A Novel ADA Screening Assay for Immunogenicity Testing of Oligonucleotide Drug Using PALSAR ® Technology

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Introduction

Anti-drug antibody (ADA) screening testing by bridging assay for oligonucleotide drug development has not been reported. Approved oligonucleotide drugs mainly used direct ELISA. This results in the detection of IgG-type ADA with relatively high sensitivity. However, detection of other isotypes, such as IgM and IgA, is limited. Furthermore, although the sensitivity of the ADA screening assay recommended by FDA for protein therapeutics is at least 100 ng/mL, some of the assay methods have not attained this level.

Probe alteration link self-assembly reactions (PALSAR) technology is a method of self-assembly mediated by alternately hybridizing a pair of DNA probes with complementary sequences in 3 regions, called honeycomb probes (HCPs). In a previous study, the self-assemblies of 2 HCPs with poly-A or poly-T sequences at the 3′ end and biotinylated 5′ end were applied to amplify the fluorescence intensity (FI) in a Luminex-based assay. Notably, the phycoerythrin-labeled avidin bound to the biotin of self-aggregates in large numbers, thereby amplifying FI.

In this study we aimed to assess the feasibility of using PALSAR technology as a signal amplifier by developing high sensitivity antibody bridging assay model. We describe the application of PALSAR technology for the amplification of electrochemiluminescence (ECL) signals in Meso Scale Discovery (MSD) ECL-based immunoassays.

Method

We applied PALSAR technology to amplify the signal of Meso Scale ECL-based ADA bridging assay, and selected the anti-sense oligonucleotide drug GTI-2040 as a capture and detection Probe (which work as antigen). Prime end of GTI-2040 was modified by digoxigenin (Dig). Anti-Dig mouse monoclonal antibody was used as ADA surrogate antibody (ADA-sa). ADA-sa was spiked into normal human serum, and we measured the samples without acid dissociation step. We optimized the concentration of each probe and compared the bridging assay model with and without signal amplification. Finally, we determined cut-point and analytical sensitivity, and evaluated accuracy, precision, and drug tolerance.

MSD ECL-based antibody bridging assay
- Plate preparation
- Sample preparation
- Sample reaction
- Antibody bridging reaction
- Signal measurement

PALSAR technology : Assay Format

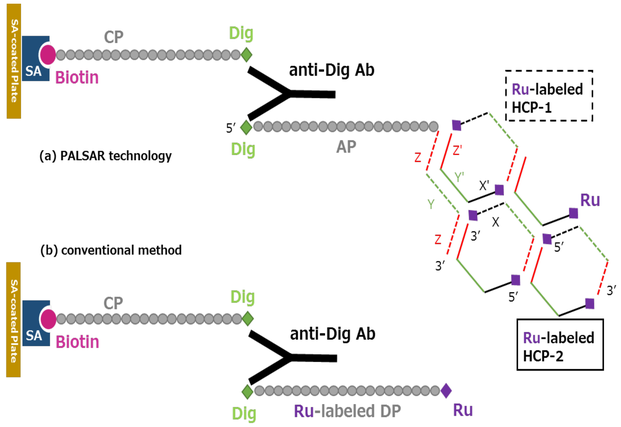

Figure 1. Principle of antibody bridging assay with (a; PALSAR Technology) and without (b; conventional method) signal amplification. SA: Streptavidin; CP: Capture Probe; DP: Detection Probe; AP: Assist Probe; HCP: Honeycomb Probe; Dig: Digoxigenin; Ru: MSD GOLD SULFO-TAG NHS-Ester

Results

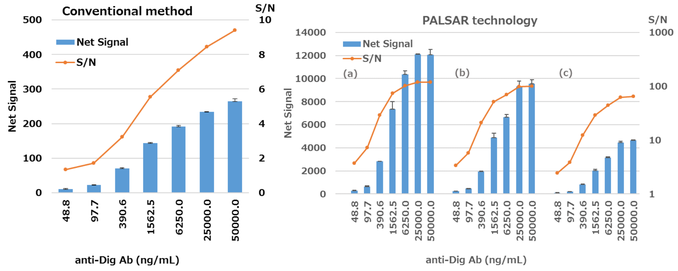

Figure 2. Effect of signal amplification. Antibody bridging assay with PALSAR signal amplification (right, concentration of AP; 0.1 pmol/μL (a), 0.2 pmol/μL (b), 0.4 pmol/μL (c)), and without signal amplification (left, concentration of Ru labeled DP; 0.2 pmol/μL).

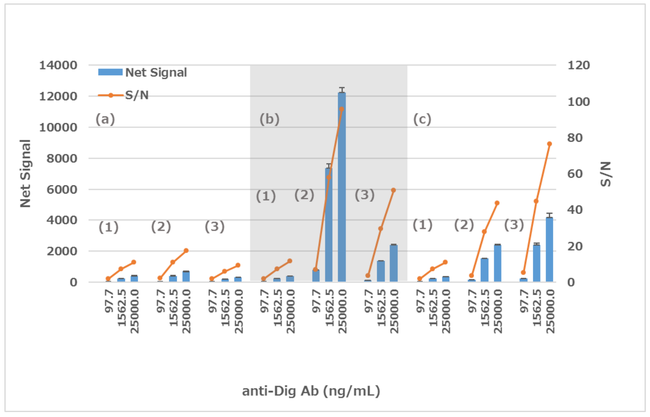

Figure 3. Optimization of Ru-labeled HCP concentration with PALSAR signal amplification.
Concentration of HCP1: 0.2 fmol/μL (a), 1 fmol/μL (b), 5 fmol/μL (c).
Concentration of HCP2; 0.2 fmol/μL (1), 1 fmol/μL (2), 5 fmol/μL (3).

Results

Table 1. Determination of cut-point.

Sample No.	Mean signal		
	Day 1	Day 2	Day 3
Negative control	124.0	120.0	121.5
Individual 1	116.0	88.0	94.5
Individual 2	127.5	120.0	119.5
Individual 3	223.5	204.0	205.0
Individual 4	108.5	94.0	107.5
Individual 5	148.0	109.5	141.5
Individual 6	144.0	119.0	136.0
Individual 7	168.5	160.0	174.5
Individual 8	140.5	102.0	120.0
Individual 9	165.0	130.5	145.5
Individual 10	94.5	100.5	106.5
Mean of individual samples	143.6	122.8	135.1
SD of individual samples	36.7	35.3	33.8
Cut-Point of each run (CPr)†	204.0	180.9	190.7
Fixed CP		191.9	

We found the mean and variability between runs. One-way ANOVA gave a P value of 0.43, and a Bartlett test a P value of 0.97, neither of which indicated a significant difference. Using the mean of CPr for each measurement, we calculated the fixed cut-point to be 191.9.

Reference) Ishii-Watabe A, Shibata H, Nishimura K, et al. Immunogenicity of therapeutic protein products: current considerations for anti-drug antibody assay in Japan. Bioanalysis 10(2), 95-105, (2018).

†CPr was calculated by the formula, Mean + 1.645 × SD.

Table 2. Analytical sensitivity and accuracy.

Positive standard (ng/mL)	Day 1				Day 2				Day 3			
	Mean signal		Back calculated conc. (ng/mL)	Accuracy (%)	Mean signal		Back calculated conc. (ng/mL)	Accuracy (%)	Mean signal		Back calculated conc. (ng/mL)	Accuracy (%)
	gross	net			gross	net			gross	net		
48.8	565.0	418.0	52.2	112.8	483.0	351.5	48.0	98.3	525.0	401.0	52.2	107.0
97.7	1056.0	909.0	99.0	97.6	923.0	791.5	95.0	97.3	1037.0	913.0	99.0	101.5
195.3	2005.0	1858.0	192.0	92.6	1933.5	1802.0	201.6	103.3	1976.0	1852.0	192.0	98.4
390.6	3956.0	3809.0	367.5	103.0	3480.5	3349.0	384.3	98.4	3459.0	3335.0	367.5	94.1
781.3	6200.0	6053.0	830.0	100.8	5986.0	5854.5	800.0	102.4	6078.5	5954.5	830.0	106.2
1562.5	8692.0	8545.0	1586.6	101.7	8222.0	8090.5	1477.0	94.5	8348.5	8224.5	1586.6	101.5
3125.0	10538.5	10391.5	2834.8	93.7	10575.0	10443.5	3455.7	110.6	10096.0	9972.0	2834.8	90.7
6250.0	12209.0	12062.0	6833.4	106.0	11437.0	11305.5	5802.8	92.8	11863.5	11739.5	6833.4	109.3
Negative control	147.0	N.A.	N.A.	N.A.	131.5	N.A.	N.A.	N.A.	124.0	N.A.	N.A.	N.A.

Gross signal of the 48.8 ng/mL was above the cut-point on all runs. Furthermore, 4-parameter logistic regression analysis of the net signal showed the accuracy of the back-calculated concentration ranged from 90.7 to 112.8 % across 3 separate days, indicating the analytical sensitivity of this assay was 48.8 ng/mL. No hook effect was observed.

Table 3. Intra-assay precision

Positive standard (ng/mL)	Mean gross signal								
	run-1	run-2	run-3	run-4	run-5	run-6	total mean	SD	CV (%)
48.8	543.0	500.5	602.0	565.5	569.5	496.0	546.1	41.6	7.6
125.0	1169.5	1312.0	1405.0	1238.0	1227.5	1237.0	1264.8	82.3	6.5
625.0	5841.0	5267.5	5088.0	5517.0	5992.0	5399.5	5517.5	343.7	6.2
6250.0	11441.5	11294.5	12613.0	11768.5	12373.5	12207.5	11949.8	530.4	4.4

Table 4. Inter-assay precision.

Positive standard (ng/mL)	Mean gross signal					
	run-1	run-2	run-3	total mean	SD	CV (%)
48.8	573.5	543.0	516.0	544.2	28.8	5.3
125.0	1285.0	1169.5	1263.0	1239.2	61.3	4.9
625.0	5614.5	5841.0	5014.0	5489.8	427.4	7.8
6250.0	12074.0	11441.5	10678.0	11397.8	699.0	6.1

The intra-assay precision was calculated to be 4.4–7.6 % by 6 runs . Meanwhile, the inter-assay precision was calculated to be 4.9–7.8 % by 3 runs .

Table 5. Drug tolerance.

Positive standard (ng/mL)	Drug (nmol/L)	Mean Signal	Interpretation (Fixed CP = 191.8)
48.8	0	**574.0**	positive
	4	**259.5**	positive
	8	189.0	negative
	16	149.0	negative
	32	127.0	negative
	64	101.5	negative
	128	92.0	negative
125.0	0	**1429.0**	positive
	4	**510.5**	positive
	8	**338.0**	positive
	16	**223.5**	positive
	32	161.5	negative
	64	124.0	negative
	128	94.0	negative
625.0	0	**5489.0**	positive
	4	**3931.0**	positive
	8	**2392.5**	positive
	16	**1166.0**	positive
	32	**504.0**	positive
	64	**258.5**	positive
	128	147.0	negative
6250.0	0	**11106.5**	positive
	4	**10314.5**	positive
	8	**9716.5**	positive
	16	**9308.0**	positive
	32	**8123.0**	positive
	64	**6808.5**	positive
	128	**2294.5**	positive

The PALSAR bridging assay was tolerant to >128, 64, 16, and 4 nmol/L of drug when 6250, 625, 125, and 48.8 ng/mL of antibody were present, respectively.

Conclusion

- PALSAR signal amplification demonstrated a remarkable increase of ECL signal compared with that by the conventional assay.
- An analytical sensitivity of ADA 48.8 ng/mL was achieved in human serum.
- PALSAR bridging assay model showed 48.8–6250 ng/mL of positive standard range with adequate accuracy, precision, and drug tolerance.
- PALSAR technology could feasibly be used to develop an antibody bridging assay as a signal amplifier.

Acknowledgement

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