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MSD GOLD™ Streptavidin and Small Spot Streptavidin Plates: Multi-year Reproducibility and Application to Pharmacokinetic Assay Development

Angelina Anderson, John Joern, Samarth Chugh, Jon Buhrman, Laure Moller, Brunah Otieno, David Stewart, and Jacob N. Wohlstadter

Meso Scale Discovery, Rockville, Maryland, USA

CONTACT INFORMATION: jbuhrman@meso-scale.com

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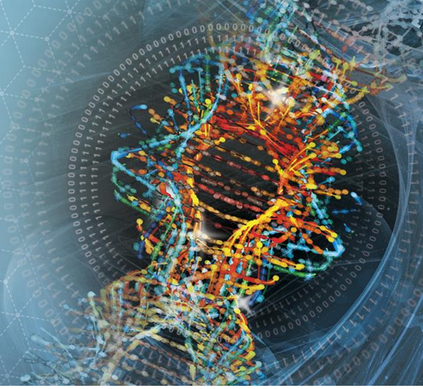
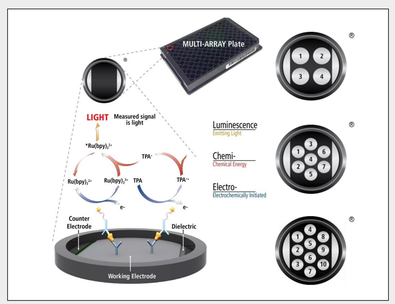


PURPOSE

Streptavidin-coated plates are a widely-used tool for creating assays to measure biomarkers including traditional sandwich immunoassays, bridging immunogenicity assays, and pharmacokinetic (PK) assays. To assure reproducible results for long-term studies, it is essential that streptavidin plates are rigorously characterized for consistent performance and stability. MSD offers streptavidin-coated plates in two different 96-well formats, MSD GOLD Streptavidin and MSD GOLD Small Spot Streptavidin. Here we review the quality control performance of both plate formats spanning multiple years, as well as the results of a 4°C stability study for the MSD GOLD Streptavidin plate format. We also demonstrate the utility of the MSD GOLD Small Spot Streptavidin plate as a tool in the early development phase of a pharmacokinetic (PK) assay for the biopharmaceutical product Adalimumab.

METHODS

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Technology

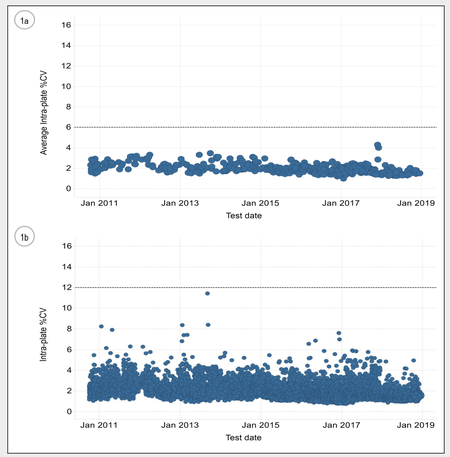
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

RESULTS

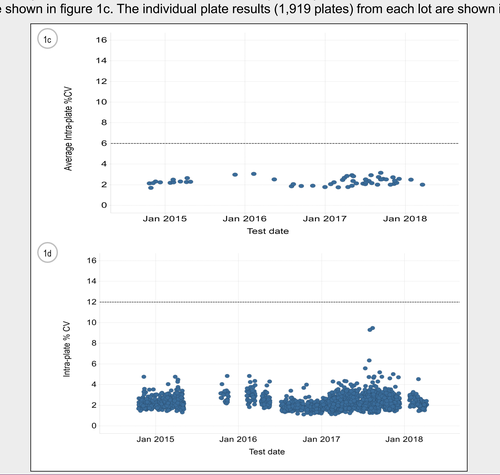
Uniformity Quality Control Results

Uniformity Measurements
Uniformity measurements for MSD GOLD Streptavidin and Small Spot Streptavidin 96-well plates are made by running whole plates with a constant amount of biotin-tagged IgG (BTI) at 0.2 pmoles of IgG per well. The mean signal and coefficient of variation (CV) is calculated for each plate (intra-plate %CV) and across plates (inter-plate %CV). Mean intra-plate CVs must be less than 6% with no plate having an intra-plate CV greater than 12%.

The mean intra-plate %CVs from 407 MSD GOLD Streptavidin lots tested between 10/2010 and 11/2018 are shown in figure 1a. The individual plate results (14,917 plates) from each lot are shown in figure 1b.



The mean intra-plate %CVs from 51 MSD GOLD Small Spot Streptavidin lots tested between 11/2014 and 3/2018 are shown in figure 1c. The individual plate results (1,919 plates) from each lot are shown in figure 1d.

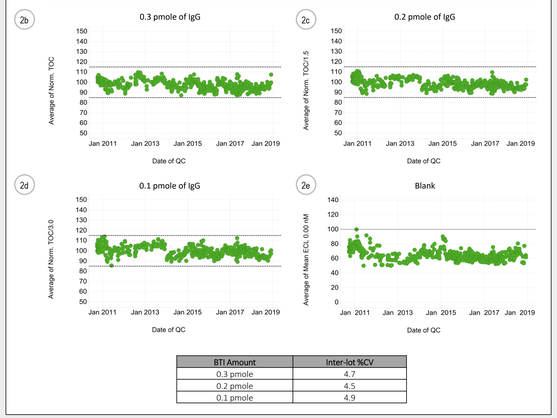


Uniformity Quality Control Results (continued)

Inter-Lot Reproducibility
To verify the inter-lot reproducibility of MSD GOLD Streptavidin 96-well plates, the BTI is measured at 0.3, 0.2, 0.1, 0.0025, and 0 pmole per well. These values correspond to typical capture antibody concentrations used in immunogenicity and PK assays (25 µL of 1 µg/mL of an antibody is 0.1667 pmoles of capture IgG). A minimum of three plates are run with the plate layout shown in figure 2a. The signal specifications at 0.3 to 0.1 pmole BTI is defined as within 15% of the established target. A reference plate lot is run with each new test lot as a control to verify proper execution of the test. The results from lots produced from 10/2010 to 11/2018 are shown in figures 2b-2e.

2a

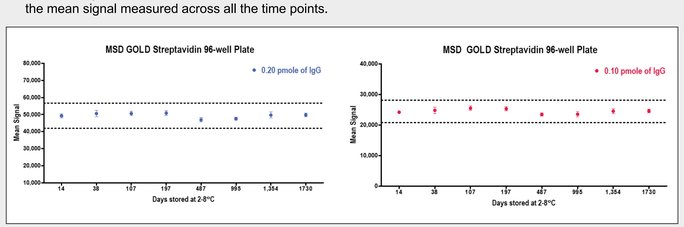
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.3	0.2	0.1	0.0025	0	Reserved	0.3	0.2	0.1	0.0025	0	Reserved
B	0.3	0.2	0.1	0.0025	0	Reserved	0.3	0.2	0.1	0.0025	0	Reserved
C	0.3	0.2	0.1	0.0025	0	Reserved	0.3	0.2	0.1	0.0025	0	Reserved
D	0.3	0.2	0.1	0.0025	0	Reserved	0.3	0.2	0.1	0.0025	0	Reserved
E	Reserved	0	0.0025	0.1	0.2	0.3	Reserved	0	0.0025	0.1	0.2	0.3
F	Reserved	0	0.0025	0.1	0.2	0.3	Reserved	0	0.0025	0.1	0.2	0.3
G	Reserved	0	0.0025	0.1	0.2	0.3	Reserved	0	0.0025	0.1	0.2	0.3
H	Reserved	0	0.0025	0.1	0.2	0.3	Reserved	0	0.0025	0.1	0.2	0.3



BTI Amount	Inter-lot %CV
0.3 pmole	4.7
0.2 pmole	4.5
0.1 pmole	4.9

Real Time Stability at 2-8°C - BTI Titration Study

Stability Measurements
A real-time stability study was performed on the 96-well MSD GOLD Streptavidin plate format. The study was conducted over a 57-month period by using a plate layout that consisted of multiple BTI concentrations tested within a single plate, with a total of 3 plates being used for each time point. The graphs below show the mean signals (n=48 replicates) collected for two different concentrations at each time point with the error bars representing the standard deviation across the replicates. The dotted lines represent a +/-15% window around the mean signal measured across all the time points.



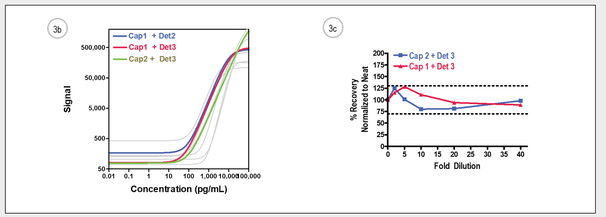
Adalimumab PK Assay Development

Primary Screening
Unbiased screening of 8 anti-Adalimumab antibodies was performed on MSD GOLD Small Spot Streptavidin plates by testing pair-wise combinations of all antibodies labeled with either biotin or MSD GOLD SULFO-TAG. The heat map shown in figure 3a is a visual representation of the signal intensities produced by each pairing. Green represents pairs with the highest signal, yellow represents pairs with signal in the 50th percentile and red represents pairs with little to no signal. From this study, 11 antibody pairs were selected based on high signal and low background (data not shown) for continued screening.

3a

	Detection Antibody							
Capture Antibodies	Detect-1	Detect-2	Detect-3	Detect-4	Detect-5	Detect-6	Detect-7	Detect-8
Capture-1	21468	270165	305754	187088	735	4088	2889	8080
Capture-2	24032	273379	283582	167445	291	5821	3902	11629
Capture-3	36719	346313	364889	204416	331	6917	4548	13596
Capture-4	8013	149696	131045	71906	757	18217	10617	33198
Capture-5	1433	3197	4237	3690	904	238	592	1137
Capture-6	4162	14598	17679	16013	156	772	1450	3777
Capture-7	4193	16330	21756	18904	344	1083	2116	5317
Capture-8	6159	23649	31127	33285	305	2915	2565	8111

Secondary Screening
The 11 antibody pairs selected during primary screening were evaluated further by testing full calibrator curves. The pairs that produced low background signals, hillslopes < 2.0, and wide dynamic ranges are highlighted in figure 3b. These 3 antibody pairs were then tested for dilution linearity, which was performed by spiking Adalimumab into pooled serum (Neat) and then serially diluting it 2, 5, 10, 20, and 40 fold. Data for 2 out of 3 final pairs is shown in figure 3c.



CONCLUSIONS

We demonstrated the lot-to-lot reproducibility of the MSD GOLD Streptavidin and MSD GOLD Streptavidin Small Spot plate by presenting uniformity results across >350 lots and >45 lots, respectively. In addition, the robustness of the MSD GOLD Streptavidin 96-well plate format, when stored at 2-8°C, was highlighted by the consistent signal generation captured in the real-time stability study conducted over a 4 year time period. For application work, we demonstrated the utility of the MSD GOLD Streptavidin plate as an effective assay development tool that could be used during the antibody selection stage of assay design.

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