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Pushing the Envelope: Detection of Generic Human IgG in Cynomolgus Monkey Serum via Quantitative ELISA

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NOVEL ASPECTS

The method presented here can support drug development programs by quantitatively detecting generic human IgG in NHP serum without the need for a full method development targeting specific monoclonal IgGs. Alternatively, the method can also be used to speed development by substituting a specific IgG1 test article in place of generic IgG1 isotype control in a fraction of the time and cost. These developments may help to enhance the drug discovery and approval process for future human antibody-derived drug products such as monoclonal antibodies and anti-drug conjugates.

INTRODUCTION

As of 2024, there has been FDA approval for over 100 monoclonal antibody drugs and 11 antibody-drug conjugate products. These products were often human-derived drug products (test articles) but modified to avoid ADA responses in human testing. Even so, within NHPs, as a common large animal model used during preclinical stages of development, these human-derived molecules can potentially cause an immune response. One way in which this ADA generation is detected is via a truncated PK dose-response curve after multiple dose administrations. This can prove difficult given the time and cost of a full test article-specific PK method development at these early stages in the drug development pipeline. To address this issue, we developed a generic IgG1 ELISA targeting the FC region common to all human antibodies to determine if NHPs have detectable levels of human IgG as a direct result of test article administration independent of client-specific test article. This serves to generate preliminary PK information and any initial indication of potential ADA response earlier than a full GLP toxicology study.

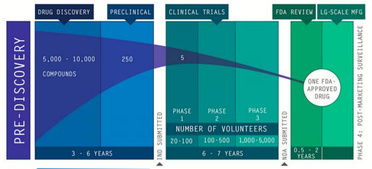

Figure 1. Drug Discovery and Development Pipeline
Image Source: Pharma, 2007. Link: https://medium.com/bioai/drug-discovery-and-development-31758bf6cc72

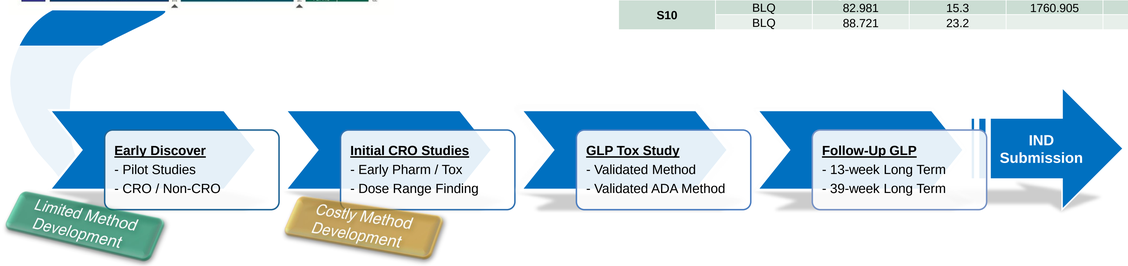

Figure 2. Expanded Drug Discovery and Development Pipeline

METHOD

A colorimetric ELISA-based assay was developed to detect generic human IgG1. In brief, the assay involves overnight incubation of coating reagent, followed by blocking in 3% BSA in 1x PBS. After washing the plate, standards, QCs, and samples were diluted to the minimum required dilution of 20-fold using low-cross buffer and incubated for 60 minutes at room temperature while on a plate vortexer/shaker at 700 rpm. Incubation using an HRP conjugated detection antibody was performed for 60 minutes also while on a plate vortexer/shaker at 700 rpm. Colorimetric output was determined using TMB reagent incubation for 12-14 minutes before the addition of acidic stop solution. Results were determined using a Synergy H1 plate reader at 450 nm.

Evaluations during qualification included precision and accuracy, hook effect and dilution linearity, specificity and selectivity, and both short- and long-term stability. Stability of coated and blocked plates was also established to increase the ease of use and decrease the day-to-day workload during sample analysis. Reagents were carefully screened and selected to minimize non-specific binding and detection of endogenous immunoglobulins as elevated background noise. We observed that lot-to-lot variability of NHP serum was a critical evaluation due to confirmed detectable levels of an unknown molecule in a single naïve unspiked NHP sample. Subsequent testing supported assay precision and accuracy due to background subtraction from spiked samples falling within acceptable recovery ranges when spiked at both HQC and LLQC levels. And finally, while the assay was developed using a generic human IgG1 isotype molecule, there was detectable cross-reactivity for the other three human subtypes: IgG2, IgG3, and IgG4 to varying degrees.

Table 1. Specificity and Selectivity Evaluations: 6 Individual Lots of Serum (3 Males and 3 Females)

Serum Specificity and Selectivity					
Matrix	Blank Result	LLOQ		HQC	
		Mean	%Bias	Mean	%Bias
S7	BLQ	72.188	-0.4	2307.819	13.7
S8	BLQ	85.433	17.8	2372.267	16.9
S9	BLQ	72.719	0.3	2302.954	13.4
S10	*163.605*	246.586	*240.1*	2001.024	-1.4
	147.846	236.567	*240.1*		
S11	BLQ	72.093	-0.6	2154.659	6.1
S12	BLQ	77.892	7.4	2481.669	22.2
After Background Subtraction of Blank Results					
S10	BLQ	82.981	15.3	1760.905	-13.3
	BLQ	88.721	23.2		

RESULTS

The qualified method consists of a standard curve with a single low anchor point and seven other non-zero calibrators across a dynamic range of 72.5 pg/mL to 2900 pg/mL. Precision and accuracy was established across eight separate runs over multiple days for generic IgG1 isotype at 22% CV for L/M/H QC levels, and 23.9% CV for LL/UL QC. Acceptance criteria for passing runs were set to 25% at L/M/H QC and 30% at the LL/UL. Dilution linearity was established at up to 480-fold in NHP serum at the minimum required dilution of 20-fold. No hook effect was observed at a concentration of IgG1 isotype at 145 µg/mL. Background subtracted specificity and selectivity met 80% threshold criteria in individual serum lots at both HQC and LLQC levels. Sample stability was established using HQC and LQC samples at -80 Celsius for up to 28 days, 24 hours under benchtop conditions, and up to 3 freeze/thaw cycles. Coated and blocked plate stability was established at up to 5 days when stored desiccated at 4˚ C.

Table 2. Back Calculated STD Performance Over 16 Runs Using Generic IgG1

Run ID	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
	29 (ng/mL)	72.5 (ng/mL)	145 (ng/mL)	435 (ng/mL)	725 (ng/mL)	1276 (ng/mL)	2088 (ng/mL)	2900 (ng/mL)
N	15	14	16	16	16	16	16	14
Ave	28.755	71.677	150.713	464.153	701.665	1271.583	2114.657	2895.283
%CV	26.5	6.5	6.1	6.5	6.4	2.3	4.5	3.2
%Bias	-0.8	-1.1	3.9	6.7	-3.2	-0.3	1.3	-0.2

Table 3. Inter-Assay Precision and Accuracy Results From 8 Runs Using Generic IgG1

Run	LLQC	LQC	MQC	HQC	ULQC
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
Exp	72.5	217.5	870	2030	2900
N	8	8	8	8	8
Mean	57.281	197.139	804.247	1842.582	2483.834
%CV	24.8	20.6	19.7	16.7	18.6
%Bias	-21.0	-9.4	-7.6	-9.2	-14.4
%TE	45.8	30.0	27.2	25.9	33.0

Table 4. Intra-assay Precision and Accuracy From N=6 Replicates Using Generic IgG1

Run	LLQC	LQC	MQC	HQC	ULQC
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
Exp	72.5	217.5	870	2030	2900
Mean	51.297	176.473	728.742	1571.228	2190.944
%CV	6.4	1.7	1.3	3.5	2.3
%Bias	-29.2	-18.9	-16.2	-22.6	-24.5
%TE	35.7	20.5	17.5	26.1	26.8

Table 5. Benchtop and Freeze/Thaw Stability of Generic IgG1

	24hr Benchtop Stability			
	HQC (2030)		LQC (217.5)	
	Mean	%CV	Mean	%CV
R1	1875.734	4.3	194.927	1
R2	1898.094	2.3	197.088	1.9
R3	1842.805	1.4	193.171	1.8
Mean	1872		195	
%CV	1.5		1.0	
%Bias	-7.8		-10.3	

	3 Freeze/Thaw Stability			
	HQC (2030)		LQC (217.5)	
	Mean	%CV	Mean	%CV
R1	1811.523	4.3	229.112	1
R2	1738.666	2.3	212.364	1.9
R3	1808.725	1.4	193.607	1.8
Mean	1786		212	
%CV	2.3		8.4	
%Bias	-12.0		-2.7	

Proof of Concept: Substituting Specific IgG1 mAb

Table 6. Single Precision and Accuracy Run Substituting Client Specific IgG1 mAb [A]

Run	LLQC	LQC	MQC	HQC	ULQC
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
Exp	72.5	217.5	870	2030	2900
Mean	76.611	229.361	859.513	1982.481	2810.833
%CV	5.0	1.0	1.8	1.3	2.4
%Bias	5.7	5.5	-1.2	-2.3	-3.1
%TE	10.6	6.5	3.1	3.7	5.5

Table 7. Single Precision and Accuracy Run Substituting Client Specific IgG1 mAb [B]

Run	LLQC	LQC	MQC	HQC	ULQC
	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)	(ng/mL)
Exp	72.5	217.5	870	2030	2900
Mean	59.221	212.104	886.136	1828.046	2354.430
%CV	12.7	11.6	5.9	14.5	14.3
%Bias	-18.3	-2.5	1.9	-9.9	-18.8
%TE	31.0	14.1	7.8	24.4	33.1

Analysis of two different client supplied monoclonal antibody targets in single Precision and Accuracy runs (5 QC levels, N=3 each) was performed via direct method application as with generic IgG1 reagent. In each case, generic IgG1 reagent was substituted with client specific molecules [A and B] for Standard and QC preparation. Results indicate successful direct bridging with specific molecules without need for optimization of any other method reagents, ranges, or conditions.

Table 8. Qualification Summary

Qualification Parameter Tested	Summary of Results
Calibration curve	Met acceptance criteria within the range of: 72.5 ng/mL (LLOQ) to 2,900 ng/mL (ULOQ) Low Anchor point of 29 ng/mL.
Intra-Assay Accuracy and Precision	Met acceptance criteria: 25% (30% at LLOQ/ULOQ)
Inter-Assay Accuracy and Precision	Met acceptance criteria: 25% (30% at LLOQ/ULOQ)
Sensitivity	72.5 ng/mL (LLOQ)
Specificity	Met acceptance criteria: 83% (5/6) of Lots BLQ
Selectivity	Meets criteria: at 100% (6/6 Lots with background correction) at LLQC, 100% (6/6) lots at HQC
Hook Effect	Met acceptance criteria: Not observed
Dilutional Linearity	Met acceptance criteria up to 480-fold dilution
Short term stability (Benchtop)	Met acceptance criteria: ±20% Bias up to 24 hours at RT
Freeze/Thaw	Met acceptance criteria: ±20% Bias up to 3 freeze/thaw cycles
Long-term Stability	Met acceptance criteria: ±20% Bias up to 28 days at -80° C nominal

CONCLUSION

The qualification of this novel IgG1 colorimetric ELISA serves to increase the robustness of early drug development results using generic IgG1 isotype targeting for capture and detection. Application of this qualified method will aid in the generation of early development PK data of many IgG1-based test articles earlier in development pipelines in support of current and future safety and efficacy testing. This work will also accelerate and decrease risk in early drug development timelines for IgG1-based molecules by reducing the time and cost needed to generate TA-specific antibodies and develop specific PK/TK methods prior to validation.