W3081

A 3-Year Longitudinal Study of Streptavidin Plate Reproducibility

Pankaj Oberoi, John Joern, Nicolas Sammons, Danielle Russell, James Wilbur, and Jacob N. Wohlstadter

1 Abstract

Purpose: Streptavidin plates are a critical component of biomarker, bridging immunogenicity, and pharmacokinetic (PK) assays performed using MSD's electrochemiluminescence MULTI-ARRAY® technology. We present a review of the performance of over 150 lots of MSD® STREPTAVIDIN GOLD plates tested with both functional assays and quality control techniques.

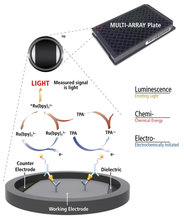
Methods: Quality control methods for releasing STREPTAVIDIN GOLD™ plates use biotin-conjugated IgG to measure capacity and uniformity of the plates. Tests were performed within binding capacity specifications (<0.3 pmole of IgG) and above the specified capacity. Six lots of plates manufactured over 12 months were tested using multiple biomarker and immunogenicity assays.

Results: The average intra-plate %CV across 4000 plates tested was less than 3.0%, with all plates below 6%. Average inter-plate %CV was 3.7%. Fewer than 0.02% of wells (20 out of 100,000) were classified as outliers (measured signals more than 20% higher or lower than the plate mean). Inter-lot %CVs for the QC data was less than 10%. All plates showed consistent capacity across lots. A real- time stability study showed no change in binding capacity or signal variability out to 36 months. Assays optimized with capture antibody outside the specifications of the plate capacity (> 0.3 pmole per well) showed lot-to-lot variability as high as 30–40%. However, all assays developed within the specifications of the binding capacity showed lot-to-lot %CV of less than 10% (typically less than 5%). The results were consistent for both bridging immunogenicity assays and biomarker assays.

Conclusion: Lot-to-lot reproducibility of MSD STREPTAVIDIN GOLD plates was demonstrated for assays developed within the plate's binding capacity specifications. Assays developed outside plate capacity specifications may exhibit lot-to-lot variability due to the introduction of excessive biotinylated capture protein or free biotin in the capture solution.

2 The MSD Platform

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.

3 Quality Control Methods

The following procedures are followed during incoming quality control and in-process quality control:

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 quality control test to determine both the uniformity and capacity of STREPTAVIDIN GOLD plates. The functional test mimics the format of common immunogenicity and homogenous assays.

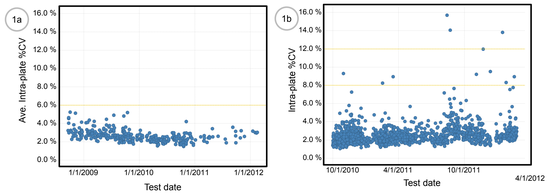
Protocol

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

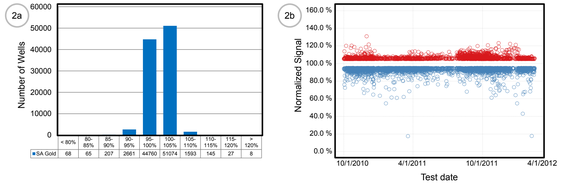
Uniformity Measurements

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 316 lots manufactured between 10/2008 and 1/2012 are shown Figure 1a. The results for 1299 plates tested after the specifications were established in 10/2010 are shown in Figure 1b. Of these 1299 plates, 3 plates have CVs greater than 12%. This triggered lot re-tests using twice as many plates as originally tested from that lot. The retest found no plates with an intra-plate %CV greater than 12%.



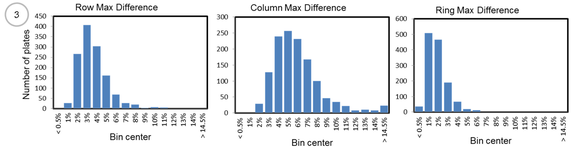
Outlier Detection

Any wells with signals greater than 20% from the plate mean are flagged. To pass the quality control test, no 2 plates may have the same well with a signal that is greater than 20% away from their respective plate means. A single well greater than 50% away from the plate mean would cause a re-test of the lot. A histogram of all wells from the 1048 plates (100,608 wells) tested is shown in Figure 2a. Only 4.7% of the wells were greater than 5% from the plate mean. These wells are shown in Figure 2b. Only 76 wells out of 100,608 wells (0.075%) were greater than 20% from the plate mean. The standard deviation of all normalized signals, across plates, was 2.67%.



Patterns

To show uniformity and lack of patterns, the median and CVs were computed for rows, columns, and rings. The difference between the maximum and minimum medians for each must be less than 10% with no more than 1 out of 12 plates falling between 10 and 15%. Histograms of the differences for the 1299 plates is shown in Figure 3. The percentage of plates with > 10% maximum difference was 0.85%, 6.7%, and 0.08% for rows, columns, and rings, respectively.



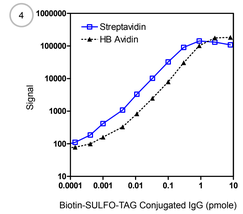
Metric	Specification
Mean intra-plate CV	≤ 6%
Number of plates with intra-plate CV >12%	0 plates
Inter-plate CV	≤ 8%
Intra-plate CVs (92% of plates tested)	≤ 8%
Plates where signal > 20% from plate mean occurs in same well on multiple plates	0 plates
Wells with signal > 50% from plate mean	0 plates
Median signal for concentric rings, max to min range	≤ 10%
Median signal for rows, max to min range	≤ 10%
Median signal for columns, max to min range	≤ 10%
Number of plates sampled (distributed across lot)	1-2 %

5 Binding Capacity and Inter-Lot Reproducibility

Plate Binding Capacity

The binding capacity of the plates was determined by titrating the amount of BTI. Typical curves from STREPTAVIDIN GOLD and High Bind AVIDIN GOLD™ plates are shown in Figure 4. The table below shows the titration across 6 lots of STREPTAVIDIN GOLD plates. The plate specifications are 0.3 and 0.9 pmoles for Streptavidin and High Bind Avidin plates respectively.

BTI (pmole)	Inter-Lot Average	Inter-Lot %CV
1	143544	5.11%
0.8	137940	4.40%
0.6	129306	4.08%
0.4	104465	1.27%
0.3	83271	1.52%
0.2	57548	1.34%
0.1	28918	2.89%
0.0025	710	6.21%

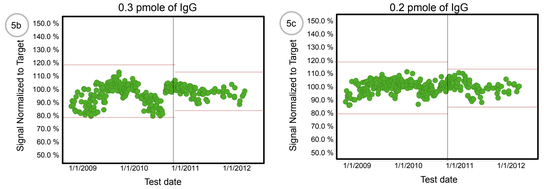


Inter-Lot Reproducibility

To verify the inter-lot reproducibility, the BTI is measured at 0.3, 0.2, 0.1, 0.05 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). A minimum of 3 plates are run with the plate layout shown figure 5a. With the release of the STREPTAVIDIN GOLD specification on 10/2010 (indicated by the vertical dashed line), the signal specifications at the 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 10/2008 to 3/2012 are shown in the figures 5b-5e.

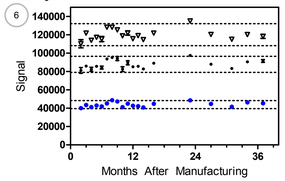
5a

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.3	0.2	0.1	0.025	0	Reserved	0.3	0.2	0.1	0.025	0	Reserved
B	0.3	0.2	0.1	0.025	0	Reserved	0.3	0.2	0.1	0.025	0	Reserved
C	0.3	0.2	0.1	0.025	0	Reserved	0.3	0.2	0.1	0.025	0	Reserved
D	0.3	0.2	0.1	0.025	0	Reserved	0.3	0.2	0.1	0.025	0	Reserved
E	Reserved	0	0.025	0.1	0.2	0.3	Reserved	0	0.025	0.1	0.2	0.3
F	Reserved	0	0.025	0.1	0.2	0.3	Reserved	0	0.025	0.1	0.2	0.3
G	Reserved	0	0.025	0.1	0.2	0.3	Reserved	0	0.025	0.1	0.2	0.3
H	Reserved	0	0.025	0.1	0.2	0.3	Reserved	0	0.025	0.1	0.2	0.3



6 Real Time Stability of Plates

Real time stability was performed over a 37 month period. At regular intervals, binding capacity measurements were made to assess the performance of the plates. The signals at 0.3, 0.2, and 0.1 pmoles of IgG were within 10% of the mean signal during the stability study (dashed lines) and well within the 15% specifications of new plates. The graph shows the signals over the 37 month period with the dotted lines representing ±10% from the mean signals.



7 Functional Assay Performance

Pharmacokinetics and Pharmacodynamic Assays

A biomarker assay for rat BNP was developed using the STREPTAVIDIN GOLD plates. The assay format consists of a biotin-conjugated monoclonal antibody used at 0.167 pmoles (25 ul of 1 µg/ml), calibrator or serum based control, and detection antibody at 25 µl of 1 µg/ml. The assay performance was tested across 6 lots of STREPTAVIDIN GOLD plates manufactured over a 2-year period. Inter-lot %CVs for both the calibration curve signals and control concentrations are well below 10%.

	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5		Lot 6		
Assigned Conc. (pg/mL)	Ave. Counts	% CV	Ave. Counts	% CV	Ave. Counts	% CV	Ave. Counts	% CV	Ave. Counts	% CV	Ave. Counts	% CV	% Inter-lot CV
2000	114637	2.8	117896	1.5	116974	2.0	103318	2.1	108256	0.9	114056	1.4	5.0
667	31952	2.9	34065	2.0	33977	0.8	29575	1.0	31519	1.9	32823	1.8	5.2
222	9335	1.9	9934	2.0	9963	1.5	8921	1.6	9395	2.3	9962	0.9	4.5
74.1	2950	2.4	3173	2.4	3205	1.3	2808	1.9	3011	1.8	3097	2.2	4.9
24.7	1022	1.0	1101	1.1	1107	2.8	975	1.5	1055	2.7	1098	0.8	5.0
8.23	400	1.6	427	0.9	430	2.6	377	2.1	406	0.9	421	1.0	4.9
2.74	177	0.6	194	0.6	193	1.3	172	2.9	178	6.3	193	1.4	5.4
0	68	7.6	79	2.5	79	2.2	73	7.6	78	5.3	78	4.5	5.9

	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5		Lot 6			
	Assigned Conc. (pg/ml)	Calc. Conc. (pg/mL)	% CV	Calc. Conc. (pg/mL)	% CV	Calc. Conc. (pg/mL)	% CV	Calc. Conc. (pg/mL)	% CV	Calc. Conc. (pg/mL)	% CV	Calc. Conc. (pg/mL)	% CV%	% Inter-lot CV
Control 1	426	440.9	1.5	443.8	3.1	440.3	2.3	458.0	2.8	448.5	2.4	440.6	2.4	1.6%
Control 2	73	79.6	2.2	81.6	2.2	80.7	2.0	84.8	2.1	81.2	2.5	81.2	2.2	2.1%
Control 3	25	26.8	2.5	27.0	1.9	26.8	0.6	28.2	1.2	27.4	1.7	25.9	9.7	2.8%

Immunogenicity Assays

A common source of assay variability is excessive biotin-conjugated capture. We developed a bridging immunogenicity assay and titrated the capture antibody on several lots of STREPTAVIDIN GOLD Plates. (Two lots are shown below, Z0020365 and Z0020367.) When the amount of capture material is below 0.3 pmoles, the signals between the lots are within 10% of one another. Above the specified capacity of the plates, the signals can vary between lots.

	Lot Z0020365				Lot Z0020367				Ratio 365/367			
	Amount of Biotin Drug (pmole)				Amount of Biotin Drug (pmole)				Amount of Biotin Drug (pmole)			
	0.67	0.33	0.16	0.08	0.67	0.33	0.16	0.08	0.67	0.33	0.16	0.08
Conc ADA (ng/ml)	Mean Signal	Mean Signal	Mean Signal	Mean Signal	Mean Signal	Mean Signal	Mean Signal	Mean Signal	% Signal	% Signal	% Signal	% Signal
3000	672799	921584	697940	326944	905496	1015713	725475	225,368	74%	91%	96%	145%
1000	238954	354370	341486	248177	307582	386388	342331	241,492	78%	92%	100%	103%
100	23900	38518	38503	30657	32890	40968	37021	29,773	73%	94%	104%	103%
10	3081	4362	4192	3348	4065	4649	4040	3,110	76%	94%	104%	108%
1	977	947	727	521	1199	991	689	487	81%	96%	106%	107%
0.1	777	615	387	240	930	638	362	219	84%	96%	107%	110%
0.01	763	579	359	205	896	604	375	188	85%	96%	96%	109%
0	772	573	348	212	866	598	325	184	89%	96%	107%	115%

8 Conclusions

The plates are a critical reagent for immunogenicity and pharmacokinetics assays. We have demonstrated reproducibility within plates (<6% intra-plate CV) and lots (<8% inter-plate CV) as well as between lots (<15% inter-lot). The performance of these plates has been demonstrated over several hundred lots of plates with more than 10,000 plates tested. The plates exhibit real-time stability over more than 36 months.

Inter-lot and inter-plate variability may be related to specific biological interactions, variability in critical reagents, or diluents used. Well-developed assays can produce single-digit %CVs across multiple lots. Excessive capture material (greater than specifications) is one potential source of inter-lot variability.

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Metric	BTI Amount	STREPTAVIDIN GOLD Plate Average Signal	STREPTAVIDIN GOLD Plate Average Signal (SECTOR PR®)	High Bind AVIDIN GOLD Plate Average Signal
Control Lot R0010192	0.9 pmole)	N/A	N/A	134201 +/- 15%
	0.6 pmole	N/A	N/A	101374 +/- 15%
	0.3 pmole	82211 +/- 15%	69960 +/- 15%	54876 +/- 15%
	0.2 pmole	55593 +/- 15%	48229 +/- 15%	N/A
	0.1 pmole	26330 +/- 15%	23837 +/- 15%	N/A
Zero biotin	0	≤ 100 counts	≤ 235 counts	≤ 100 counts