Altasciences, 575 Armand-Frappier, Laval, Québec, H7V 4B3, Canada
Altasciences.com | contact@altasciences.com

Design and Validation of a Bioanalytical Method to Support a Clinical Pharmacokinetic Study Involving the Use of Multiple Lots of the Biological Therapeutic Drug

Houtan Moshiri, Vasken Parsekhian, and Danielle Salha
Altasciences, Laval, Canada

Click here to listen to the recorded poster presentation

ABSTRACT

Therapeutic recombinant proteins are expressed exogenously and purified from a wide range of organisms. Reproducible production of biological therapeutics poses unique challenges for bioanalysis. For example, the use of different expression systems to produce recombinant protein therapeutics may result in subtle variations in post-translational modifications, protein conformation, and immunogenicity. Glycoproteins are one of the most common protein therapeutics, typically manufactured through cell culture systems, which undergo post-translational modifications known as glycosylation. The level of glycosylation may vary among manufactured lots and may have an impact on tertiary conformation and antigenicity. In addition, heterogeneity in glycosylation patterns is more evident when using non-human systems for manufacturing glycoprotein drug products. Herein, we describe a case study and present mitigation strategies to design an effective bioanalytical method where multiple lots of therapeutic recombinant glycoprotein were used. The presence of the endogenous form of this therapeutic presents an additional challenge.

METHOD

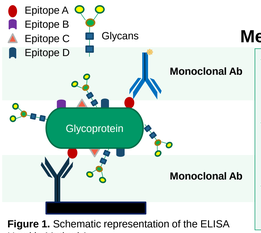

Figure 1. Schematic representation of the ELISA Used in Method 1

Method 1

- Sandwich enzyme-linked immunoassay (ELISA) technique was employed for quantifying the glycoprotein therapeutic in human serum.
- A 200-fold minimum required dilution (MRD) was applied to account for the high endogenous levels present in human serum.
- Standards and QCs prepared in human serum over a concentration range of 60.0 to 2000 µg/mL.

Method 2

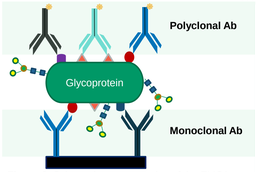

Figure 2. Schematic representation of the ELISA used in Method 2

- In order to optimize the assay performance and reproducibility among three lots, two monoclonal antibodies were evaluated as capture and one polyclonal antibody as detection.

Method 3

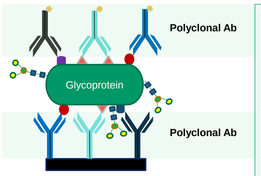

Figure 3. Schematic representation of the ELISA used in Method 3

- Method employed polyclonal antibodies for capture and detection.
- Standards prepared in a surrogate matrix over the concentration range of 50.0 to 1500 ng/mL.
- The quality control (QC) samples prepared in human serum.
- An additional MRD increase to of 10,000-fold in the surrogate matrix was established for the QC and study samples to reduce assay background.

RESULTS

Method 1

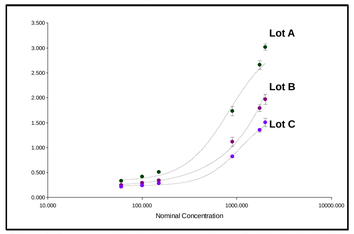

Figure 4. Comparison of calibration curve between 3 different lots of reference material

Table 1. % Differences of the instrumental signals between all three lots with Method 1

Sample ID	Nominal Conc (µg/mL)	Lot A Signal	Lot B Signal	Lot C Signal	% Difference Lot A vs. Lot B	% Difference Lot B vs. Lot C	% Difference Lot A vs. Lot C
STD1	60.0	0.330	0.247	0.213	29	15	43
STD2	100	0.421	0.295	0.241	35	20	55
STD3	150	0.511	0.342	0.553	40	47	8
STD4	900	1.730	1.120	1.082	43	3	46
STD5	1750	2.658	1.791	1.350	39	28	65
STD6	2000	3.019	1.968	1.503	42	27	67
Overall %					**38**	**23**	**47**

Table 2. The signal-to-noise ratio (SNR) is calculated with the LLOQ over the blank for each lot

	Signal to Noise Ratio (SNR) LLOQ/BL
Lot A	2.1
Lot B	1.6
Lot C	1.3

- It was suspected that monoclonal antibodies bind with different affinity across lots of glycoprotein. Thus different combinations of monoclonal and polyclonal antibodies were tested.

Method 2

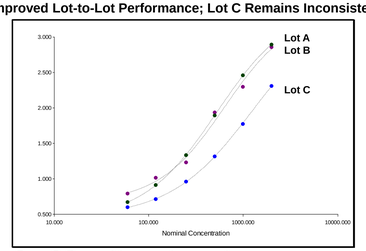

Figure 5. Representative calibration curve: two monoclonal antibodies (mAbs) used as a capture and a polyclonal antibody (pAb) as a detection

Table 3. % Differences of the instrumental signals between all three lots with Method 2

Sample ID	Nominal Conc (µg/mL)	Lot A Signal	Lot B Signal	Lot C Signal	% Difference Lot A vs. Lot B	% Difference Lot B vs. Lot C	% Difference Lot A vs. Lot C
STD1	60.0	0.669	0.791	0.597	17	28	11
STD2	100	0.915	1.016	0.715	10	35	25
STD3	150	1.333	1.229	0.959	8	25	33
STD4	900	1.894	1.935	1.319	2	38	36
STD5	1750	2.463	2.301	1.775	7	26	32
STD6	2000	2.893	2.857	2.311	1	21	22
Overall %					**8**	**29**	**27**

- The updated method shows comparable instrument response between Lot A and Lot B but not Lot C.
- As switching the detection from monoclonal antibody to polyclonal antibody improved the inter-lot performance of the assay between Lot A and Lot B, we hypothesized that using a polyclonal antibody as both capture and detection should increase reproducibility between all these lots.

Method 3

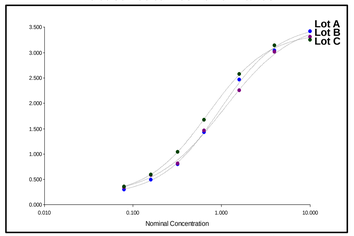

Figure 6. Representative calibration curve: polyclonal antibodies used as detection and capture

- The final method shows comparable instrument response between all three lots.

VALIDATION EVALUATIONS

- The curve range was from 50.0 to 1500 ng/mL.
- Lot B of analyte used in all the validation evaluations.
- Lot B and Lot C were further cross-verified since Lot B and Lot C were used interchangeably for dosing in clinical studies.

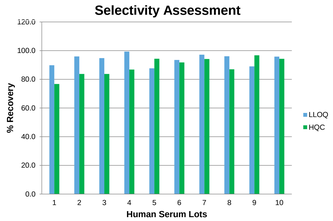

Figure 7. Selectivity assessment in 10 human serum lots

Table 4. Validated Stability Evaluations. Evaluations of freeze-thaw, short-term, and long-term stabilities

Stability Evaluation		%CV		%Bias	
		LQC	HQC	LQC	HQC
Freeze-Thaw	3 cycles (-80˚C)	2.4	7.1	-1.1	-5.7
Short-Term	7 hours (bench top)	8.7	4.9	-15.7	-14.8
Long-Term (-80˚C)	50 days	4.5	2.0	1.9	0.0

Table 5. Dilution linearity evaluation. Linearity evaluation performed successfully between 3- to 10-fold diluted samples

DILUTIONAL LINEARITY	Dilution factor	%CV	%Bias
DIL1	3	11.3	3.5
DIL2	5	6.6	15.2
DIL3	10	7.1	5.5

Table 6. Precision and Accuracy (Lot B), Result below is from 10 independent Runs

	LLOQ QC	LQC	MQC	HQC	ULOQ QC
Inter-run %CV	23.7	8.8	15.5	12.8	15.2
Inter-run %Bias	9	-3	-10.5	-8.2	-11.3
Inter-run %Total Error	32.7	11.8	25.9	21	26.5
n	58	60	60	60	60

Table 7. Lot B and Lot C cross-verification. 3 runs with 5 levels of QC (LLOQ QC, Low QC, Medium QC, and High QC, and ULOQ QC) prepared with Lot C and back-calculated against standard curved prepared from Lot B

	LLOQ QC	LQC	MQC	HQC	ULOQ QC
Inter-run %CV	7.5	5.6	4.6	4.3	5.1
Inter-run %Bias	-4	-6.3	-11	-11.5	-13.2
Inter-run %Total Error	11.5	11.9	15.6	15.8	18.3
n	15	15	15	15	15

CONCLUSIONS

- Biological therapeutics that meet specification criteria for lot release may still be prone to slight variations among multiple manufactured lots. These variations need to be considered when developing bioanalytical assays to support a pharmacokinetics (PK) clinical study.
- Subtle lot-to-lot variations (such as the degree of glycosylation) may impact bioanalytical assay performance.
- For example, as shown here, polyclonal antibodies provide superior recognition of heterogeneous reference material at higher sensitivity, as multiple epitopes are involved in antibody interaction.
- Polyclonal sera also ensures consistent bioanalytical method performance, regardless of the multiple lots used in the PK clinical study.
- Therefore, it is imperative to develop a bioanalytical method that consistently measures the therapeutic drug product in a precise and accurate manner when several drug product lots are used in the clinical study.