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Evaluation of a multiplexed Anti Drug Antibody (ADA) assay for immunogenicity testing in support of combination biologic therapies

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ABSTRACT

Purpose

Clinical development of biotherapeutics in combination requires monitoring immunogenicity to both molecules. Currently, ADAs to each biotherapeutic are monitored in assays which detect antibodies to one biotherapeutic at a time. The ability to detect antibodies to both biotherapeutics in the same sample is beneficial in that it provides more data in the same time as a single analyte assay. We evaluated two biologics, A and B, in ADA assays using MSD® streptavidin (single) and U-PLEX® (multiplex) plates.

Methods

The same lot of biotinylated and ruthenylated biologics A and B and positive controls (anti- A,anti-B) were used in both single and multiplex assays. ADAs in human serum were mixed with biotinylated and ruthenylated drug master mix. For the multiplex assay, each biotinylated capture antibody was initially coupled to a unique linker, then ruthenylated drug added to create a master mix. Sample and master mix were incubated overnight and the immunocomplexed samples added to blocked MSD streptavidin-coated or U-PLEX plates. Unbound material was removed; MSD read buffer added and the bound complexes detected by reading electrochemiluminescence signals on the MSD SECTOR® 2400.

Results

Positive controls were evaluated at 0-1000 ng/mL in the absence and presence of drug (0-100 µg/mL). In single assays, Drug A and B had sensitivity of 25 ng/mL and 12.5 ng/mL for detection of the respective positive control ADAs. ADA levels of 500 ng/mL were detected in the presence of 50 µg/mL of Drug A and approximately 25 µg/mL of Drug B. Several U-PLEX master mix combinations were evaluated and the best combinations yielded a sensitivity of 12.5 ng/mL for both biologics. ADA levels of 500 ng/mL were detected in the presence of 100 µg/mL Drug A and 25 µg/mL Drug B. Additional experiments to evaluate lot-lot reproducibility for U-PLEX plates and linker preparations will be conducted.

Conclusion

Evaluation of a multiplex ADA assay shows comparable performance with ADA specific detection to each biologics at levels similar to that of the single assay. Drug tolerance assessment also shows comparable assay performance across the two assays suggesting a potential savings in sample volume, analysis time and cost.

INTRODUCTION

There has been an increase in the number of combination therapies that are either approved or in development over the past decade. Understanding of multiple causal factors has led to rational targeting of redundant pathways to enhance efficacy. In some instances, the rationale for combination therapy is to reduce adverse drug reactions of each individual active substance by counteracting compensatory mechanisms or by synergistic mechanisms. Clinical development of biotherapeutics in combination requires monitoring immunogenicity to both molecules. Currently, anti-drug antibodies (ADA) to each biotherapeutic are monitored in separate assays which detect antibodies to one biotherapeutic at a time. The ability to detect antibodies to both biotherapeutics with one single assay means savings in time and sample volume, and can be potentially cost saving. Bridging assay format with MSD streptavidin (SA) plates has been widely used for ADA detection. The new MSD U-PLEX product provides a simple, convenient way for creating customized multiplex assays. We evaluated two monoclonal antibody therapeutics, A and B, in ADA assays using MSD SA (single) and U-PLEX (multiplex) plates.

This is the first study which evaluates the potential use of U-PLEX technology for multiplexing ADA detection, and suggests its possible application in combination therapy bioanalysis.

METHOD

The same lot of biotinylated and SULFO-TAG™ biologics A and B and positive controls (anti-A, a mouse monoclonal antibody; anti-B, a monkey polyclonal antibody) were used in both single and multiplex assays. A bridging format was used to measure antibody response to each biotherapeutic. Anti-drug antibodies in human serum were mixed with biotinylated and SULFO-TAG drug master mix. For the multiplex assay, each biotinylated capture antibody was initially coupled to a unique linker, then SULFO-TAG-drug was added to create a master mix. Sample and master mix were incubated overnight to allow the formation of molecular complexes. The complexed samples were added to wells of a blocked MSD streptavidin-coated or U-PLEX plates. After incubation the unbound material was removed by plate washing, MSD read buffer added and the bound complexes detected by reading electrochemiluminescence signals on the MSD SECTOR® 2400.

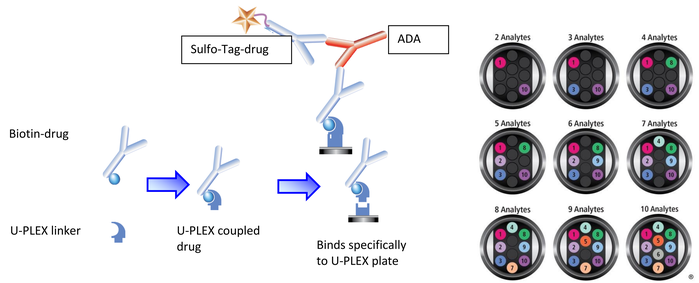

Figure 1. U-PLEX Multiplex Procedure. Each master mix of the biotin-drug and SULFO-TAG-drug was incubated with a unique Linker x (1-10) for 30 minutes at room temperature and the reaction was terminated by the addition of U-PLEX Stop Solution. The "Multiplex Linker-Coupled Master Mix" solution and sample were incubated overnight. The immune complexes were added the following day to blocked U-PLEX 2-Assay, 96-well SECTOR Plates and read on MSD SECTOR Imager 2400.

RESULTS

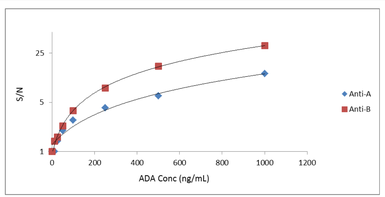

Figure 2. ADA SinglePlex Sensitivity. Samples were prepared by spiking pooled human serum with anti- Drug A and anti-Drug B (ADA positive controls) at 8 different concentrations: 0-1000 ng/mL. The sensitivity is the lowest concentration of the positive control antibody with a signal (mean ECL count normalized to the ECL count of the plate NC) greater than the screening assay cutpoint. In single assays, Drug A and B had sensitivity of 25 ng/mL and 12.5 ng/mL for detection of the respective positive control ADAs.

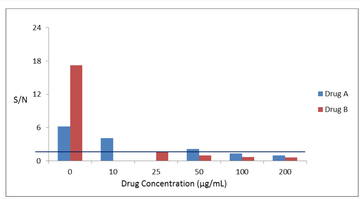

Figure 3. SinglePlex Drug Tolerance. Positive controls were evaluated at 0-1000 ng/mL in the absence and presence of drug (0-200 µg/mL). In single assays, ADA levels of 500 ng/mL were detected in the presence of 50 µg/mL of Drug A and approximately 25 µg/mL of Drug B.

	U-PLEX Mastermix					
	10µg/mL	5µg/mL	2.5 µg/mL	1.25 µg/mL	2.5 µg/mL	1.25 µg/mL
anti-A (ng/mL)	S:N	S:N	S:N	S:N	ECL Counts	ECL Counts
1000	13.8	37.2	58.2	35.3	13222	4435
500	11.3	30.3	50.5	32.3	11465	4048
250	7.2	17.3	30.8	20.7	7003	2604
100	3.0	6.6	11.1	7.8	2510	981
50	1.8	3.2	4.8	3.4	1079	428
25	1.2	1.6	2.2	1.5	496	189
12.5	1.0	1.3	1.4	1.6	312	198
0	1.0	1.0	1.0	1.0	227	126
anti-B (ng/mL)	S:N	S:N	S:N	S:N	ECL Counts	ECL Counts
1000	15.1	19.6	28.5	45.0	2463	2383
500	7.4	9.1	12.7	18.5	1097	982
250	3.9	4.7	5.9	9.3	508	495
100	2.2	2.4	3.0	4.3	257	230
50	2.3	1.6	1.8	2.8	155	150
25	1.6	1.2	1.6	1.7	141	88
12.5	1.4	1.3	1.5	1.5	131	81
0	1.0	1.0	1.0	1.0	87	53

Table 1. U-PLEX Optimization. Several U-PLEX master mix combinations were prepared using biotinylated biologics A and B and evaluated in bridging assay format. The best combinations yielded a sensitivity of 12.5 ng/mL for both biologics.

	U-PLEX Lot #				
	Drug A		Drug B		
	S:N		S:N		
ADA (ng/mL)	1	3	1	2	3
1000.0	58.2	74.3	45.0	43.4	61.5
500.0	50.5	71.9	18.5	17.7	24.3
250.0	30.8	42.2	9.3	8.1	11.9
100.0	11.1	12.2	4.3	3.1	5.0
50.0	4.8	5.1	2.8	1.9	3.1
25.0	2.2	2.6	1.7	1.5	1.9
12.5	1.4	1.6	1.5	1.1	2.0
0.0	1.0	1.0	1.0	1.0	1.0

Table 2. Reproducibility- Lot- Lot Variability
Three U-PLEX "Multiplex Linker-Coupled Master Mix" solutions were prepared independently using three lots of U-PLEX kits. ADA spiked into pooled human serum were compared using the three different lots. Comparable data (Sensitivity) were obtained across three different lots.

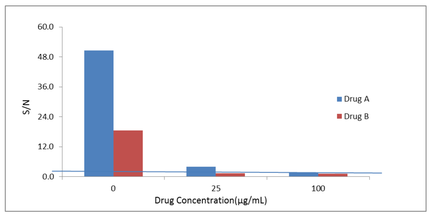

Figure 4. MultiPlex Drug Tolerance. Positive controls (0-1000 ng/mL) in the absence and presence of drug (0-100 µg/mL) were incubated overnight (ON) with U-PLEX "Multiplex Linker-Coupled Master Mix" solution. ADA levels of 500 ng/mL were detected in the presence of 100 µg/mL of Drug A and approximately 25 µg/mL of Drug B.

	SinglePlex		MultiPlex	
	Drug A	Drug B	Drug A	Drug B
MRD	30	20	30	30
Sensitivity (ng/mL)	25	12.5	12.5	12.5
Drug Tolerance (µg/mL)	50	<25	100	<25

Table 3. Comparison between Single and U-PLEX Multiplex Assays. Comparable assay performance was observed across both assays. Preliminary data suggest that the U-PLEX may be more sensitive than single assay. However, more testing is needed to show this definitively.

CONCLUSIONS

This is the first publication which shows drug tolerance and performance of immunogenicity in a multiplex format. Evaluation of a U-PLEX multiplex ADA assay with the single assays shows comparable performance with the ability for detection of ADA specific to each of the biologics at levels similar to that of the single assay. Drug tolerance assessment also shows comparable assay performance across the two assays suggesting a potential savings in sample volume, analysis time and cost. This study sheds light on the potential use of U-PLEX plates in combination studies.