Establishment of an Immunoassay to Quantify Total TL1a Target in Monkey Serum Samples

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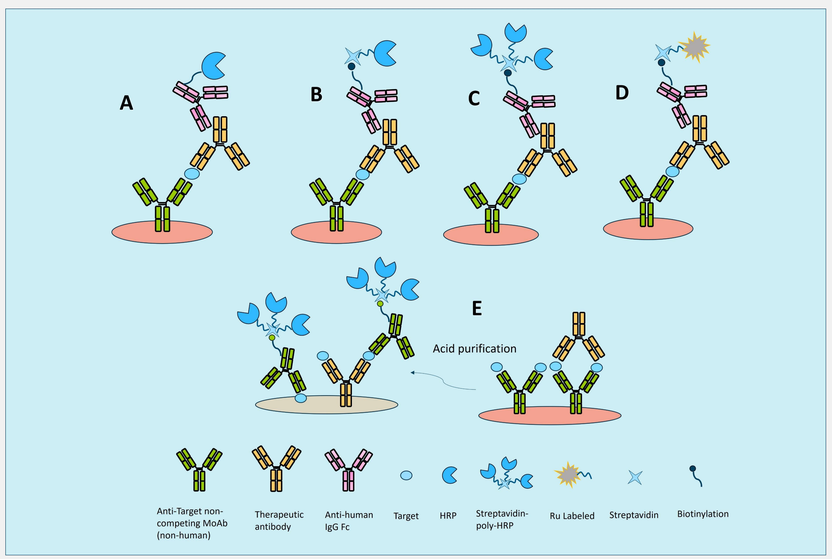
PURPOSE

Quantification of target is frequently required for bioanalysis during therapeutic antibody development. However, it is challenging to quantify a target accurately when target levels are lower and/or when levels of therapeutic antibody are relatively higher. To develop an assay for total (free plus bound) target TL1a measurement with sufficient sensitivity (<40 pg/mL) and drug tolerance (up to 50 µg/mL anti- TL1a antibody), we evaluated several assay formats for their sensitivity and tolerance to drug interference for quantification of total TL1a in the presence of an excess amount of anti-TL1a antibody in monkey sera.

METHOD(S)

Four Sandwich assay formats employ a non-competitive mouse anti-human TL1a monoclonal antibody as the capture reagent, and an anti-human IgG-HRP (Format A of Figure 1) or biotinylated anti-human IgG (Formats B, C, D of Figure 1) as the detection reagent after a saturation step by the excessive drug (a human anti-TL1a antibody). Additionally, a direct immunoassay is built with sample pre-treatment with purification and acid dissociation steps, followed by detection with a biotinylated mouse anti-human TL1a non-competitive monoclonal antibody (Format E of Figure 1). Two detection technologies are used in these assays - Except electrochemiluminescence detection system for the 4th assay format (Format D of Figure 1), all other assay formats use optical density detection technology. Assay performance was evaluated and compared among these assay formats in terms of assay sensitivity and drug tolerance. Based on assay performance, one assay format is selected for validation.

Figure 1. Assay design and formats



RESULT(S)

Compared to the direct assay, all four Sandwich assays exhibited greater sensitivity (36-105 pg/mL versus 1216 pg/mL). Specifically, the sensitivity ranks in the order: format C ≈ format D > format A ≈ format B > format E (Table 1). Among these assays, the ability to tolerate drug interference ranks in the order: format C ≈ format D ≈ format B > format A > format E (Table 2). The final selected assay (format C) has the capability to accurately quantify TL1a as low as 36 pg/mL in the presence of the anti-TL1a antibody at a level of up to 50 µg/mL, which meet the pre-set acceptance criteria.

A total of 3 accuracy and precision runs were performed by 2 analysts over 3 different days. The calculated intra- and inter-run precision (%CV), accuracy (%Bias), and total error are listed in Table 3. Accuracy, precision and total error are all within the respective acceptance ranges.

Table 1. Determination of assay sensitivity

Target Conc. (pg/mL)	Format A		Format B		Format C		Format D		Format E	
	Signal Value	SNR	Signal Value	SNR	Signal Value	SNR	Signal Value	SNR	Signal Value	SNR
640000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	2.459	131.5
160000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.321	70.6
40000	3.603	185.7	3.623	230.8	3.565	46.5	175494	2017.2	0.400	21.4
10000	2.996	154.4	2.180	138.8	3.442	44.9	41988	482.6	0.116	6.2
2560	0.862	44.4	0.622	39.6	2.724	35.5	8384	96.4	0.044	2.3
640	0.170	8.8	0.123	7.8	1.206	15.7	1710	19.7	0.026	1.4
160	0.051	2.6	0.041	2.6	0.409	5.3	450	5.2	0.018	0.9
40	0.027	1.4	0.021	1.3	0.164	2.1	165	1.9	N/A	N/A
10	0.020	1.0	0.019	1.2	0.105	1.4	112	1.3	N/A	N/A
Blank	0.019	1.0	0.016	1.0	0.077	1.0	87	1.0	0.019	1.0
Estimated sensitivity (pg/mL)	100		105		36		45		1216	

Note: The Excel 'FORECAST' function was utilized for sensitivity calculation. A signal-to-noise ratio (SNR) of 2 was used as the sensitivity cut-off value.

Table 2. Drug tolerance assessment of total target assay

Drug tolerance Sample	Drug conc. (µg/mL)	Format A		Format B		Format C		Format D		Format E	
		Signal value	SNR	Signal value	SNR	Signal value	SNR	Signal value	SNR	Signal value	SNR
DT-Target (5.12 ng/mL)	50	1.329	0.76	1.039	0.94	3.129	1.00	16202	0.85	0.050	0.72
	5	1.676	0.95	1.098	1.00	3.183	1.02	18049	0.95	0.051	0.74
	0	1.755	N/A	1.101	N/A	3.12	N/A	19097	N/A	0.069	N/A
Estimated drug tolerance (µg/mL)		40.3		≥50		≥50		≥50		3.8	

Note: a signal to noise ratio (SNR) of 0.8 was used as the drug tolerance cut-off value, and the 'FORECAST' function was utilized for the drug tolerance calculation

Table 3. Precision and accuracy results for format C assay validation

Item	ULOQ 5120 pg/mL	HQC 3840 pg/mL	MQC 360 pg/mL	LQC 40 pg/mL	LLOQ 20 pg/mL
Intra-run %CV	1.5~1.6	2.0~2.4	1.0~2.2	2.6~7.1	6.7~11.3
Intra-run %Bias	-11.0~-9.2	-0.4~2.3	-13.7~4.0	-16.0~-4.0	-10.2~-7.3
Inter-run %CV	1.8	2.4	10.3	8.9	8.4
Inter-run %Bias	-10.1	1.0	-4.9	-10.0	-8.8
Inter-run %Total Error	11.9	3.4	15.2	18.9	17.2

Note: Intra- and inter-run accuracy (%Bias) met the acceptance criteria: ±20% (±25% for ULOQ and LLOQ). Intra- and inter-run precision (%CV) met the acceptance criteria: %CV ≤20% (≤25% for ULOQ and LLOQ), and total error also met the acceptance criteria: ≤30% (≤40% for ULOQ and LLOQ).

CONCLUSION(S)

Among the 5 assays tested for total TL1a target analysis in this study, formats C and D have been found to display greater assay sensitivity as well as the drug tolerance when compared to other assays. The selected assay format C demonstrated sufficient assay sensitivity, precision, accuracy, and drug tolerance for the intended application.