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Streptavidin Plate Performance: A Five-Year Longitudinal Study of Biological Applications

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1 Abstract

Purpose: Streptavidin plates are a critical component of biomarker assays, bridging immunogenicity assays, and pharmacokinetic (PK) assays that are performed with the use of MSD's electrochemiluminescence MULTI-ARRAY® technology. MSD GOLD™ plates exhibit consistent performance across lots and over time. These plates can be utilized in a wide variety of applications and with a wide variety of biological reagents. In this study, we present a performance review of quality control results for multiple lots of MSD GOLD plates produced over the last five years. We also tested over 40 different functional assays across multiple lots of plates. Our objective was to better understand potential variability due to the different biological components used with streptavidin plates and how this variability may correlate with quality control testing.

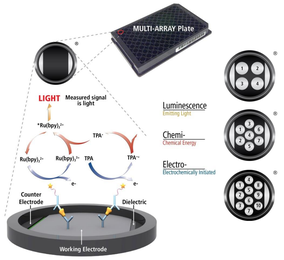
Methods: Quality control methods for releasing MSD GOLD Streptavidin plates use biotin and SULFO-TAG™ conjugated to IgG (BTI) to measure capacity and uniformity of the plates. Intra-plate reproducibility was measured by testing the entire plate with a constant amount of BTI near the plate's binding capacity (0.2 pmole of IgG). Inter-lot reproducibility was determined from BTI titration measurements across multiple plate lots. To understand variability due to different biological reagents, over 40 biomarker assays were run across six plate lots using multiple concentrations of biotinylated reagent, ranging from below to above binding capacity. Measurements included background signals as well as assay signals at low to medium analyte levels relevant for sensitive applications.

Results: The average intra-plate %CV across plates was less than 3%, with all lots below 6%. Inter-lot %CVs for the BTI titration data were less than 10% and all plates showed consistent capacity across lots. Inter-lot signal performance for BTI titration at all levels was +/-15% of the established target. When used at or below the specified plate capacity, the biological reagents exhibited comparable performance, with typical variability below 10%. Consistent backgrounds and signals across MSD GOLD Streptavidin plate lots indicated that they have the sensitivity and utility to be used with different biotinylated reagents.

Conclusion: Lot-to-lot reproducibility over an extended period of time demonstrates the longitudinal stability of MSD GOLD Streptavidin plates. Consistent lot-to-lot performance across a wide variety of biological reagents illustrates their use for a broad range of applications.

2 Methods

MSD's electrochemiluminescence (ECL) 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.

3 Quality Control Methods

The following procedures are followed during incoming material quality control (QC) and in-process QC:

Incoming Material QC

- Electrical conductivity of each plate
- Positional accuracy of the plate bottom and electrode
- Functional testing of each streptavidin and plate lot with a pilot production run to ensure:
  - Generation of electrochemiluminescence
  - Performance within final streptavidin functional QC specifications

In-Process QC

- Environmental control (humidity, temperature, and cleanliness)
- Barcode tracking throughout manufacturing process
- Automated coating and assembly
- Visual detection of dispensed fluids

Biotin and SULFO-TAG labeled IgG (BTI) is used for the functional QC test to determine both the uniformity and capacity of MSD GOLD Streptavidin plates. The functional test mimics the format of common immunogenicity and homogenous assays.

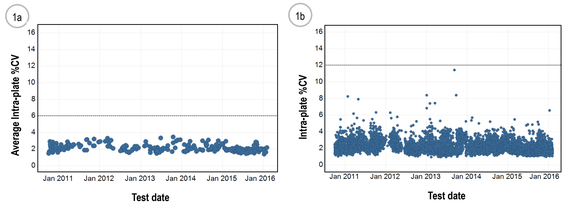
Protocol

1. Add 150 µL MSD® Blocker A. Incubate overnight at room temperature.
2. Wash with PBS-T. Add 50 µL of BTI. Incubate for 2 hours with shaking at room temperature.
3. Wash with PBS-T. Add 150 µL of Read Buffer T (2X). Read on MSD SECTOR® Imager.

4 Uniformity Quality Control Results

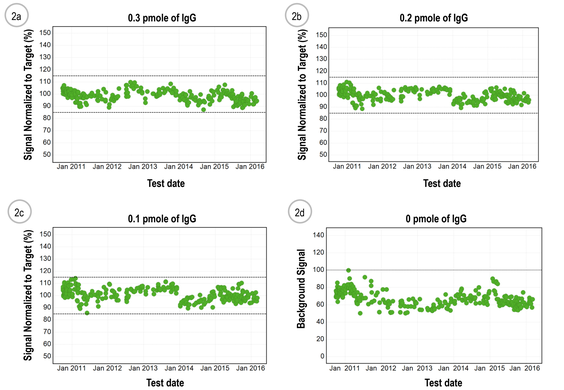
Uniformity Measurements

Uniformity measurements are made by running whole plates with a constant amount of BTI at 0.2 pmoles of IgG. 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 220 lots tested between October 2010 and February 2016 are shown Figure 1a. The results for 7905 plates tested are shown in Figure 1b.



Inter-Lot Reproducibility

To verify the inter-lot reproducibility, the BTI is measured at 0.3, 0.2, 0.1, 0.0025 and 0 pmole. 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). The signal specifications at 0.3 to 0.1 pmole BTI is defined as within 15% of the established target. The signal specification at 0 pmole is <100 ECL counts. 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 October 2010 to March 2016 are shown in Figures 2a-2d. The inter-lot %CVs across the BTI titration data was <10% (Figure 2e).



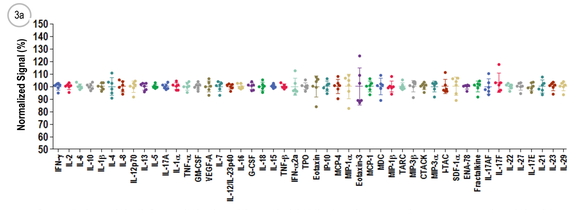
2e

Inter-lot %CVs	
BTI Amount	Inter-lot CV
0.3 pmole	4.5%
0.2 pmole	4.5%
0.1 pmole	5.2%

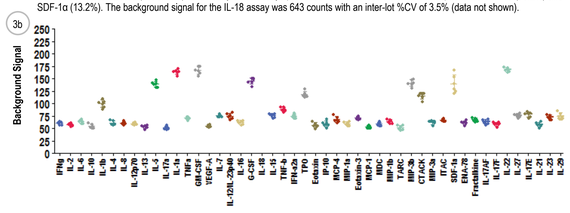
5 Inter-Lot Reproducibility using Biological Reagents

A total of 48 biomarker assays were tested across six lots of MSD GOLD Streptavidin plates. The assay format consists of a biotin-conjugated capture antibody, calibrator, and SULFO-TAG-conjugated detection antibody. Mid-to-low calibrator levels were tested to demonstrate the utility of the platform when working with low abundance analytes and the consistency of signals in the lower part of an assay's dynamic range are presented (Figure 3a). Similarly, the reproducibility of background signals and signal/background ratios are shown in Figures 3b and 3c, respectively. The amount of capture antibody used for all assays was 0.30 pmol/well, which is the recommended binding capacity of MSD GOLD Streptavidin Plates.

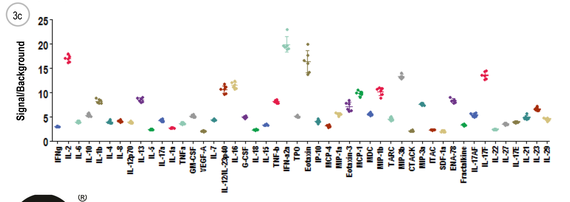
Calibrator signals (ranging from 200 to 22,000 ECL counts across all assays) were normalized to the average signal measured across six plate lots for each assay and the normalized signal from each plate lot is shown below. Each data point represents the average of three replicates on a plate, with the error bars representing the inter-lot %CVs. All assays tested produced inter-lot %CVs of less than 10% with the exception of Eotaxin-3 (14.8%).



Average background signals from each plate lot and the standard deviation (error bars) across those averages, are shown below. Inter-lot %CVs were calculated (not shown), and nearly all 48 assays produced %CVs of less than 10%. Exceptions were IP-10 (10.2%) and SDF-1α (13.2%). The background signal for the IL-18 assay was 643 counts with an inter-lot %CV of 3.5% (data not shown).

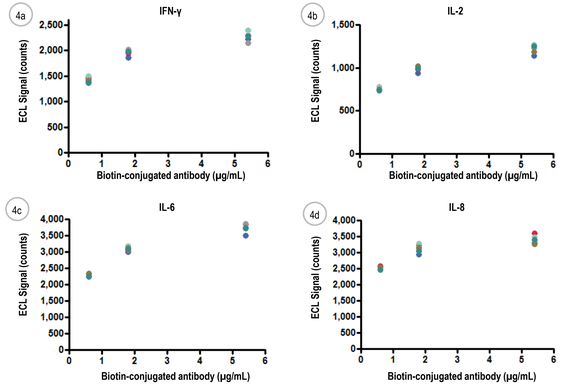


The reproducibility of the signal/background ratios is shown in Figure 3c. The average calibrator signal was divided by the average background signal from each plate and plotted below; calibrator levels used for this calculation were the lowest calibrator level tested that yielded a signal/background ratio of at least 2. The error bars represent the standard deviation across the six plate lots. Inter-lot %CVs were calculated and nearly all 48 assays produced %CVs of less than 10%. Exceptions were Eotaxin (14.1%) and Eotaxin-3 (13.1%).



6 Inter-Lot Reproducibility: Below and Above Binding Capacity

The binding capacity of MSD GOLD Streptavidin plates is 0.30 pmol/well, which equates to a biotin-conjugated capture concentration of 1.8 µg/mL when adding 25 µL to the well. To characterize the performance at different biotin-conjugated capture concentrations, a collection of biomarker assays was tested using three different capture concentrations (0.60 µg/mL, 1.8 µg/mL, and 5.4 µg/mL). Each concentration was tested across six MSD GOLD Streptavidin plate lots and the impact of capture concentration on signal is plotted in Figures 4a-4d.



The table below represents the entire titration data set collected for 24 different biomarker assays. The signal from replicates (n=3) tested across six MSD GOLD Streptavidin plate lots were averaged and inter-lot %CVs were calculated. When assays were tested below or at the binding capacity of MSD GOLD Streptavidin plates (≤ 1.8 ug/mL), the average inter-lot %CVs were 4.3% and 3.4%, respectively. For assays tested above binding capacity (5.4 ug/mL), the average inter-lot %CV increased to 6.5%.

[Biotin-capture] µg/mL	IFN-γ		IL-6		IL-1β		IL-8		IL-13		IL-17A	
	Ave. Signal	%CV	Ave. Signal	%CV	Ave. Signal	%CV	Ave. Signal	%CV	Ave. Signal	%CV	Ave. Signal	%CV
0.60	1,430	3.7	2,293	2.4	6,248	3.2	2,504	2.3	380	7.9	2,144	3.7
1.8	1,966	2.9	3,082	2.2	11,513	2.9	3,132	4.2	454	3.3	2,806	2.7
5.4	2,230	5.3	3,728	4.0	16,722	3.6	3,410	3.6	503	5.8	4,006	4.5
[Biotin-capture] µg/mL	IL-2		IL-10		IL-4		IL-12p70		IL-5		IL-1ra	
	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV
0.60	750	2.6	3,313	5.2	2,961	4.9	2,078	4.1	2,586	6.9	3,707	2.6
1.8	988	2.6	3,846	3.3	2,829	7.0	2,802	4.5	3,306	1.8	4,631	3.0
5.4	1,223	3.9	4,507	3.1	1,708	7.9	3,010	7.4	3,867	4.0	4,571	5.5
[Biotin-capture] µg/mL	TNF-α		VEGF-A		IL-12/IL-23p40		G-CSF		IL-15		IFN-α2a	
	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV
0.60	1,704	5.5	1,185	4.8	8,103	3.1	9,137	2.9	2,039	5.4	18,143	8.7
1.8	3,116	3.2	1,095	4.7	11,399	2.2	9,291	2.5	2,981	2.1	22,193	7.2
5.4	4,316	4.2	1,156	41.2	13,113	3.9	10,504	4.2	3,877	4.2	23,167	6.1
[Biotin-capture] µg/mL	GM-CSF		IL-7		IL-16		IL-18		TNF-β		TPO	
	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV	Ave. Signal	CV
0.60	9,266	4.8	3,319	3.0	5,482	3.4	13,078	4.0	7,171	4.4	5,967	3.3
1.8	11,266	2.7	4,139	3.8	10,446	2.5	14,410	4.5	10,329	2.2	8,165	3.4
5.4	11,492	5.0	4,369	4.4	13,427	5.0	13,121	6.6	13,010	5.6	8,704	7.2

7 Conclusion

The reproducibility of the MSD GOLD Streptavidin plates was demonstrated using internal quality control test methods and numerous biomarker assays.

Over a five-year time period, plates tested across over 200 lots produced average intra-lot uniformity %CVs below the specification of 6.0%, with over 97% of plates producing intra-plate %CVs below 4.0%. Titration data sets collected during this same time period also verified that the binding capacity across multiple manufactured lots remained consistent.

Further performance characterization of the MSD GOLD Streptavidin platform provided data supporting the diverse biological application of these plates. Over 40 different biological assays were characterized by testing multiple analyte and biotin-conjugated capture levels across six plates. Reproducibility within and across plates lots was assay rather than run dependent, demonstrating the utility of the platform over several applications.

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