

# MSD GOLD™ Streptavidin Products: Demonstration of Reproducibility and Extended Stability on Multiple Plate Formats

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## 1 Abstract

**Purpose:** Streptavidin-coated plates are a widely-used tool for use in biomarker measurements 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. In past studies, we have demonstrated the uniformity, reproducibility and stability of 96-well MSD GOLD Streptavidin plates for a variety of biological applications. Here we present new data demonstrating consistent performance over even longer durations when stored at 4°C as well as stability studies showing the feasibility of storing streptavidin and avidin plates at room temperature for multiple plate formats (96- and 384-well), which will allow for more flexibility in usage.

**Methods:** Precision, accuracy, binding capacity, and uniformity were evaluated for 96-well MSD GOLD Streptavidin plates. Quality control tests utilized biotin and SULFO-TAG™ label conjugated to IgG (BTI) as a surrogate for an assay using a biotinylated capture reagent. 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) and inter-lot reproducibility was determined from BTI titration measurements across multiple plate lots. Real time stability was determined by conducting quality control tests at regular intervals over an extended period of storage time: 45 months at 2-8°C and 24 months at room temperature.

**Results:** Based on quality control data collected over more than six years, the average intra-plate %CV (coefficient of variation) across plate lots was less than 3% (> 10,000 plates tested), with all lots below 6% (> 270 lots tested). Inter-lot %CVs for the BTI titration data were less than 10% and all plates showed consistent binding capacity across lots. Inter-lot signal performance for BTI titration at all levels was ± 15% of the established target. In stability studies, BTI signal at several levels across the assay range remained constant, indicating equivalent performance throughout the duration of the study at both 2-8°C and room temperature.

**Conclusion:** Lot-to-lot reproducibility as well as consistent performance of individual lots over an extended period of time demonstrated the longitudinal stability of MSD GOLD Streptavidin plates and related MSD GOLD plate formats. These results confirmed the robustness of the coated plates, which directly impacts users, enabling greater flexibility, utility, and consistency for a wide variety of biological applications.

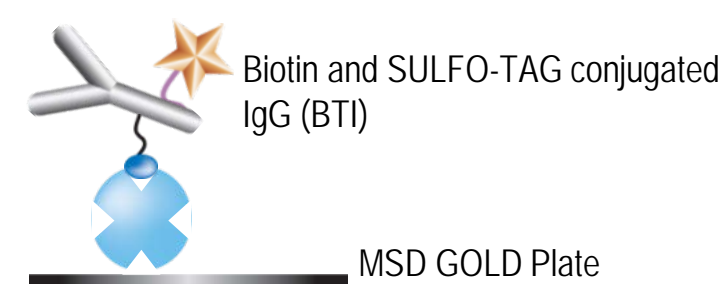
## 2 Methods

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. MSD GOLD 96-well plates conform to rigorous specifications for precision, accuracy, binding capacity, and uniformity. The procedures followed during incoming quality control and in-process quality control for the MSD GOLD plates are summarized below.

- Incoming Material QC:**
- Electrical conductivity of each plate
  - Positional accuracy of the plate bottom and electrode
  - Functional testing of each plate lot with a pilot production run to ensure:
    - Generation of electrochemiluminescence
    - Performance within final 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 MSD 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 instrument.

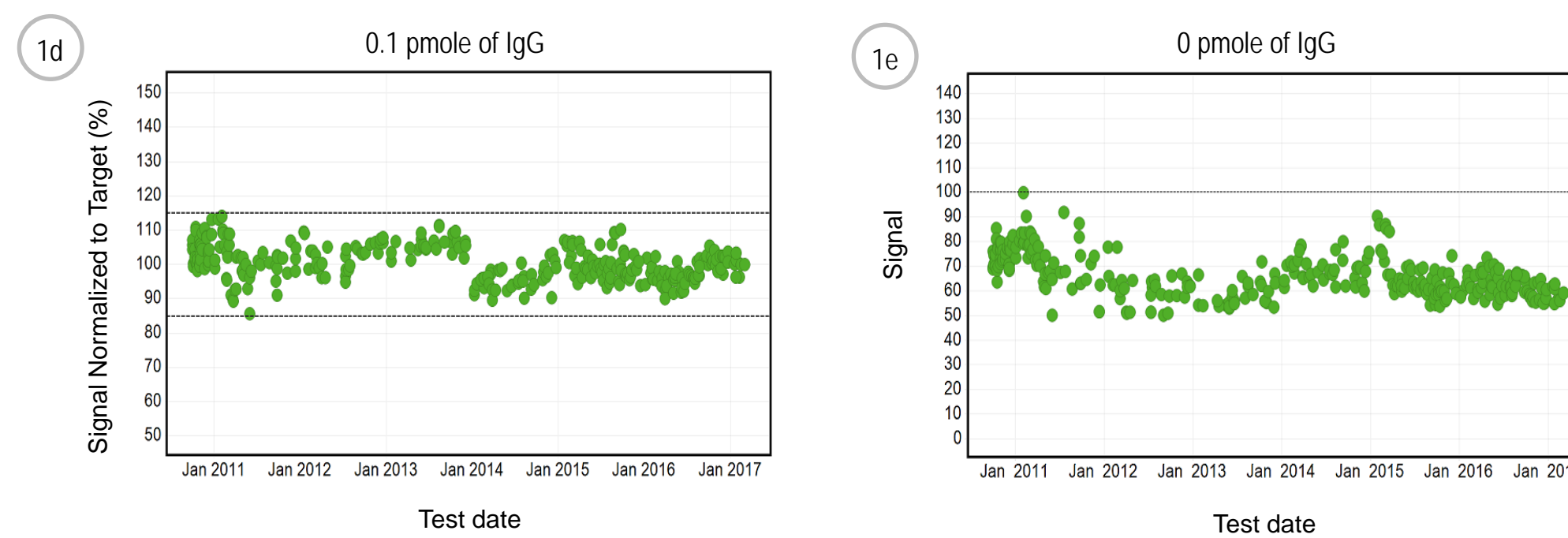
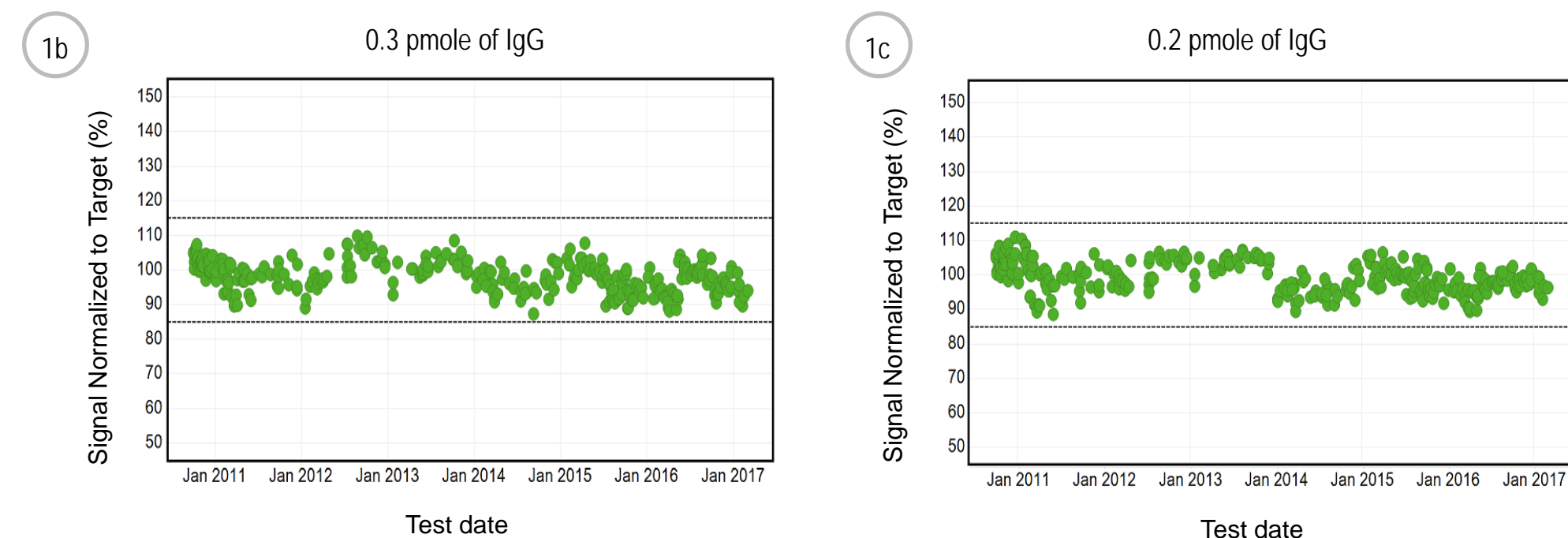
## 3 Uniformity Quality Control Results

### Inter-Lot Reproducibility

To verify the inter-lot reproducibility of MSD GOLD Streptavidin 96-well plates, the BTI was 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). A minimum of three plates were run with the plate layout shown in figure 1a. 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 was 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 01/2017 are shown in figures 1b-1e. The inter-lot %CVs across the BTI titration data was <10% (figure 1f).

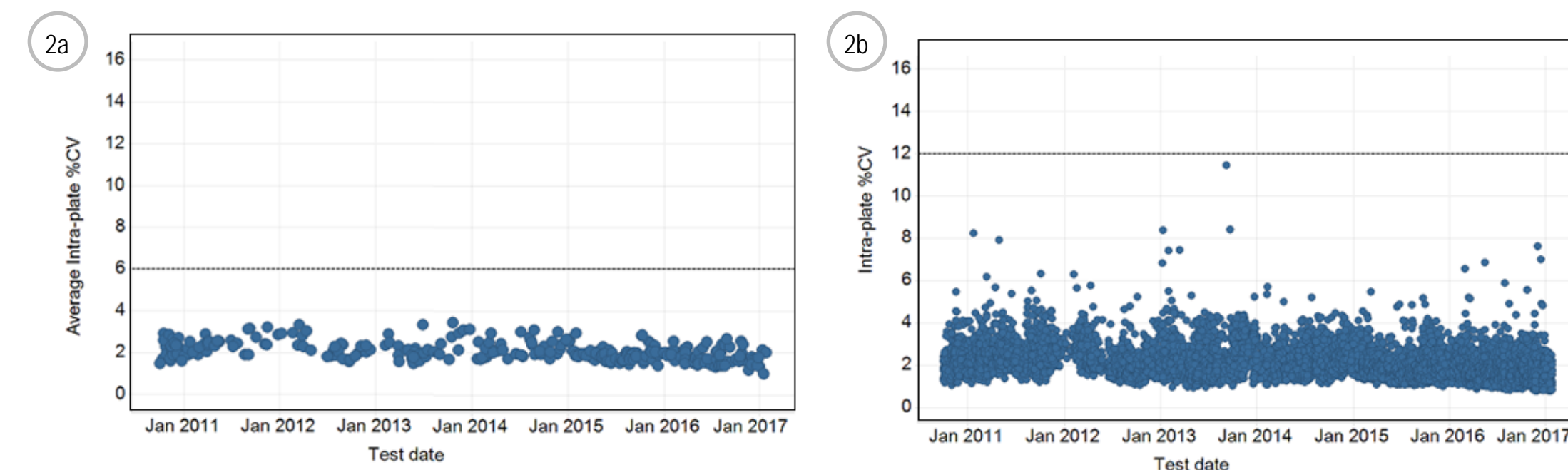
1a

	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



### Uniformity Measurements

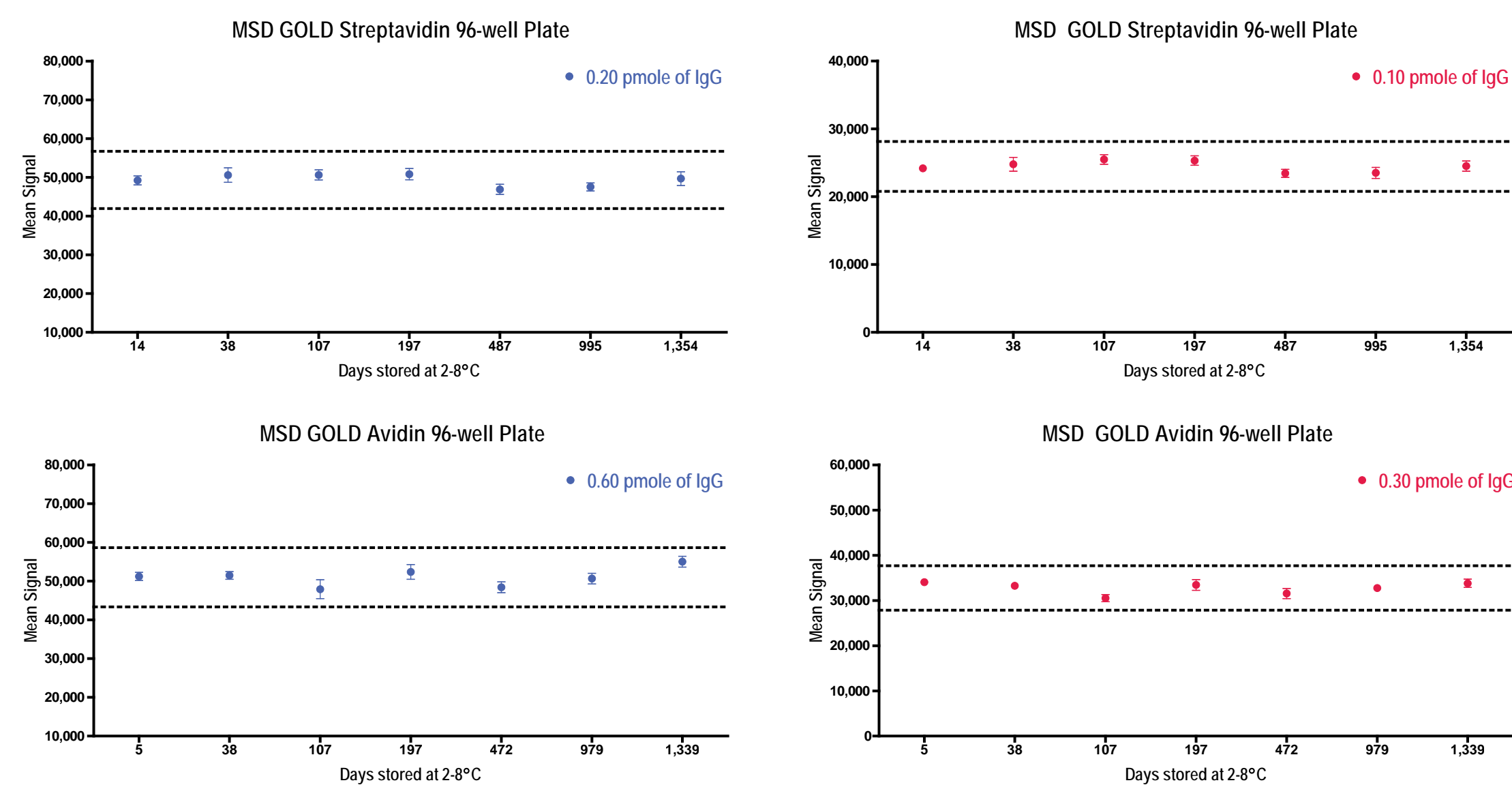
Uniformity measurements for MSD GOLD Streptavidin 96-well plates were made by running whole plates with a constant amount of BTI at 0.2 pmoles of IgG. The mean signal and %CV was calculated for each plate (intra-plate %CV) and across plates (inter-plate %CV). Mean intra-plate CVs must be less than 6% with no plate having an intra-plate CV greater than 12%. The mean intra-plate %CVs from 286 lots tested between 10/2010 and 01/2017 are shown in figure 2a. The results for 10,715 plates tested are shown in figure 2b. The overall %CV was 2.0 for both intra-plate and inter-plate data.



## 4 Real Time Stability at 2-8°C - Titration Study

### Stability Measurements

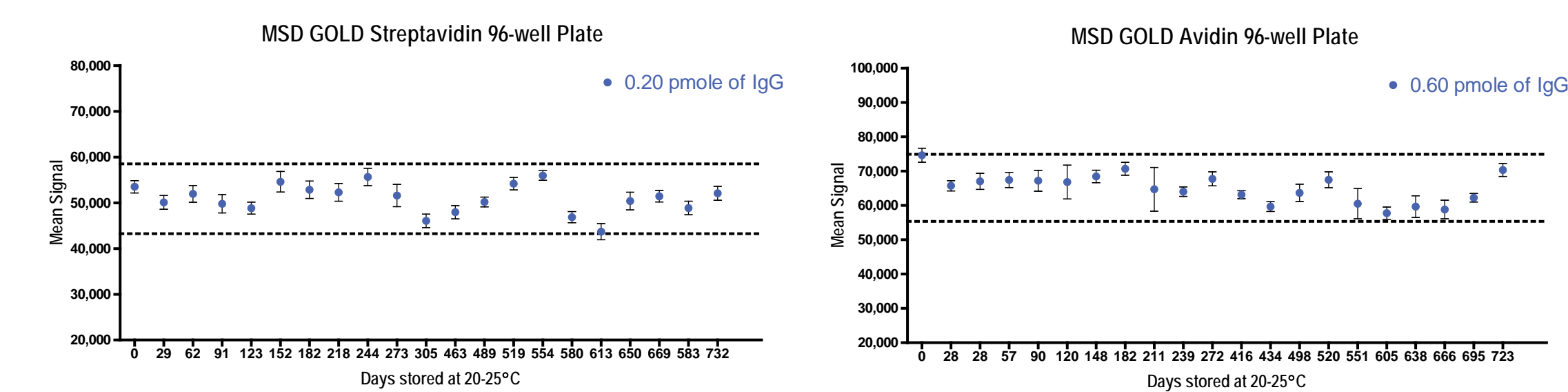
Real-time stability studies were performed on the 96-well plate formats for the MSD GOLD product line. The study was conducted over a 45-month period by using a plate layout that consisted of multiple BTI concentrations tested within a single plate, with a total of three plates used for each time point. 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.



## 5 Real Time Stability at Room Temperature – Whole Plate Study

### Stability Measurements

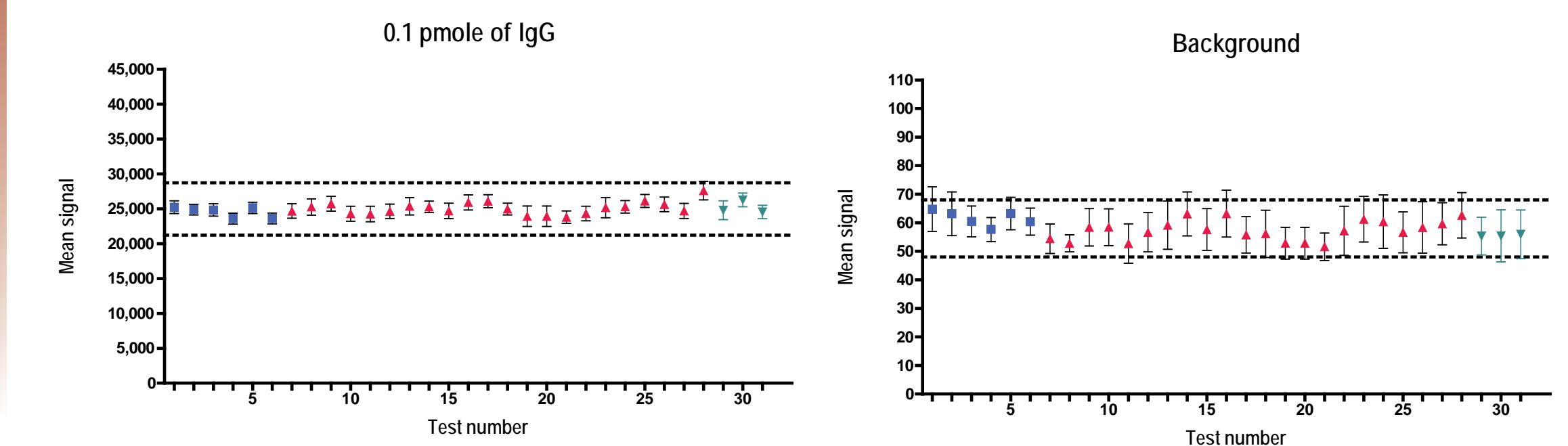
Real-time stability studies were performed on the 96-well plate format for the MSD GOLD product line. The study was conducted over a 24-month period using a plate layout that consisted of a single BTI concentration tested across the entire plate, with a total of three plates used for each time point. The graphs below show the mean signals (n=288 replicates) collected 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. The average laboratory temperature recorded during the study was 22.3°C, with minimum and maximum recorded temperatures of 16°C and 28.8°C, respectively.



## 6 Performance of MSD GOLD Read Buffer

### Signal Reproducibility

The MSD GOLD designation for the coated plate product line certifies that the plates meet high specifications for reproducibility. MSD GOLD Read Buffer is an essential component of MSD immunoassays and has stringent quality control release criteria that ensure lot-to-lot reproducibility. The data sets below demonstrated the signal reproducibility of three independent lots of MSD GOLD Read Buffer when tested on a single MSD GOLD Streptavidin plate lot across multiple test days. Each data point is the mean signal measured across three plates and the error bars represent the standard deviation across all the replicates (n=48 replicates). The dotted lines represent a +/-15% window around the mean signal measured across the entire data set.



## 7 Conclusion

The MSD GOLD Streptavidin and Avidin product line continues to demonstrate a high level of consistency across multiple test methods. We demonstrated the lot-to-lot reproducibility of the plates by presenting uniformity and binding capacity results across six years of plate production (>270 lots). In addition, the steady performance of individual plate lots tested under MSD's Real-Time Stability Program highlighted the robustness of both the streptavidin- and avidin-coated plates when stored at either 2-8°C or room temperature. Signal reproducibility data for MSD GOLD Read Buffer also demonstrated the same uniform performance that is essential for critical assays and longitudinal studies.



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