, a suspension of high-capacity streptavidin coated magnetic beads (~ 1 mg) with immobilized biotinylated affinity purified chicken IgY (~ 0.025 mg), and cynomolgus monkey serum or diluted standards (e.g. 10 – 100 µL) containing the candidate mAb therapeutic.

After incubation, the beads are washed 3 times with a high salt buffer (20 mM Tris + 750 mM NaCl + 0.1% Tween-20 pH 7.4) to remove unwanted serum proteins. The final bead wash is performed using 20 mM Tris + 150 mM NaCl + 0.02% NaN_3 pH 7.4. The washed beads are stored refrigerated in this same buffer prior to LC-MS/MS evaluation.

LC-MS/MS Assay

Following capture of Adalimumab (model mAb), on-bead digestion was performed using Trypsin/LysC (0.1 mg/mL, 50 mM ammonium bicarbonate) to digest the captured human IgG1 into signature peptides to be analyzed by LC-MS/MS. Previously adopted workflows[1-3] for universal peptide approach were implemented with modifications. Two signature peptides (VVSVLTVLHQSWLNGK and DSTYSLSSTLTLSK) are followed with MRM transitions 603.7/805.4 and 752.0 to 836.0, respectively.
A stable isotope labeled version of Adalimumab was used as the internal standard (IS), which produced heavy labeled signature peptides that aligned with the peptides used for quantitation. MRM transitions of 606.3/809.4 and 756.0/844 were used to monitor the IS. Quantitation was performed using peak area ratios. A 6500+ Sciex mass spectrometer was used coupled with a 30 series UPLC and autosampler from Shimadzu. Chromatographic separation of the peptides was performed using a Phenomenex Kinetex Biphenyl (2.1x30 mm, 2.6 µm). The column was eluted using a gradient mobile phase mixture of acetonitrile/water and 0.1% formic acid at a flow rate of 0.5 mL/min. Signature peptides were quantitated down to a lower limit of quantitation of 0.1 µg/mL.

RESULTS

Following affinity purification, the purity and reactivity of the chicken IgY reagent were assessed (**Figure 1**). The titer assessment against both human IgG and cynomolgus IgG indicated that the reagent was highly specific for human IgG (10.4 ng/mL EC_{50}) vs. cynomolgus IgG (3490 ng/mL EC_{50}, ~ 0.3% residual cross-reactivity). Purity analysis by SDS-PAGE showed a single band at approximately 180 kDa which is consistent with chicken IgY molecular weight and demonstrating high purity. Signature peptides monitored by MRM were linear, reproducible, and appropriate for accurate quantitation of the intact mAb down to 0.1 µg/mL. Six replicates of quality control samples at 0.3, 3, and 30 µg/mL were analyzed in parallel with a calibration curve ranging from 0.1 – 50 µg/mL. The results are presented in **Table 1** below.

Table 1. P&A of Adalimumab Quality Control Samples in NHP serum

	LQC 0.300 µg/mL	% bias	MQC 3.00 µg/mL	% bias	HQC 30.0 µg/mL	% bias
	0.257	-14.3	2.73	-9.0	36.1	20.3
	0.250	-16.7	3.13	4.3	32.7	9.0
	0.243	-19.0	3.18	6.0	34.6	15.3
	0.286	-4.7	3.50	16.7	36.4	21.3
	0.248	-17.3	NA	NA	36.6	22.0
	0.282	-6.0	2.99	-0.3	31.6	5.3
Intra-run Mean	0.261		3.11		34.7	
Intra-run SD	0.0184		0.281		2.10	
Intra-run % CV	7.1		9.1		6.1	
Intra-run % Accuracy	87.0		103.5		115.6	
n	6		5		6	

NA: Peak not observed for analyte or internal standard.

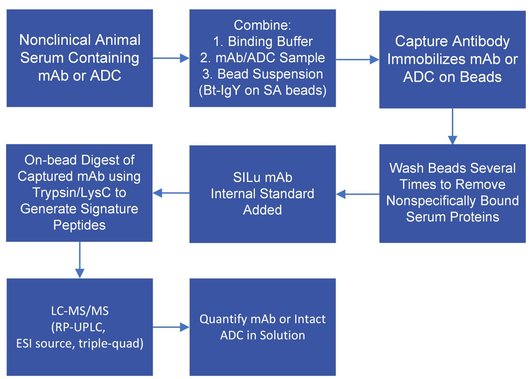

Chart 1. Graphical representation of Method workflow including sample preparation and analytical methods.

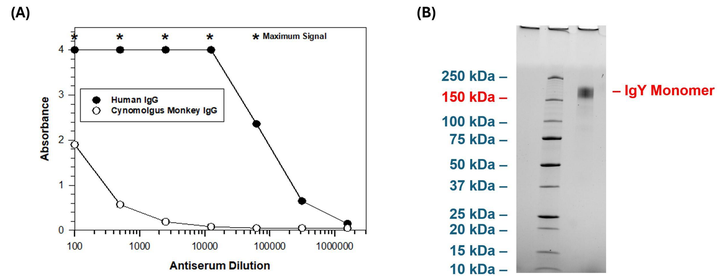

Figure 1. (A) ELISA cross-reactivity assessment to human and cynomolgus IgG and (B) Purity of the reagent chicken IgY by SDS-PAGE.

Figure 2. LC-MS/MS chromatogram of VVSVLTVLHQSWLNGK (left panel) at the LLOQ of 0.1 (A) and the ULOQ of 50 µg/mL (B) and corresponding SIL-VVSVLTVLHQSWLNGK used as internal standard (right panel) at 5 µg/mL.

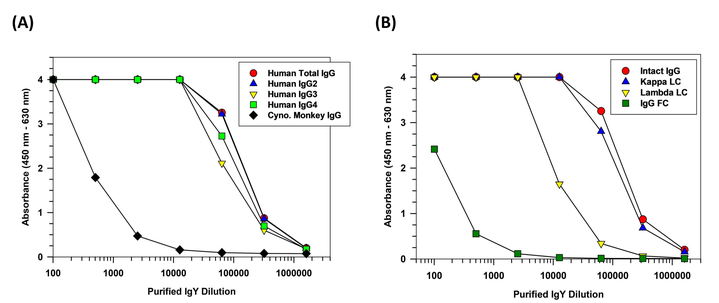

Figure 3. (A) ELISA titer assessment of purified IgY versus human total IgG, human isotype controls, and cynomolgus monkey total IgG and (B) ELISA titer assessment of purified IgY versus total human IgG and human IgG fragments (FC, λ-LC and κ-LC).

RESULTS (CONTINUED)

Having demonstrated initial success of the purified IgY antibody for the analysis of an IgG1 mAb, we performed additional characterization of the reagent to estimate its applicability to other biomolecules (**Figure 3**). The reactivity pattern resembled a reagent we previously prepared, it and revealed that the dominant epitope for this polyclonal IgY is located on the constant region of the light chain.[4] Taken together with promising LC-MS/MS results for an IgG1 model, we believe this approach will find use in preclinical PK/TK studies and is likely generalizable to biotherapeutics containing human light chain fragments e.g. mAb, fusion proteins, or ADCS.

CONCLUSIONS

The new hybrid LC-MS/MS assay will permit rapid and sensitive quantitation of human mAbs in serum from sera of nonclinical species, including cynomolgus monkey. Moreover, the broad specificity of the chicken antiserum for IgG subclasses will allow this assay to function in a plug-and-play manner with minimal optimization for efficient support of nonclinical PK of different monoclonal-based therapeutics and ADCs across nonclinical species. This novel workflow will drastically decrease the lead time for bioanalysis since the LC-MS/MS workflow can be applied to all human IgG based therapeutics.

FUTURE WORK

- Expand scope to include additional mAbs and biotherapeutics containing human light chains..
- Investigate additional signature peptides by LC-MS/MS corresponding to conserved sequences of IgG2, IgG3.

REFERENCES

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