Development and Validation of Pharmacokinetic (PK) Assay for a Biparatopic Antibody

Day1-P06

Y. Joyce Huang[1,*], Erwin Bankston Jr[1], Brian Feldmann[1], Yvonne Liu[2], Harshad S. Ugamraj[2], Kevin Dang[2], Ute Schellenberger[2], Andrew Martens[1], Ruqi Wang[1], Erik Jerks[1]

[1]Eurofins BioPharma Services, St. Charles, Missouri, USA, [2]Teneobio, Newark, California, USA

eurofins BioPharma Services

ABSTRACT

Novel Aspect
Application of a bridging immunological assay in PK analysis for a bispecific antibody drug – a viable option.

Introduction
TNB-738 is a newly developed, fully human anti-CD38 biparatopic antibody that pairs two non-competing heavy chain-only antibodies in a bispecific format (Ugamraj et al., 2022, MABS, Vol. 14, No. 1, e2095949; https://doi.org/10.1080/19420862.2022.2095949). It is generated as a combination of two UniAbs, F11A and F12A, that bind to two different epitopes on CD38 and synergize to achieve maximum CD38 inhibition without depletion of CD38+ cells. Given its long half-life, safety profile, specificity, and high potency, TNB-738 has a broad therapeutic potential for metabolic and inflammatory diseases associated with NAD+ deficiencies. In this study, we sought to develop, optimize, and validate a PK method for quantitation of TNB-738 in human serum.

Methods
An Electrochemiluminescence (ECL)-based immunoassay using Meso Scale Discovery (MSD) platform was chosen to quantitatively determine TNB-738 in human serum. A sandwich assay format and a homogenous bridging assay format using the pair of the two UniAbs, F11A and F12A, were evaluated and compared. A more sensitive and robust assay format was selected for further optimization and full validation.

Results
In sandwich assay format, the pair of Anti-ID-F12A (coat Ab) and Anti-ID-F11A (detection Ab) was approximately 8-fold more sensitive than the pair of Anti-ID-F11A (coat Ab) and Anti-ID-F12A (detection Ab). Among the three types of assay buffers tested, SuperBlock (from Scytek) displayed the best signal to noise ratio. However, sporadic high %CV values of duplicated wells were observed across the plates, which occurred frequently. This resulted in failures in the recovery of quality controls during assay optimization with targeted detection range between 0.188 µg/mL (LLOQ) and 10 µg/mL (ULOQ).
In homogenous bridging assay format, of all the antibody pairs tested, plating the mixture of analytes and the antibody MasterMix of biotinalyted Anti-ID-F12A and ruthenylated Anti-ID-F11A onto the Streptavidin SECTOR Plate (MSD) produced superior results. During assay optimization, we uncovered a potential loss of positive ECL signals in plate wash steps and during the time lapse from the addition of Read Buffer to plate reading on the MSD Sector Imager 600. By minimizing plate wash cycles, decreasing the stringency of the Read Buffer. and changing the order of Read Buffer addition to the plate, we were able to resolve the issues and the assay reproducibility and robustness were significantly improved. This assay format was fully validated with the detection range of 23.44 ng/mL (LLOQ) to 1500 ng/mL (ULOQ).

Conclusion
Data from the current study demonstrated that the ECL-based homogenous bridging assay format provides a viable option for PK assays for bispecific antibody therapeutics. The LLOQ of 23.44 ng/mL of TNB-738 in human serum achieved with this method was at least 8-fold lower than that with the sandwich format. It is a sensitive, reliable, and robust PK assay for the quantitative determination of TNB-738 in human serum.

INTRODUCTION

TNB-738 is a newly developed, fully human anti-CD38 (culster of differentiation 38) biparatopic antibody that pairs two non-competing heavy chain-only antibodies in a bispecific format (Ugamraj et al., 2022, MABS, Vol. 14, No. 1, e2095949; https://doi.org/10.1080/19420862.2022.2095949). It is generated as a combination of two UniAbs, F11A and F12A, that bind to two different epitopes on CD38 (Fig.1). Unlike other clinically available anti-CD38 antibodies, daratumumab, and isatuximab, the biparatopic binding of TNB-738 to CD38 enables nearly complete inhibition of CD38 enzymatic activity without depletion of CD38-expressing cells. Given its long half-life, safety profile, specificity, and high potency, TNB-738 has a broad therapeutic potential for metabolic and inflammatory diseases associated with NAD+ deficiencies. In this study, we sought to develop, optimize, and validate a PK method for quantitation of TNB-738 in human serum.
The Meso Scale Discovery (MSD) electrochemiluminescent (ECL) immunoassay platform is chosen as it provides a better sensitivity and a broader dynamic range than ELISA. Most MSD immunoassays are sandwich assays, designed to measure the concentration of specific molecules in complex biological samples. Biomolecules of interest are detected using Sulfo-Tag– conjugated reporter molecules. Electricity is applied to the plate electrodes by an MSD imager leading to light emission by Sulfo-Tag labels. Light intensity is then measured to quantify analytes in the sample.

Typical parameters are tested and optimized when developing MSD assays include:
1) **Antibody pairs:** To identify an appropriate antibody pair and optimal antibody orientation (capture and detection) for sandwich immunoassays.
2) **Detection:** Detection antibodies can be directly conjugated to Sulfo-Tag to generate ECL signals, or detection reagents can be used in conjunction with Sulfo-Tag-conjugated streptavidin or anti-species antibodies.

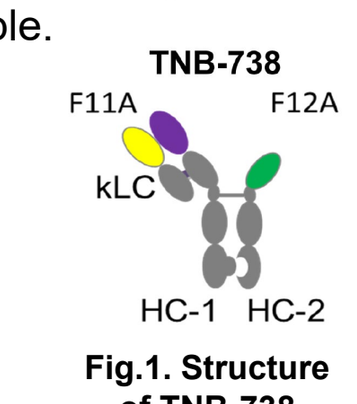


Fig.1. Structure of TNB-738

MATERIALS AND METHODS

Biological matrix (normal human serum lots from 10 males and 10 females) were purchased from BioIVT. SuperBlock (Serum-Free Protein Block) was from Scytek Laboratories and LowCross Buffer from Candor Bioscience.

An ECL-based immunoassay using Meso Scale Discovery (MSD) platform was chosen to quantitatively determine TNB-738 in human serum. A sandwich assay format and a homogenous bridging assay format (Fig.3) using pairs of the two UniAbs, F11A and F12A, were evaluated and compared. A more sensitive and robust assay format was selected for further optimization and full validation. Validation was performed according to the FDA *Guidance for Industry: Bioanalytical Method Validation*, May (2018) and the EMA *Guideline on Bioanalytical Method Validation*. Committee for Medicinal Products for Human Use (CHMP). London UK, February (2012).

- **Biotinylation:** Antibodies were labelled with EZ-Link Sulfo-NHS-LC-Biotin (ThermoFisher Scientific) using the manufacturer's protocol.
- **Ruthenylation:** Antibodies were labeled with MSD GOLD™ Sulfo-Tag™ NHS-Ester (MSD) using the manufacturer's protocol.
- **Sandwich assay format (Fig.2):** One of the two UniAbs, F11A or F12A was used as a capture antibody to coat MSD 96-well Standard Bind Plates. The other UniAb was biotinylated and used as the detection antibody in combination with the Sulfo-Tag conjugated Streptavidin (MSD) for signal amplification.
- **Bridging assay format (Fig.3):** One of the two UniAbs was biotinylated and the other UniAb was ruthenylated. Standards, QCs, or samples were first incubated with a MasterMix of biotinylated and ruthenylated antibodies in a homogenous solution phase. The mixtures were loaded onto MSD Gold 96-well Streptavidin plates.
- **Data collection:** Raw signals (ECL Units) were collected from the MSD Sector® Imager using MSD software.
- **Data analysis and statistics:** During method development, data were analyzed using both GraphPad Prism (version 9.5.1) or Watson LIMS (version 7.6). During validation, all data were imported and analyzed in Watson LIMS using the 5-Parameter Logistic regression model with a weighting factor of $1/Y^2$.
  - $\%CV = \left(\frac{\text{Standard Deviation}}{\text{Mean}}\right) * 100$
  - $\%RE = \left(\frac{\text{Measured Value} - \text{Nominal Value}}{\text{Nominal Value}}\right) * 100$

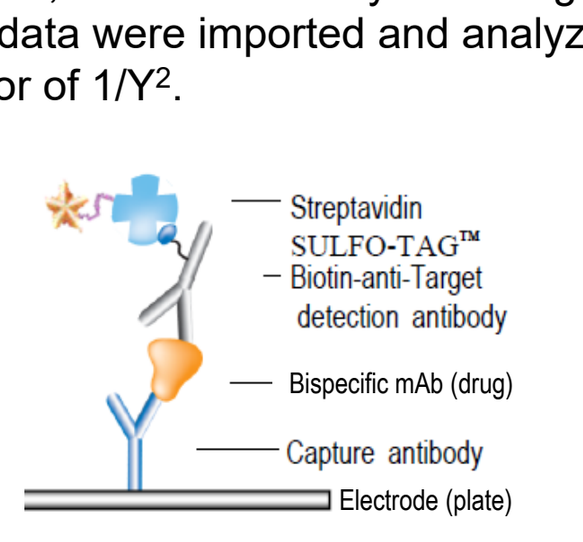


Fig.2. Sandwich assay format

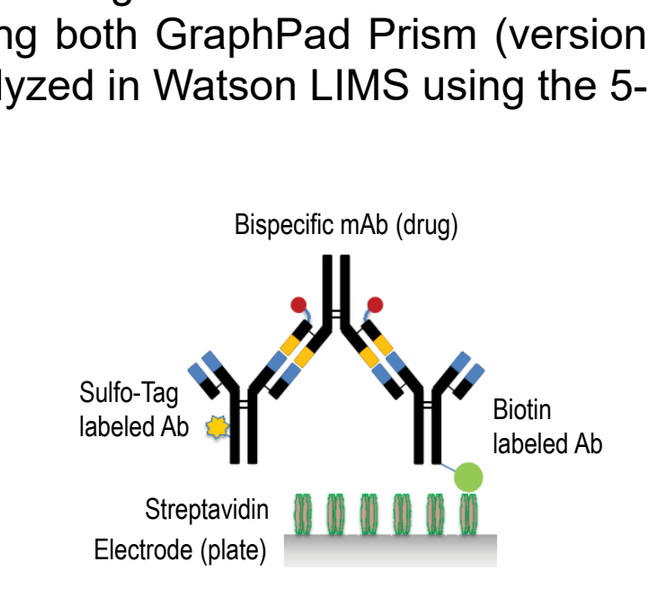


Fig.3. Bridging assay format

RESULTS & DISCUSSION

A

TNB-738 (µg/mL)		F11A (coat) / F12A (detect)				F12A (coat) / F11A (detect)			
		MRD 5	MRD 10	MRD 20	MRD 50	MRD 5	MRD 10	MRD 20	MRD 50
STD.1	800	348.9	207.3	186.7	145.7	1519.4	1191.9	935.5	548.1
STD.2	200	174.7	179.3	136.1	81.7	902.4	821.7	689.6	381.5
STD.3	50	117.7	88.5	59.9	25.6	527.6	617.9	510.0	327.9
STD.4	12.5	46.2	28.5	18.4	6.1	346.5	346.9	448.1	117.4
STD.5	3.125	12.3	8.6	4.4	2.2	145.0	166.3	244.8	26.9
STD.6	0.781	3.7	2.5	1.8	1.2	36.3	31.6	21.4	5.6
STD.7	0.195	1.7	1.5	1.2	1.0	7.5	7.7	3.9	1.5
STD.8	0.0488	1.2	1.1	1.0	0.9	1.9	1.6	1.3	1.0
NBP	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

B

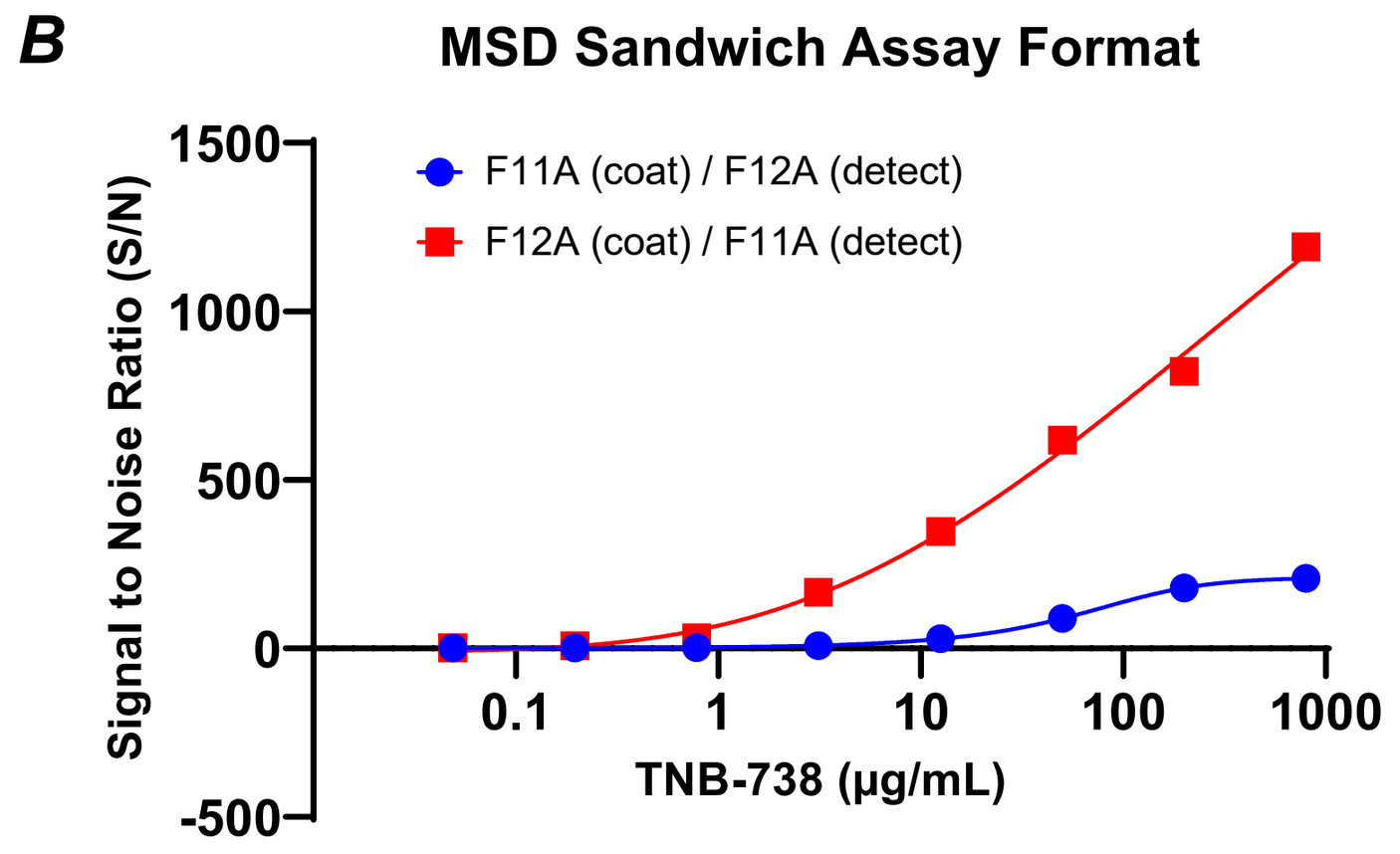


Fig.4. Evaluation of antibody pairs and optimization of Minimal Required Dilutions (MRD) using MSD sandwich assay format. (*A*) Comparison of antibody pairs. The results indicated that the pair of F12A (capture) / F11A (detection) produced robust signals, of which MRD 1:10 had the best sensitivity and dynamic range of detection. (*B*) A representative comparative plot of the standard curves using the two antibody pairs at MRD 1:10.

A

TNB-738 (µg/mL)		F12A (coat) / F11A (detect) at MRD 10		
		SuperBlock	LowCross	PBS + 0.1% BSA
STD.1	300	783.4	75.2	32.2
STD.2	100	582.9	72.1	23.5
STD.3	33.33	447.4	48.0	16.4
STD.4	11.11	274.2	29.6	11.7
STD.5	3.70	134.1	13.2	6.6
STD.6	1.23	47.9	4.4	3.0
STD.7	0.412	22.6	2.0	1.7
STD.8	0.137	5.2	1.4	1.6
STD.9	0.0457	2.0	1.3	1.0
NBP	0	1.0	1.0	1.0

B

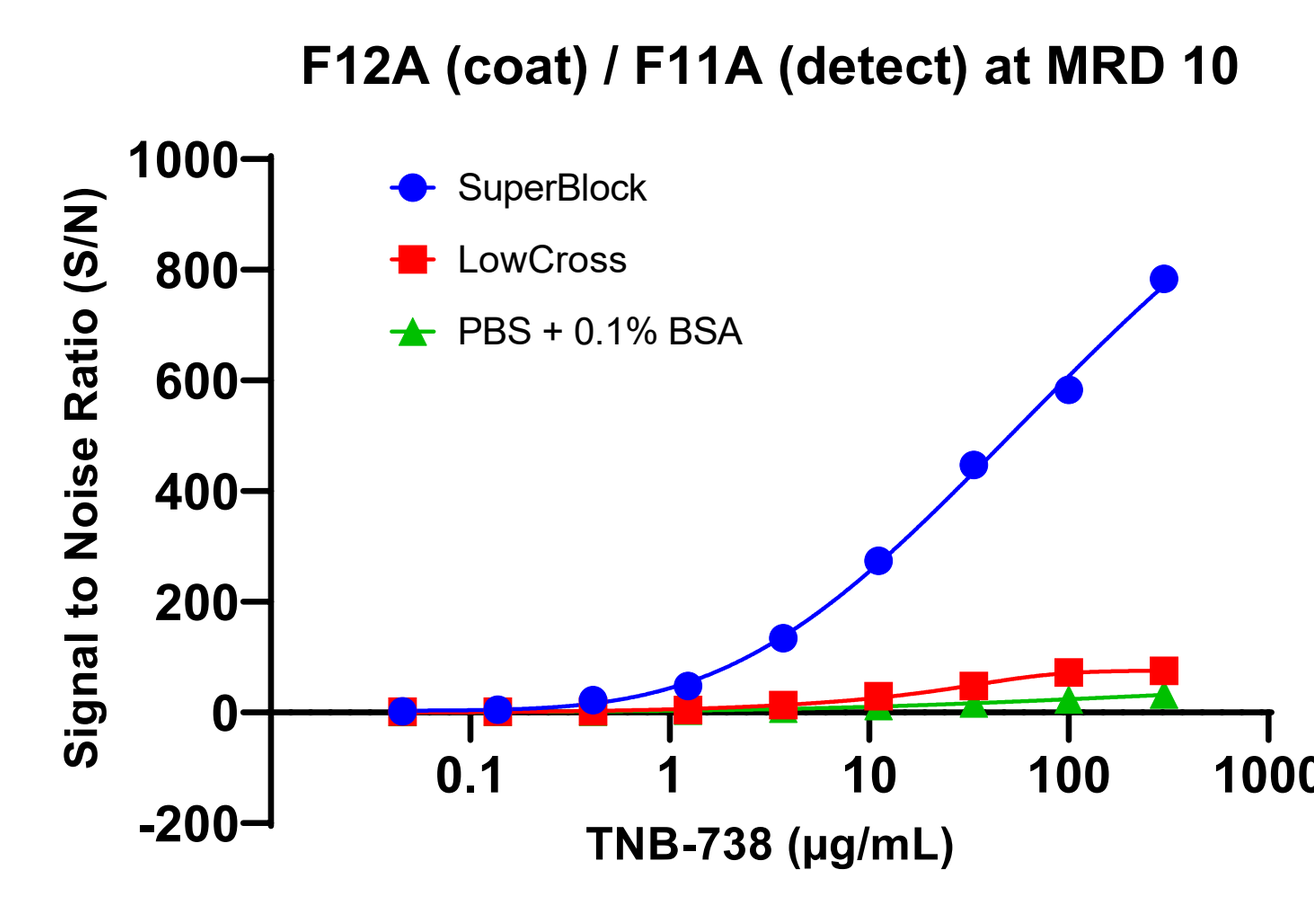


Fig.5. Optimization of assay buffers using MSD sandwich format. The antibody pair of F12A (capture) / F11A (detection) and MRD 1:10 were chosen in further optimization of assay buffers. SuperBlock, LowCross, and PBS containing 0.1% BSA buffers were compared. The results demonstrated that SuperBlock gave the best signal to noise ratio (S/N), assay sensitivity and dynamic range of detection.

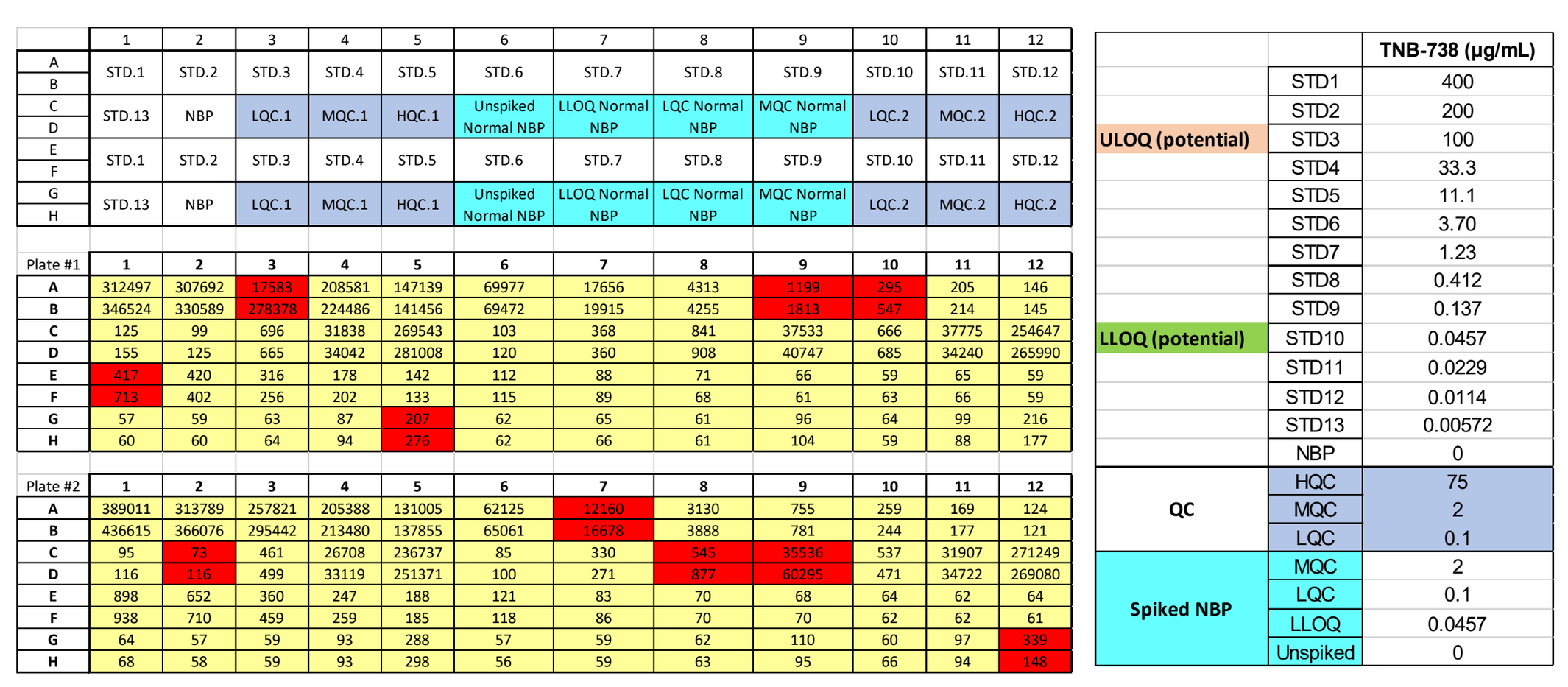


Fig.6. Sporadic high %CV in duplicates of standards and tested samples occurred across assay plates. During development stage, when testing the assay reproducibility, sporadic high %CV in duplicates across the plate were noticed. Representative experimental results are shown. (*A*) Plate map; (*B* and *C*) Raw signals (ECL units) of the two identical (repeating) plates. Red spots indicated the duplicated wells with %CV > 20%.

	Experimental Conditions	Analyst 1, Assay Date of 03/22/2022												Analyst 2, Assay Date of 03/25/2022							
		Plate 1 top		Plate 1 bottom		Plate 2 top		Plate 2 bottom		Plate 3 top		Plate 3 bottom		Plate 1 top		Plate 1 bottom		Plate 2 top		Plate 2 bottom	
	Watson Run #	Run 49		Run 50		Run 51		Run 52		Run 53		Run 54		Run 55		Run 56		Run 57		Run 58	
	Shaking spped	300 rpm		300 rpm		500 rpm		500 rpm		500 rpm		500 rpm		300 rpm		300 rpm		300 rpm		300 rpm	
	BT-mAb conc	0.5 ug/mL BT-F11A		0.25 ug/mL BT-F11A		0.5 ug/mL BT-F11A		0.75 ug/mL BT-F11A		0.5 ug/mL BT-F11A		0.25 ug/mL BT-F11A		0.25 ug/mL		0.5 ug/mL		0.37 ug/mL		0.25 ug/mL	
	Wash condition	Plate washer		Plate washer		Plate washer		Plate washer		manual wash		manual wash		Plate washer		Plate washer		Plate washer		Plate washer	
TNB-738 (µg/mL)	Identity	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE	%CV	%RE
24	STD1	20.4	-7.6		6.4	93.0	99.6		-11.3	48.2	10.5		-56.2	13.8	41.9	33.8	3.4	52.5	27.8	11.3	5.1
12	STD2	3.7	3.1	16.7	-11.3	8.2	-15.9	19.4	-14.0	45.9	24.9	60.8	4.7	16.0	-15.2	9.9	1.1	18.3	-10.8	0.5	-2.6
10	STD3 (ULOQ)	16.3	7.4	7.2	4.2	18.6	-10.5	17.5	3.7	26.9	-8.0	2.3	20.6	16.2	0.6	5.8	0.1	25.2	8.2	1.5	-2.1
6	STD4	7.0	3.8	8.4	-0.1	20.4	19.9	7.8	-0.8	14.4	8.8	6.8	-3.7	1.4	5.6	6.9	1.4	18.6	1.4	0.7	-1.3
3	STD5	1.6	-5.8	6.4	8.4	15.0	-0.4	7.8	1.1	6.0	-4.6	14.7	3.4	0.9	3.8	0.9	-0.3	9.7	4.3	8.6	7.0
1.5	STD6	2.8	-1.6	2.6	-1.8	1.7	-1.0	15.0	16.8	13.3	2.6	0.1	-2.6	3.4	3.1	9.3	-0.9	8.4	2.0	1.9	-0.7
0.75	STD7	4.7	7.3	4.5	-0.8	7.4	10.5	3.6	-1.5	19.4	-0.8	2.1	0.6	1.6	-2.4	7.3	-0.1	9.3	-11.1	4.7	-3.5
0.375	STD8	8.4	-3.4	4.7	-4.2	11.3	-10.0	8.1	-13.9	5.6	1.5	2.9	0.0	3.0	-5.9	1.4	1.6	3.0	25.0	10.8	3.6
0.188	STD.9 (LLOQ)	9.5	4.2	0.3	-3.1	7.5	8.1	19.4	6.7	15.8	-1.5	15.4	2.6	15.5	1.3	5.2	-1.0	3.9	-1.2	3.7	-5.7
0.0938	STD.10	23.3	-4.6	37.1	11.3	5.4	-0.1	29.5	10.9	3.8	0.2	6.9	-3.3	23.4	5.3	0.5	-0.1	13.1	-8.2	23.2	6.6
0.0469	STD.11	40.0	0.4	2.9	-2.9	7.2	-0.5	8.6	-2.9	25.7	-0.5	6.1	1.3	4.3	-1.4	15.4	-0.1	102.8	-0.5	2.8	-1.1
10	ULOQ 1	1.1	-37.0	2.9	-22.8	22.0	-27.0	19.3	-41.5	34.8	52.0	10.7	-51.9	8.5	-20.8	12.9	-25.7	2.1	-25.7	1.0	-13.5
10	ULOQ 2	6.4	45.0	9.2	1.9	44.0	190.8	20.6	3.0	9.3	29.1	N/A	620.5	49.2	-10.4	33.5	63.3	3.2	-20.5	0.8	3.0
7.5	HQC 1	7.2	-2.8	7.8	-13.7	5.8	-0.4	24.3	-43.4	7.0	-30.3	47.1	-25.0	28.5	47.2	31.5	64.7	3.2	-11.5	6.3	12.6
7.5	HQC 2	9.3	34.0	8.2	-1.8	19.4	246.8	0.3	-0.7	5.7	20.4	49.2	58.3	23.3	-17.7	10.6	-9.6	21.2	-33.1	5.7	-9.1
2	MQC 1	3.9	15.3	11.5	13.8	1.9	-16.7	3.4	-2.4	11.3	28.5	11.2	-23.8	0.8	-16.3	3.2	-15.9	2.5	-14.6	1.6	-5.4
2	MQC 2	4.8	1.9	6.5	22.6	3.9	18.9	8.2	24.1	3.8	7.9	0.6	-23.0	13.1	-8.2	8.9	-1.7	0.3	-3.2	1.0	10.3
0.3	LQC 1	0.9	10.8	8.5	1.7	2.7	2.5	21.1	-9.7	3.3	29.5	16.5	1.1	3.6	-22.5	47.5	38.3	4.6	-24.3	8.7	-14.9
0.3	LQC 2	2.2	0.9	5.7	9.6	4.7	13.6	1.4	5.8	0.6	10.3	10.8	10.5	6.1	-13.1	10.5	1.6	10.5	13.8	7.1	-4.4
0.188	LLOQ 1	6.7	7.7	6.2	5.3	11.5	18.2	1.8	5.3	24.1	12.9	24.3	13.2	65.7	29.4	35.2	23.3	0.5	-29.6	7.2	-19.6
0.188	LLOQ 2	16.0	8.9	15.1	20.8	11.0	7.3	0.8	-1.5	7.7	10.9	19.0	30.4	2.4	-14.8	15.5	6.3	36.9	-12.9	5.6	-8.0
300	DQC 1	5.4	18.8	5.3	-2.3	10.1	10.2	4.5	4.3	10.0	43.4	8.2	-8.1	3.0	-0.3	2.8	-0.5	4.1	-13.3	1.0	5.1
300	DQC 2	0.5	16.3	11.0	15.4	21.5	1.1	2.8	4.7	9.7	-0.5	1.5	-13.2	4.4	6.3	11.9	22.2	0.0	15.3	0.6	41.3

Fig.7. The random high %CV in duplicates resulted in the failure of quality control (QC) recovery (%RE) during optimization of MSD sandwich assay. To further optimize the assay, different analysts performed accuracy and precision tests on different days. Representative runs are shown. In each run, standard curve was fit using the 5-Parameter Logistic regression model with a weighting factor of $1/Y^2$ in Watson. All quality controls (QCs) were interpolated with the corresponding standard curve in each run. *Red blocks* indicated either %CV or %RE > 20%.

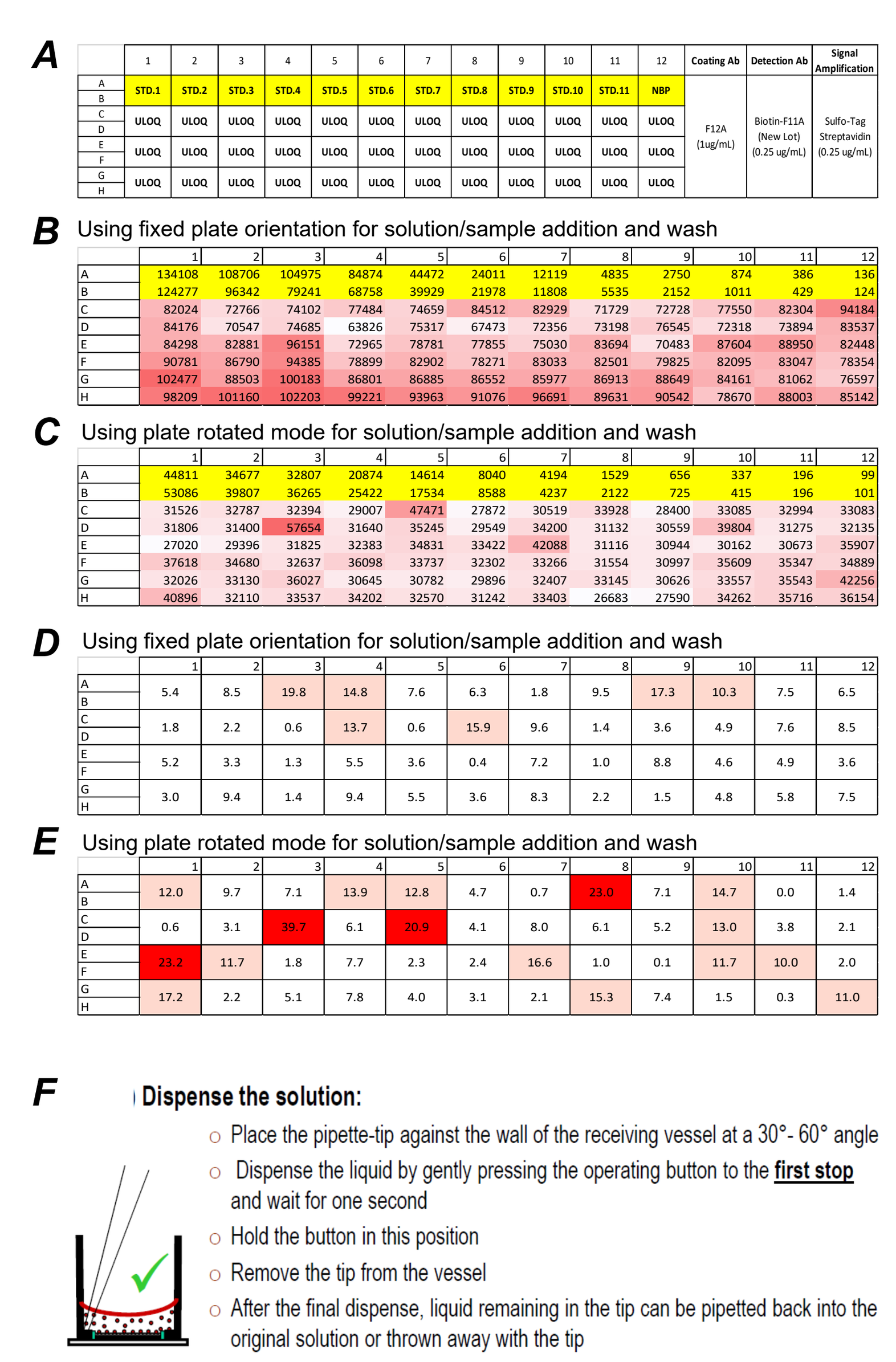


Fig.8. Using plate rotated mode for solution/sample addition and wash resulted in >60% loss in ECL signals. (*A*) Plate map; (*B* and *C*) Raw signals of the two identical plates; (*D* and *E*) %CV of duplicates. *Red spots*, %CV > 20% and *Pink spots*, %CV > 10%. (*F*) Diagram shows how to dispense the solution into the MSD plate. By rotating the plate, dispensing solutions/samples at different positions could result in more loss of low binding sites. This may explain why the rotated plate (*C*) had much less ECL signals than the plate with a fixed orientation (*B*).

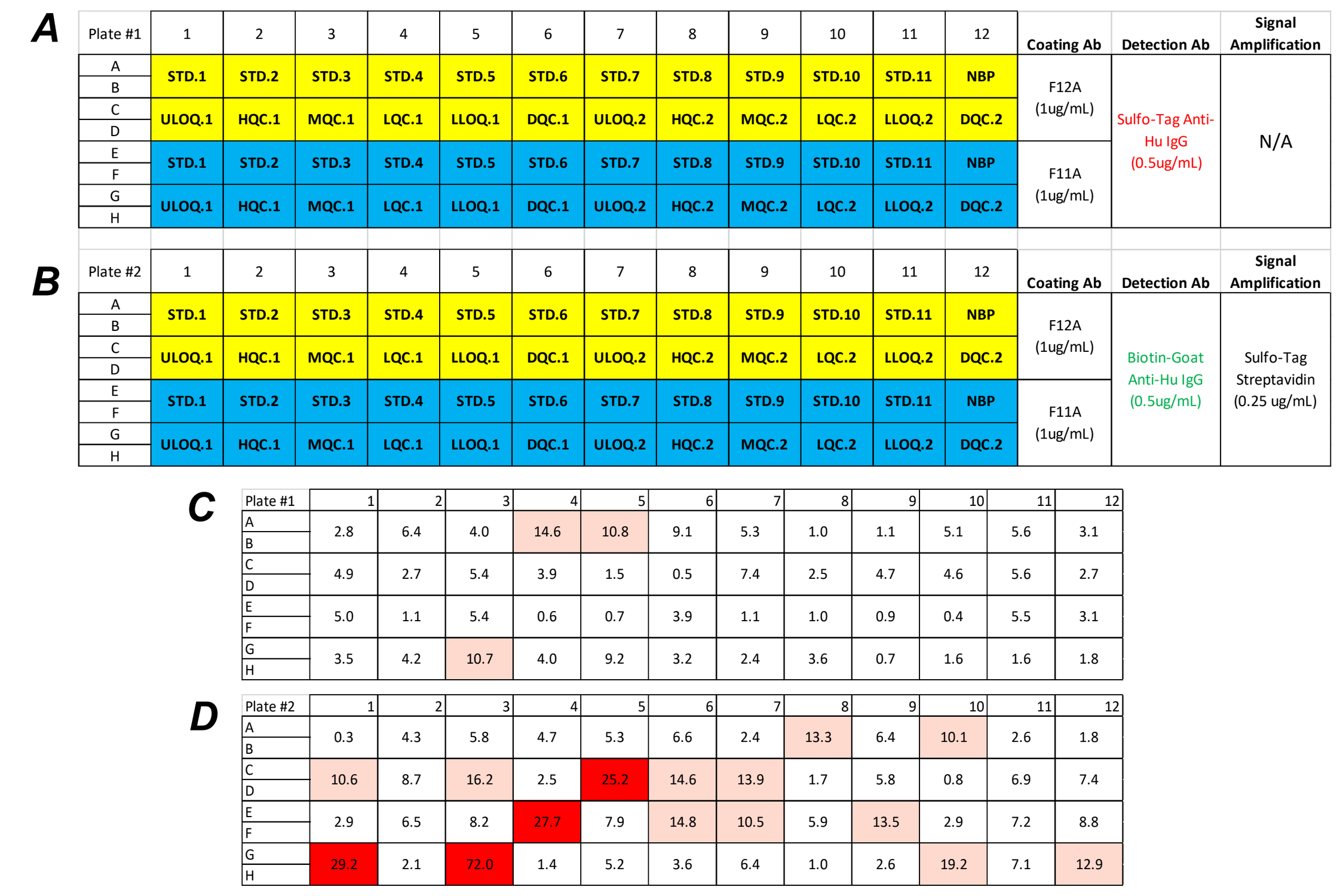


Fig.9. Troubleshooting of sporadic high %CV in duplicates. MSD sandwich assays were performed using two different detection strategies: (*A*) Plate #1: Sulfo-Tag Anti-Human IgG as detection antibody; (*B*) Plate #2 Biotinylated Goat Anti-Human IgG as detection antibody followed by signal amplification with Sulfo-Tag conjugated Streptavidin. Interestingly, Plate #2 (*D*) had significantly higher %CV (sporadic) in duplicates than Plate #1 (*C*). This further supported the hypothesis that wash step impacted the final ECL signals. The more wash cycles the assay had, the higher probability of getting higher %CV and resulting in the assay failure. *Red spots*, %CV > 20% and *Pink spots*, %CV > 10%.

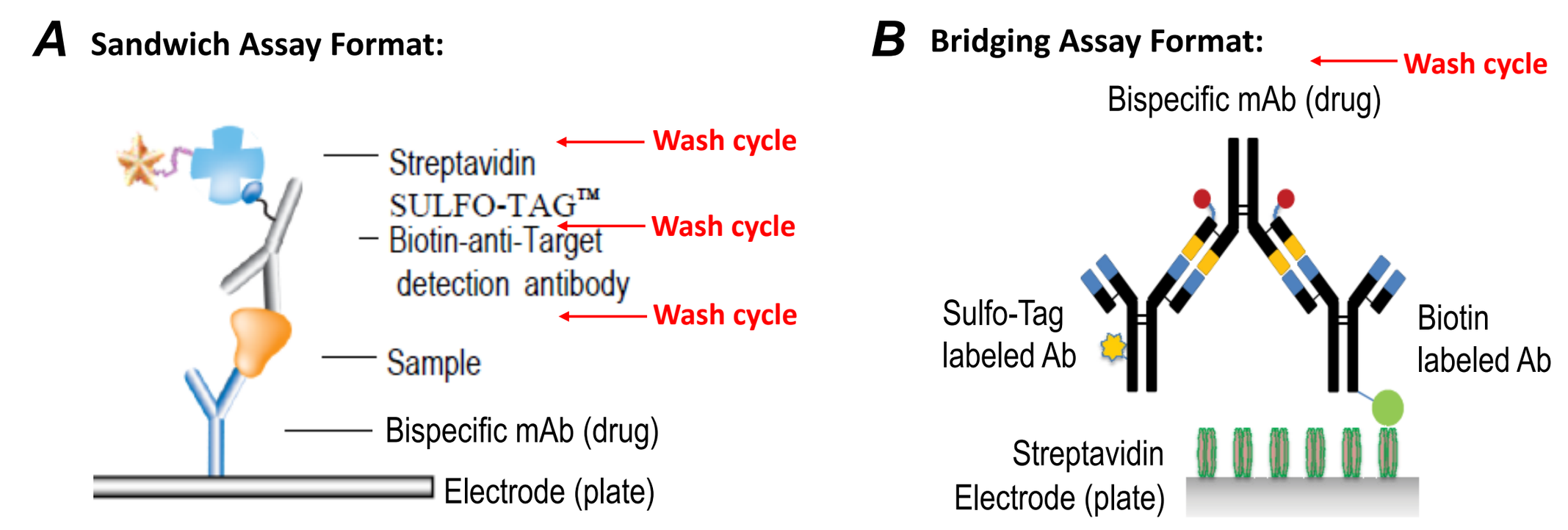
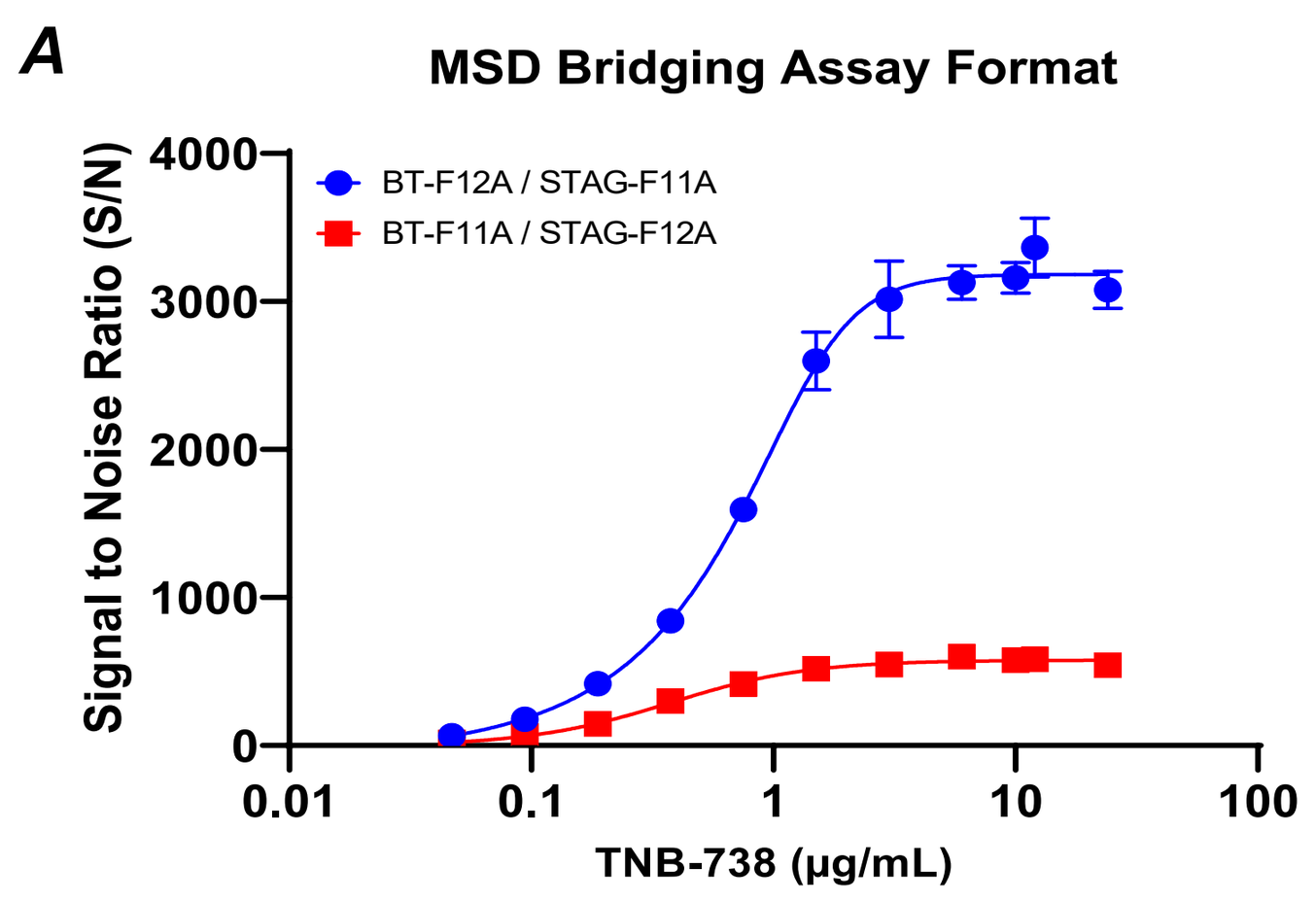


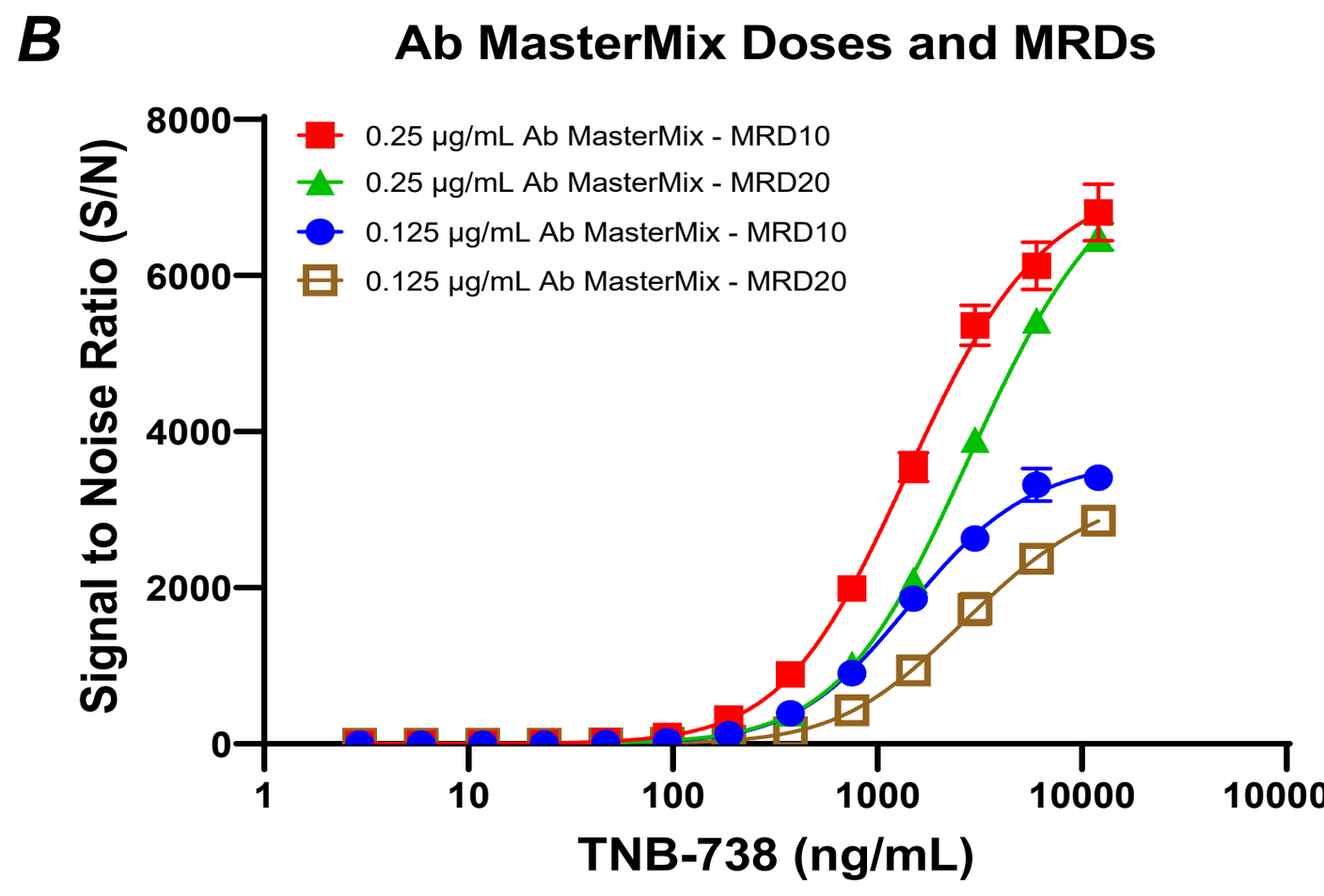
Fig.10. Comparison of MSD sandwich assay versus homogenous bridging assay. (*A*) In the Sandwich format, there are at least three wash steps after 1) sample (analyte) incubation with capture Ab, 2) incubation with biotin-labeled detection Ab, and 3) incubation with Sulfo-Tag labeled Streptavidin. (*B*) In the Bridging assay format, the sample is first incubated with a MasterMix of biotin-labeled Ab and Sulfo-Tag labeled Ab in a homogenous solution phase, and the mixture is then added onto an MSD Streptavidin plate. There is only one wash step upon completion of incubation. This can significantly reduce the probability of wash-induced signal loss and high %CV in duplicates.

A



	TNB-738 (ng/mL)
STD1	24000
STD2	12000
STD3	10000
STD4	6000
STD5	3000
STD6	1500
STD7	750
STD8	375
STD9	188
STD10	93.8
STD11	46.9
NBP	0

B



	TNB-738 (ng/mL)
STD1	12000
STD2	6000
STD3	3000
STD4	1500
STD5	750
STD6	375
STD7	187.5
STD8	93.75
STD9	46.88
STD10	23.44
STD11	11.72
STD12	5.86
STD13	2.93
NBP	0

Fig.11. Optimization of MSD homogenous bridging PK assay. (*A*) Comparison of the antibody pairs at MRD 1:10. The results indicated that the pair of BT-F12A (capture) / STAG-F11A (detection) produced robust signals with a great sensitivity and dynamic range of detection. (*B*) Optimization of antibody MasterMix concentrations and MRDs using the Ab pair of BT-F12A and STAG-F11A. The data suggests that 0.25 ug/mL of antibody each at MRD 1:10 is the best condition. BT: Biotin; STAG: Sulfo-Tag.

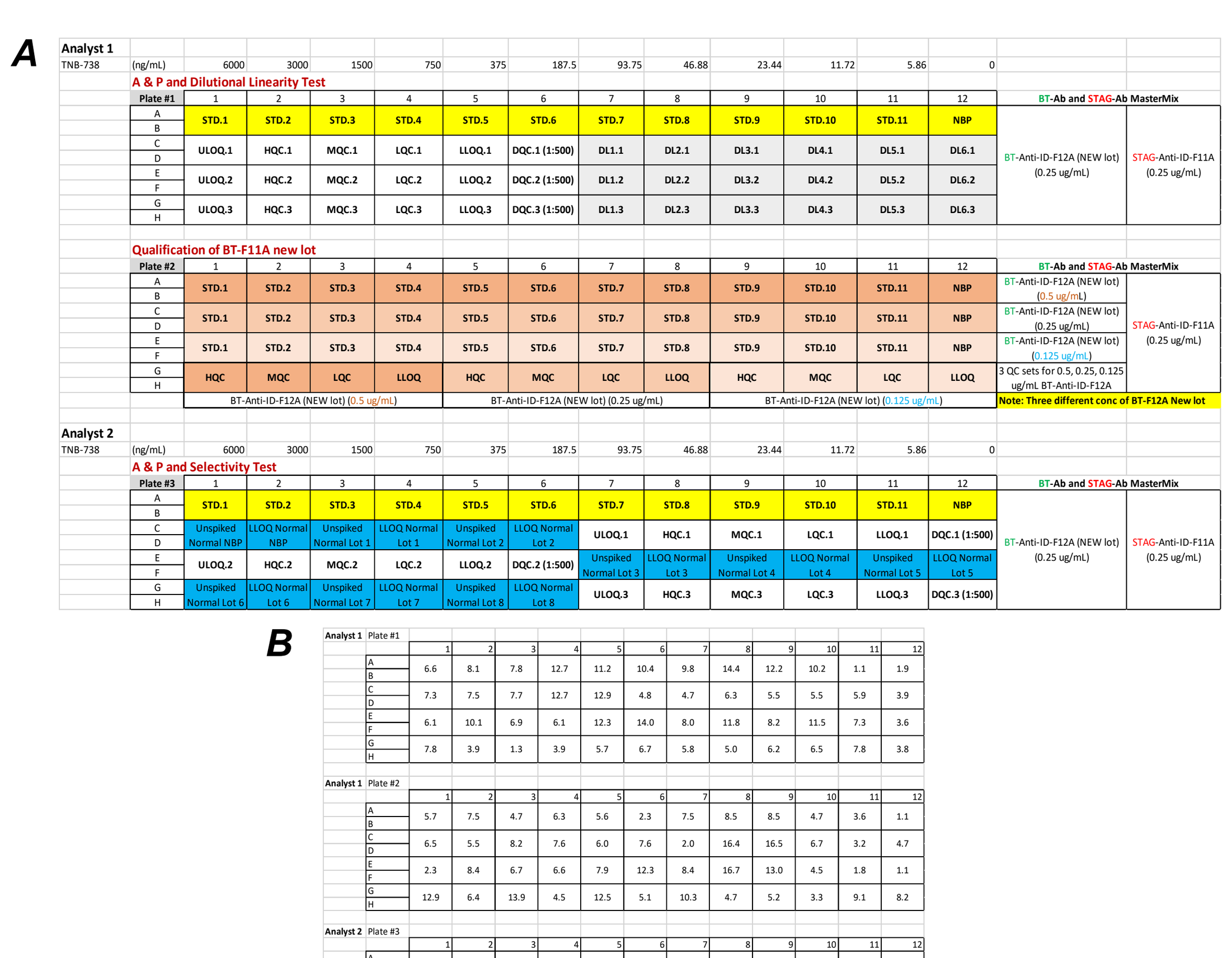


Fig.12. Precision of MSD homogenous bridging PK assay. Different analysts performed assays as shown in plate maps (*A*). The %CV values of each duplicates from all plates met the acceptance criteria, < 20% (*B*). These data demonstrated that the MSD bridging assay is indeed a viable option, which greatly improved assay precision compared to the MSD Sandwich assay format in quantification of TNB-738 in human serum.

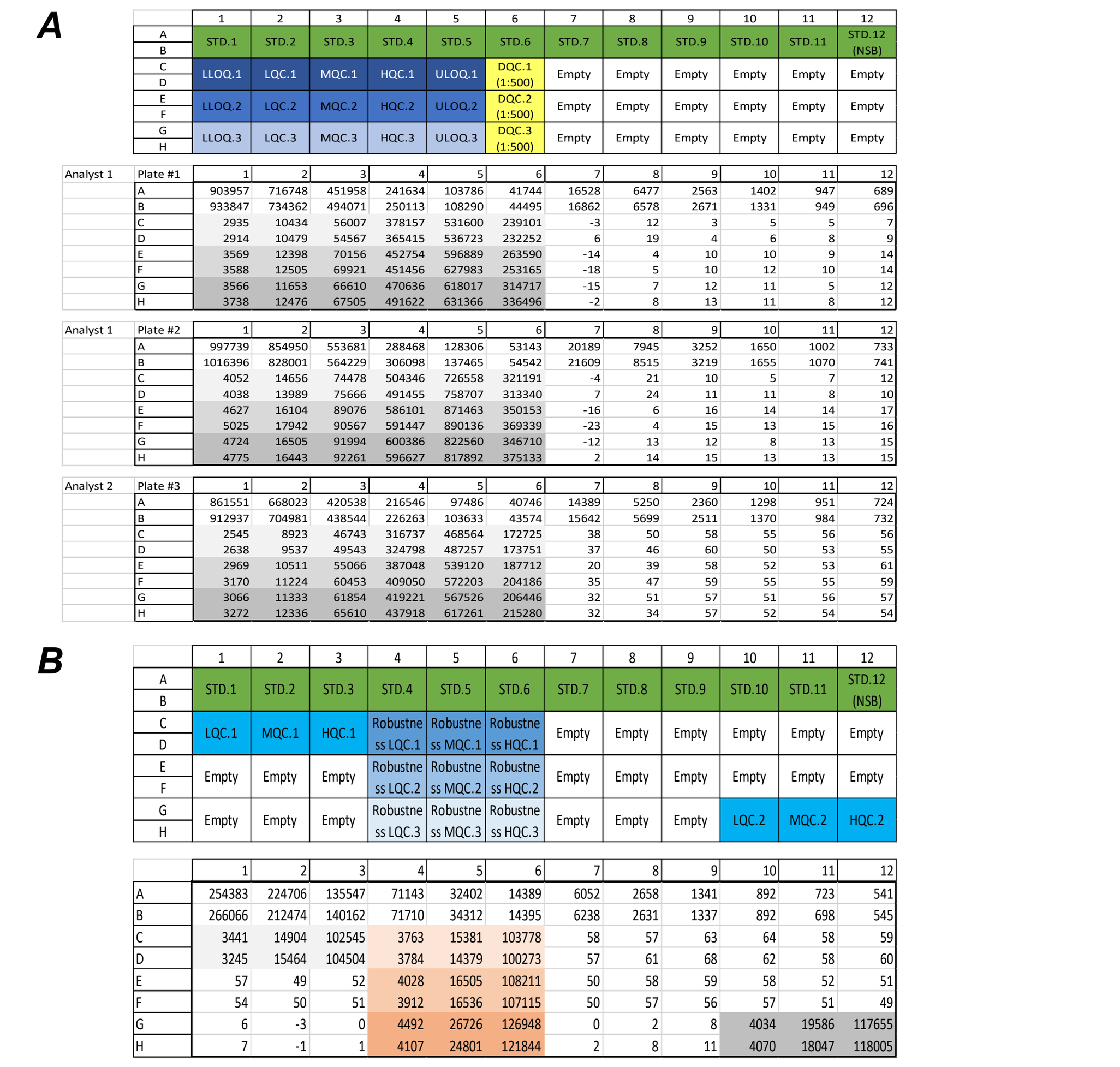


Fig.13. Position effects of MSD bridging assay. To further optimize the assay and prepare for full validation, different analysts performed assays to test several parameters of the assay, such as accuracy & precision (A & P), ruggedness & robustness, etc. (*A*) Data from representative runs. Position effects were identified. The lower set of QCs seemed to have higher ECL signals than the upper set of QCs, which resulted in the failure of A & P assessment. (*B*) When testing the assay ruggedness & robustness, soaking the sample-antibody mixtures in 2X MSD Read Buffer for 5 min prior to plate reading resulted in the loss of approx. 60-70% of ECL signals compared to reading the plate immediately after the addition of 2X Read Buffer (*B* versus *A*). These findings suggested a potential low affinity binding and a fast dissociation rate. Similar to the results shown in (*A*), the back set of QCs had slightly higher ECL signals than the front set of QCs in (*B*).

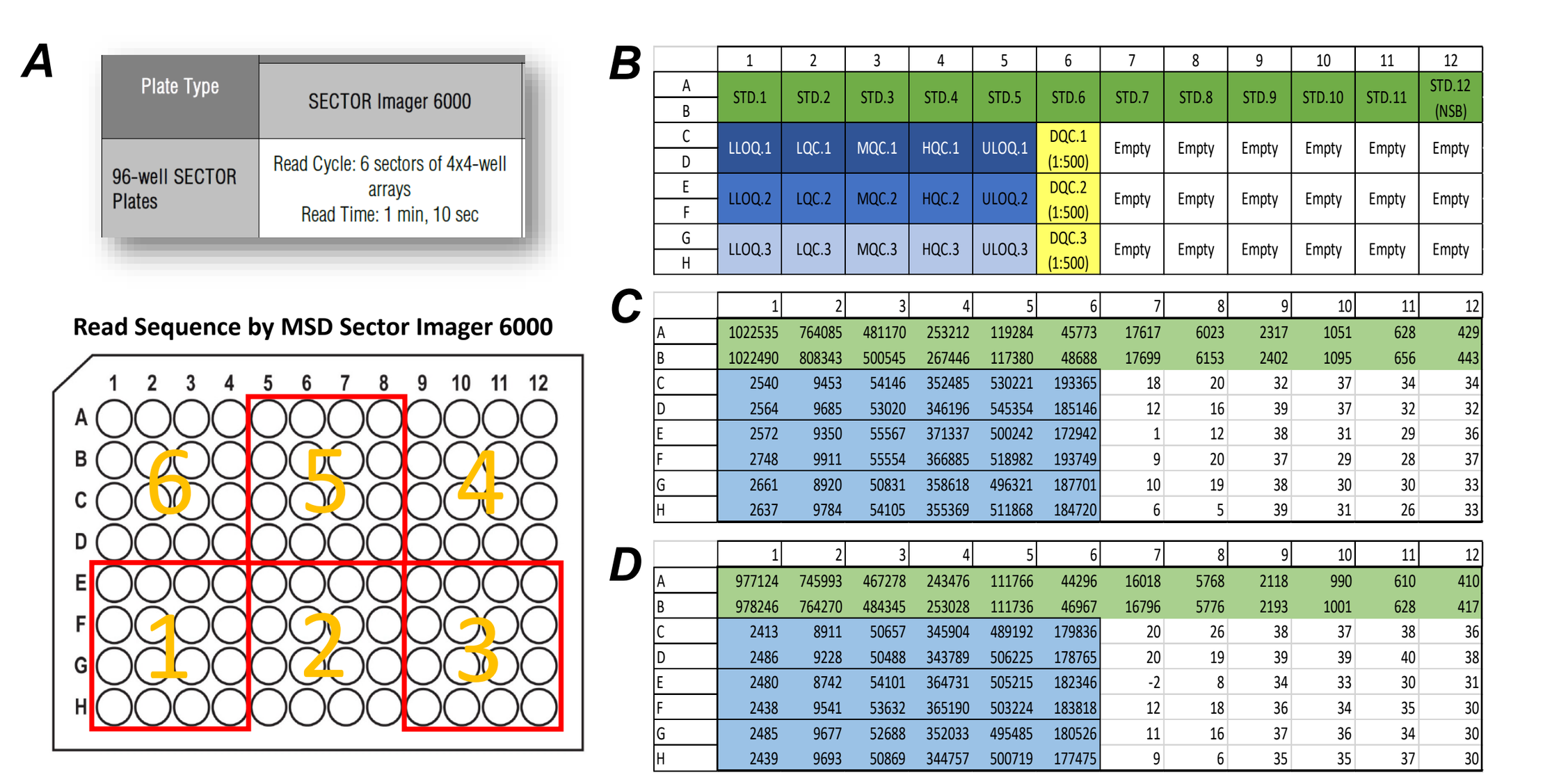


Fig.14. Optimization of MSD homogenous bridging PK assay. (*A*) Diagram depicting how the MSD Sector Imager 6000 collects ECL signals from Sector 1 through 6. The signal is generated from electrochemically stimulated Sulfo-Tag-conjugated molecules that in close proximity to the bottom of the well. Before the final wash step, the assay components are at or close to equilibrium. However, if the plate is left in wash buffer or read buffer too long, the assay components may start to dis- and re-associate, establishing a new binding equilibrium. Signal will decrease if Sulfo-Tag-conjugated antibody dissociates from the other assay components on the surface. Signal decrease will not be significant for high affinity interactions with slow off rates; however, interactions with rapid dissociation rates can result in a time-dependent signal decrease, which is also impacted by the stringency of the Read Buffer. This theory well explained the position effects shown in Fig.13. (*B-D*) To solve this problem, these remedies were executed: 1) Use 1X Read Buffer instead of 2X to decrease the stringency to minimize the dissociate rate; 2) Add 1X Read Buffer from the bottom of the plate to the top (from Row H to Row A) to allow the sector soak in Read Buffer first to be read first. Results from two representative runs (*C* and *D*) indicated that A&P assessments passed the acceptance criteria, %CV < 20% (<25% for ULOQ and LLOQ) and %RE < 20% (<25% for ULOQ and LLOQ).

ACCURACY AND PRECISION SUMMARY				
QC Identity	Nominal Conc (ng/mL)	Inter-Assay Precision (%CV)	Inter-Assay Accuracy (a) (%RE)	Total Error (b) (%TE)
LLOQ	23.44	6.2	0.9	7.1
LQC	60.00	6.6	2.2	8.8
MQC	200.00	6.1	3.3	9.4
HQC	1000.00	6.0	-0.6	6.6
ULOQ	1500.00	7.6	1.4	8.9
DQC	300000.00	9.1	-2.2	11.3
(a) %RE: Bias compared to nominal concentration.				
(b) Total Error = |%RE| + %CV.				

Fig.15. Summary of Accuracy and Precision assessments in validation. All QCs met acceptance criteria.

SUMMARY

The PK analysis for quantitative determination of TNB-738 in human serum was initiated with an MSD sandwich immunoassay format. The pair of Anti-ID-F12A (coat Ab) and Anti-ID-F11A (detection Ab) produced much more robust ECL signals than the other pair. SuperBlock used as assay buffer at MRD 1:10 produced the best signal to noise ratio. However, sporadic high %CV values of duplicated wells were observed across the plates. This resulted in failures in the recovery of quality controls (QCs) during assay optimization with targeted detection range between 0.188 µg/mL (LLOQ) and 10 µg/mL (ULOQ).

- To troubleshoot the problems, we uncovered that the high %CV values might be related to multiple wash steps in sandwich assay format. In contrast, MSD homogenous bridging assay format contains only one wash step and was thus chosen as an option for PK analysis of TNB-738.
- Similar to the results from the sandwich assay, in homogenous bridging assay format, the pair of Anti-ID-F12A (coat Ab) and Anti-ID-F11A (detection Ab) in SuperBlock (assay buffer) at MRD 1:10 produced the superior results. During assay optimization, we uncovered a plate position effect and a loss of ECL signals in plate wash steps and during the time lapse from the addition of Read Buffer to plate reading on the MSD Sector Imager. By minimizing plate wash cycles, decreasing the stringency of the Read Buffer, and changing the order of Read Buffer addition to the plate, we were able to resolve the issues and the assay reproducibility and robustness were significantly improved.
- The MSD bridging PK assay was fully validated with the detection range of 23.44 ng/mL (LLOQ) to 1500 ng/mL (ULOQ).

CONCLUSION

Data from the current study demonstrated that the ECL-based homogenous bridging assay format provides a viable option for PK assays for bispecific antibody therapeutics. The LLOQ of 23.44 ng/mL of TNB-738 in human serum achieved with this method was at least 8-fold lower than that with the sandwich format. It is a sensitive, reliable, and robust PK assay for the quantitative determination of TNB-738 in human serum.