M1037

Demonstration of U-PLEX® Assay Platform as a Tool for Rapid Immunoassay Development

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1 Abstract

Purpose: In the rapidly expanding field of biomarkers, methods to identify immunoassay reagents and optimize their use can be rate limiting for product development programs. Flexible tools for assay development, from antibody screening and selection to assay feasibility, can be used to accelerate such programs. We demonstrated the use of the MSD® U-PLEX assay platform to conduct early assay development steps in parallel in a multiplex format. This resulted in rapid identification of a multiplexed panel of compatible assays.

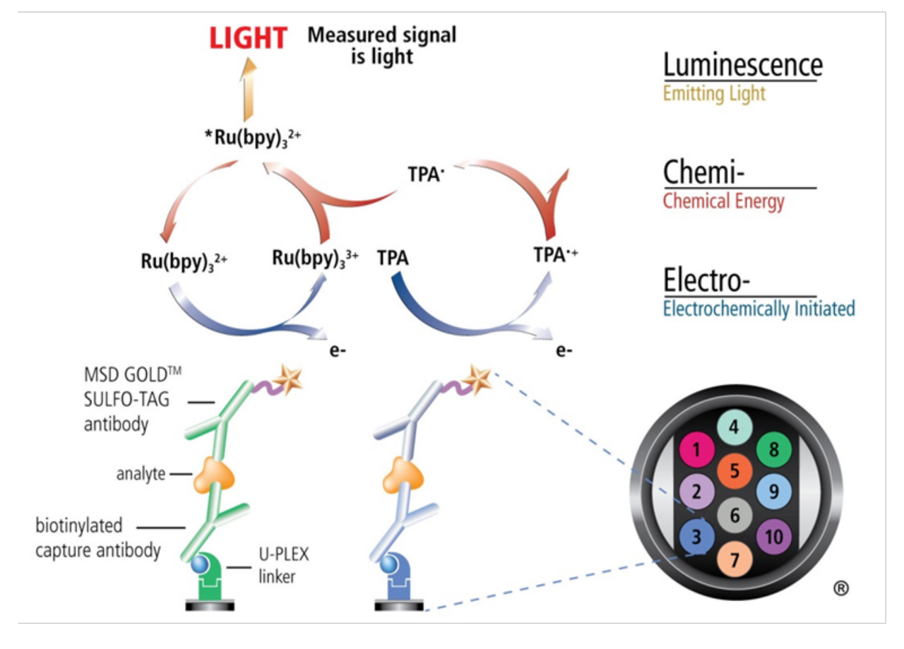
Methods: Unbiased pairwise screening of 59 antibodies for a single analyte was performed on MULTI-SPOT® U-PLEX plates using biotinylated capture antibodies and detection antibodies conjugated with SULFO-TAG™ label. Feasible antibody pairs were identified based on high signal and low background, then ranked using parameters such as dynamic range, sensitivity, specificity, sample recognition, and matrix tolerance. Due to the flexible nature of the U-PLEX platform and the ability to mix and match reagents, these same conjugated antibody pairs were used to rapidly optimize multiplex assay panels. Optimized parameters included calibration ranges, assay protocol, detection antibody concentrations, and non-specific binding.

Results: Out of a total of 3,481 possible antibody combinations tested on the U-PLEX platform, 38 antibody pairs were selected for additional feasibility testing. Evaluation of these pairs, again on the U-PLEX platform, identified two antibody pairs with broad dynamic range, high sensitivity (estimated LOD of 0.5 pg/mL), recovery of spiked samples within 20% of expected values, and the ability to recognize native analyte in several relevant matrices. The total time for development and characterization of assays from this large screen was four weeks, but this timeframe can be reduced to days for smaller screens with fewer antibodies. Further assay optimization on the U-PLEX platform ultimately yielded an immunoassay with sensitivity of less than 1 pg/mL, performance within a 3-4 log dynamic range, and less than 0.5% non-specific binding against other U-PLEX assays for biologically-related analytes.

Conclusion: We demonstrated the utility of a flexible assay development system that allows rapid screening of antibodies and optimization of subsequent assays for stand-alone use or in multiplexed combinations.

2 Principle of the Assay

Biotinylated capture antibodies are coupled to one of ten unique U-PLEX linkers, which self-assemble onto unique spots on the U-PLEX plate. Analytes in the sample bind to the capture reagents; detection antibodies conjugated with MSD's electrochemiluminescent (ECL) labels (MSD GOLD™ SULFO-TAG) bind to the analytes to complete the sandwich immunoassay (shown below). Once the sandwich immunoassay is complete, the U-PLEX plate is loaded into the MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample.


LIGHT
Measured signal is light
*Ru(bpy)₃²⁺
TPA·
Ru(bpy)₃²⁺
Ru(bpy)₃³⁺
TPA
TPA·⁺
e-
e-
Luminescence
Emitting Light
Chemi-
Chemical Energy
Electro-
Electrochemically Initiated
MSD GOLD™ SULFO-TAG antibody
analyte
biotinylated capture antibody
U-PLEX linker
1
2
3
4
5
6
7
8
9
10

U-PLEX immunoassay on the U-PLEX 10-assay plate

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 U-PLEX Assay Protocol

STEP 1: Create Individual U-PLEX Coupled Antibody Solutions
Add 200 µL of each biotinylated antibody to 300 µL of the assigned linker. Incubate at room temperature (RT) for 30 minutes.
Add 200 µL of Stop Solution. Incubate at RT for 30 minutes.

STEP 2: Prepare the Multiplex Coating Solution
Combine 600 µL of each U-PLEX coupled antibody solution. Up to 10 U-PLEX coupled antibodies can be pooled.
Bring the solution up to 6 mL with Stop Solution.

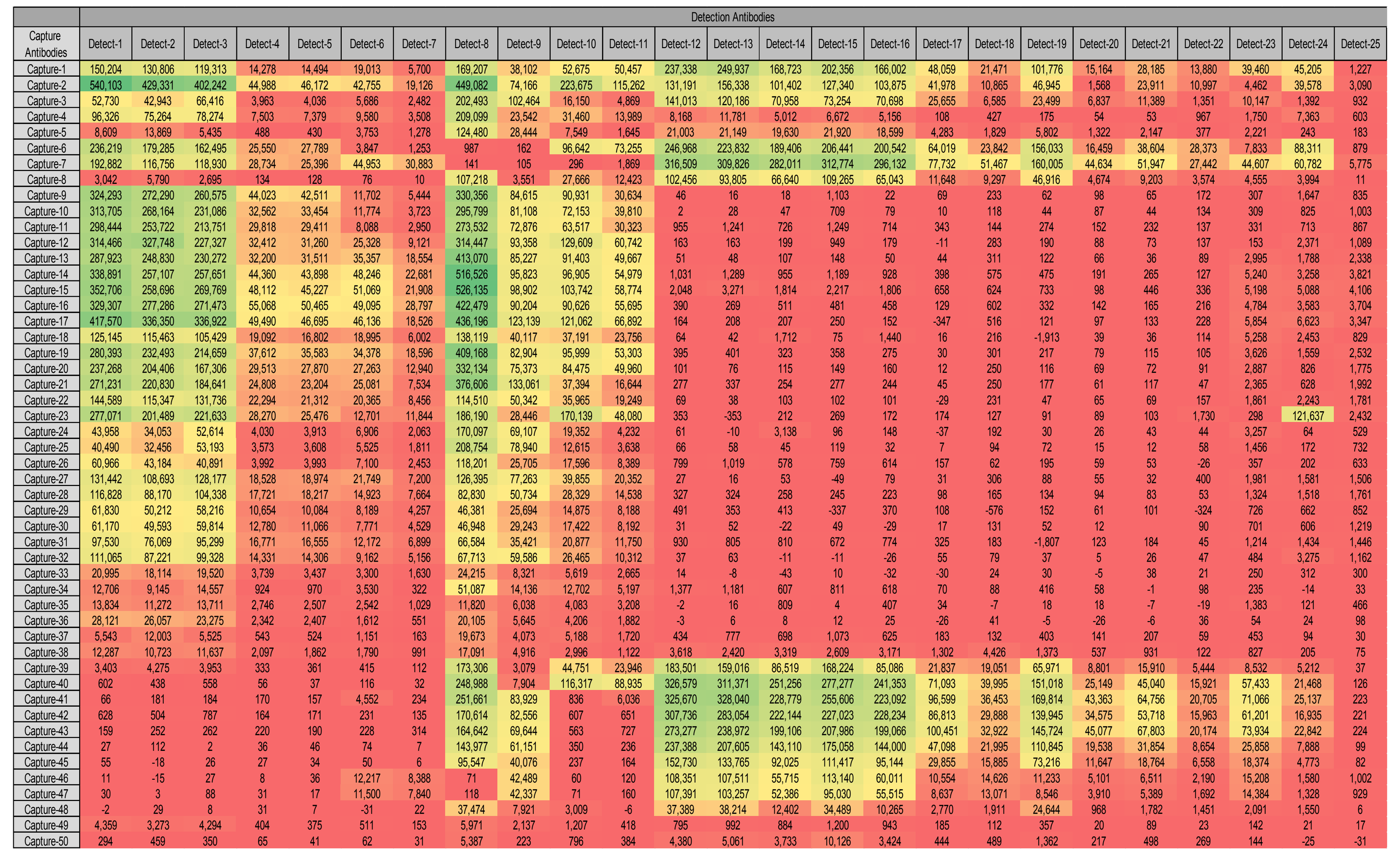
STEP 3: Coat the U-PLEX Plate
Add 50 µL of multiplex coating solution to each well. Seal the plate and incubate at RT while shaking for 1 hour.
Wash the plate 3 times with 150 µL/well Wash Buffer.

STEP 4: Add Sample or Calibrator Standard
Add 25 µL of appropriate diluent to each well. Add 25 µL of prepared sample or calibrator standard to each well.
Seal the plate and incubate at RT with shaking for 1 hour.

STEP 5: Wash and Add Detection Antibody Solution
Wash plate 3 times with 150 µL/well of Wash Buffer.
Add 150 µL of detection antibody solution to each well. Seal the plate and incubate at RT with shaking for 1 hour.

STEP 6: Wash and Read
Wash plate 3 times with 150 µL/well of Wash Buffer.
Add 150 µL 2X Read Buffer T to each well. Analyze plate on the MSD instrument.

4 Screening for Viable Antibody Pairs Using the U-PLEX Platform



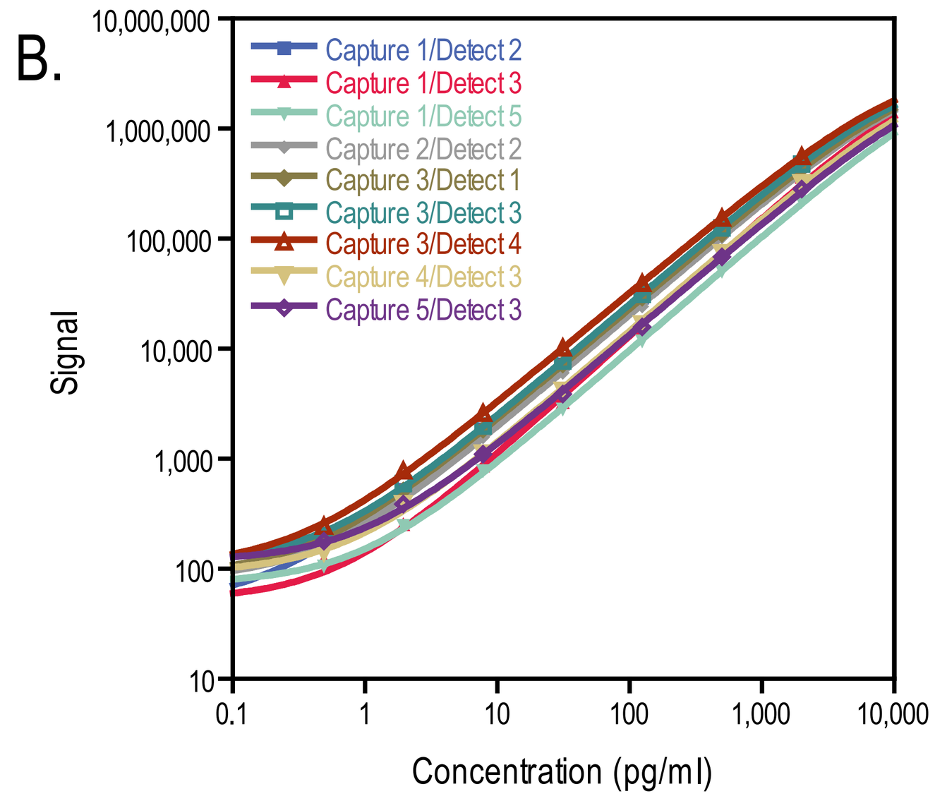
Unbiased screening of 59 antigen-specific antibody pairs on the U-PLEX platform. Pair-wise combinations of all antibodies labeled with biotin or MSD GOLD SULFO-TAG were evaluated for their ability to produce robust signal in the presence of calibrator and low signal in the absence of calibrator (diluent only). Shown is a heat map of selected signal data from this study arranged to allow visualization of antibody pairs that generated the highest signals. Green represents pairs with the highest signals, yellow represents pairs with signal in the 50th percentile and red represents pairs with low or no signal. 38 antibody pairs were selected from this screen based on high signal and low background (data not shown) for continued screening.

5 Secondary Screening of Selected Antibody Pairs on the U-PLEX Platform

A.

Captures	Detects	Hillslope	LLOD	S/B ratio at Cal-4
Capture-1	Detect-1	1.2	1.5	43
	Detect-2	1.0	0.3	157
	Detect-3	1.1	0.84	68
	Detect-4	1.3	3.2	27
	Detect-5	1.0	0.94	38
	Detect-6	1.0	1.1	76
Capture-2	Detect-1	1.2	1.9	29
	Detect-2	1.0	0.42	75
	Detect-7	1.1	1.4	63
	Detect-8	1.1	1.3	37
	Detect-4	1.3	2.8	17
	Detect-5	1.1	1.2	19
Capture-3	Detect-1	1.0	0.35	82
	Detect-9	1.3	3.5	12
	Detect-2	1.0	0.34	69
	Detect-3	1.0	0.24	96
	Detect-4	1.3	2.9	14
	Detect-10	1.2	3.4	12
	Detect-11	1.6	25.2	2
	Detect-5	1.3	4.9	6
	Detect-12	1.2	4.1	11
	Detect-13	1.1	1.6	16
	Detect-14	1.2	2.5	16
	Detect-15	1.4	6.1	9
Capture-4	Detect-1	1.3	6.4	7
	Detect-9	1.8	24.6	2
	Detect-15	1.0	0.61	46
	Detect-13	1.1	1.2	31
	Detect-14	1.2	2	18
	Detect-15	1.3	3.1	21
Capture-5	Detect-1	1.2	5.8	8
	Detect-9	1.6	19.4	3
	Detect-15	1.0	0.78	34
	Detect-13	1.1	1.4	22
	Detect-14	1.2	1.4	7
	Detect-15	1.2	2.3	7
Capture-6	Detect-16	1.0	1.3	39
	Detect-14	1.1	2.8	14

B.



Secondary screening on the U-PLEX platform was used to evaluate full standard curves of 38 selected antibody pairs. A) Summary results from secondary screening of selected antibody pairs. Individual hillslopes, lower limit of detection (LLOD), and signal to background ratio at Cal-4 were tabulated. A total of nine antibody pairs with desirable background signals, sensitivity, and hillslope were selected (highlighted in yellow). B) Standard curves for selected assays are graphically displayed demonstrating that all assays have broad dynamic range across several logs of signal.

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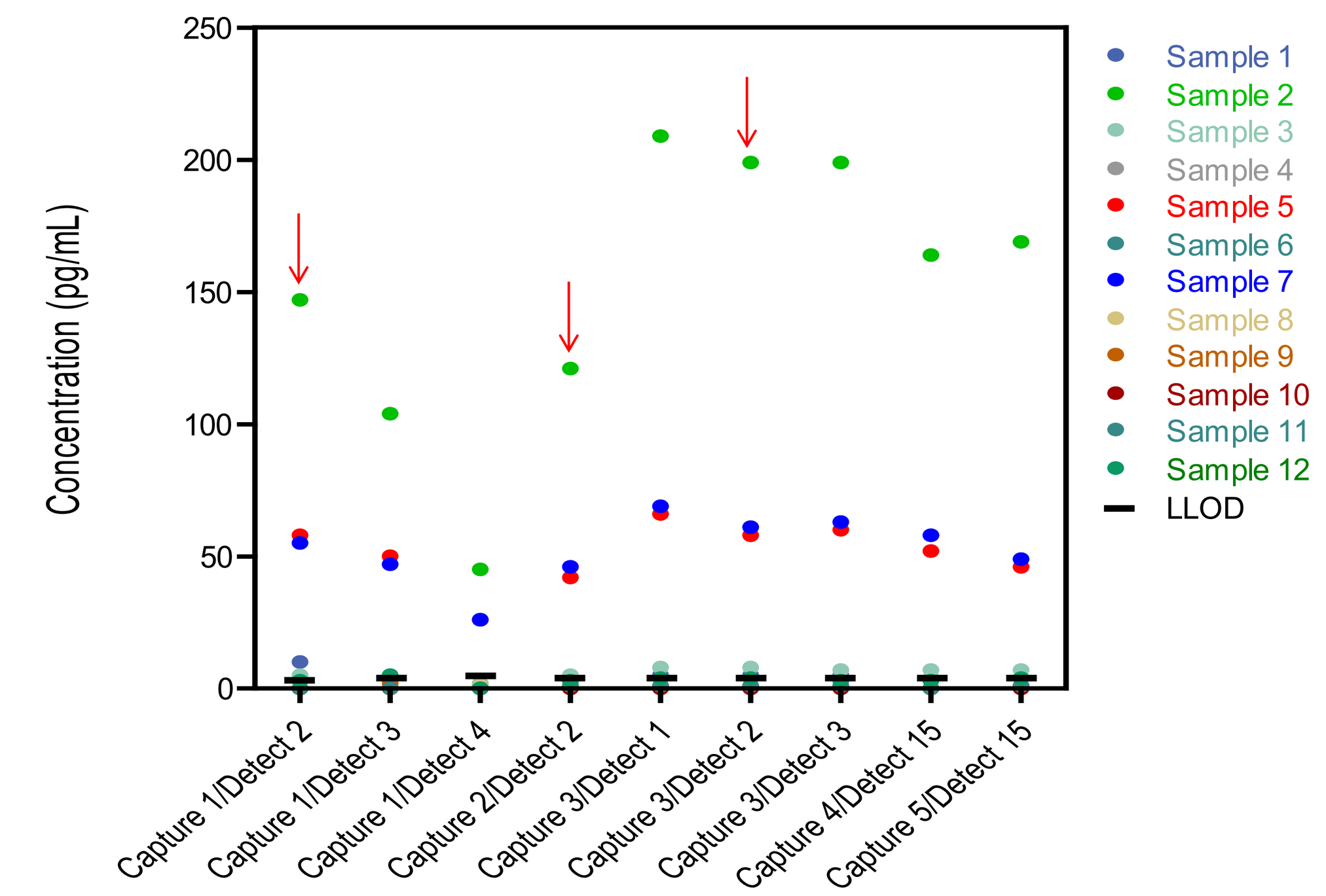
6 Sample Testing

A.


Concentration (pg/mL)
Sample 1
Sample 2
Sample 3
Sample 4
Sample 5
Sample 6
Sample 7
Sample 8
Sample 9
Sample 10
Sample 11
Sample 12
LLOD
Capture 1/Detect 2
Capture 1/Detect 3
Capture 1/Detect 4
Capture 2/Detect 2
Capture 3/Detect 1
Capture 3/Detect 2
Capture 3/Detect 3
Capture 4/Detect 15
Capture 5/Detect 15

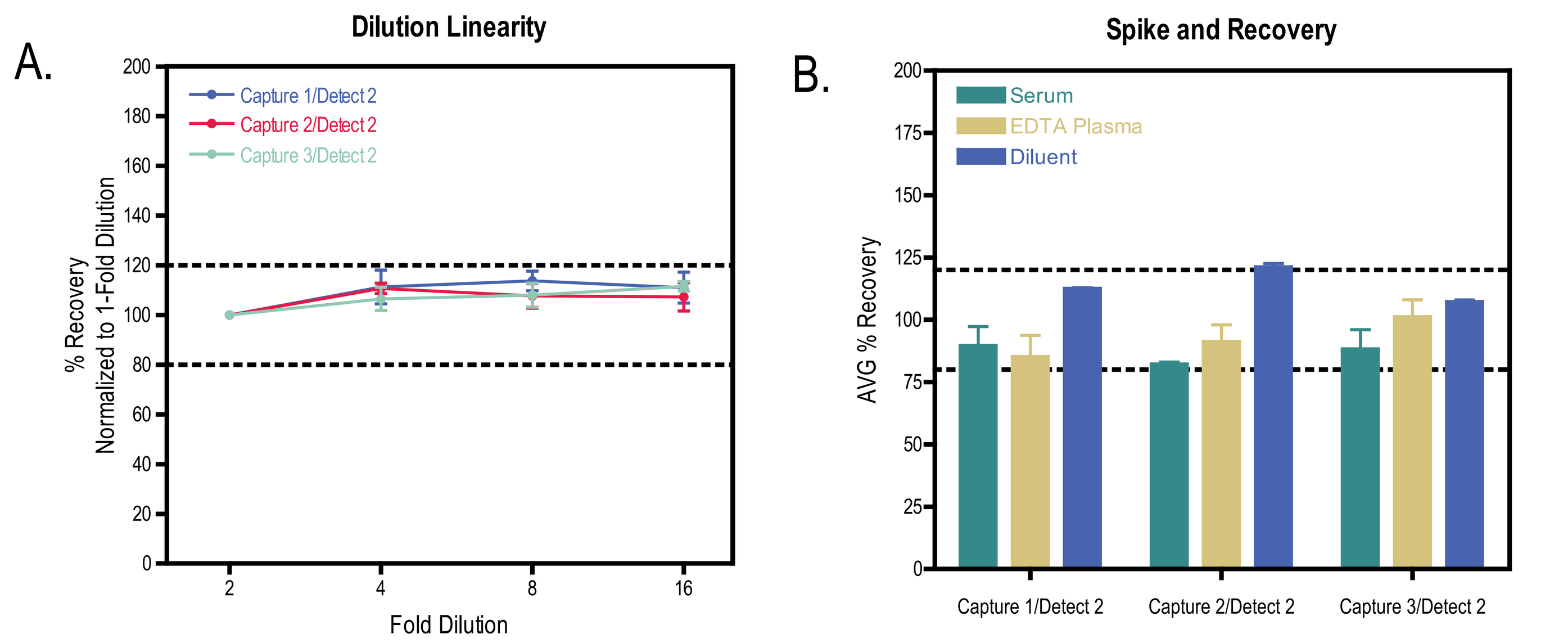
B.

Capture	1			2	3			4	5
Detect	2	3	4	2	1	2	3	15	15
Background	67	80	136	91	43	71	105	21	55
Hillslope	1.0	1.1	1.1	1.0	0.98	1.0	1.0	1.0	1.1
Rsquared	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
LLOD	0.33	0.97	1.3	0.55	0.28	0.29	0.24	0.71	1.0
S/B Cal-4	106	43	18	58	176	125	96	158	54

Sample testing on the U-PLEX platform. A) Concentrations of analytes detected by each proposed antibody pair. B) Relevant standard curve values for each antibody pair, including LLOD (selected pairs highlighted in yellow).

The nine selected antibody pairs were evaluated for their ability to detect analyte in 12 different stimulated samples. Stimulated samples were used because the analyte is not present in normal matrices. Full standard curves and stimulated samples were run in duplicate. All pairs were able to detect analyte in samples 2, 5 and 7. Three pairs (highlighted by red arrows) were selected based on best reactivity to samples and overall assay sensitivity.

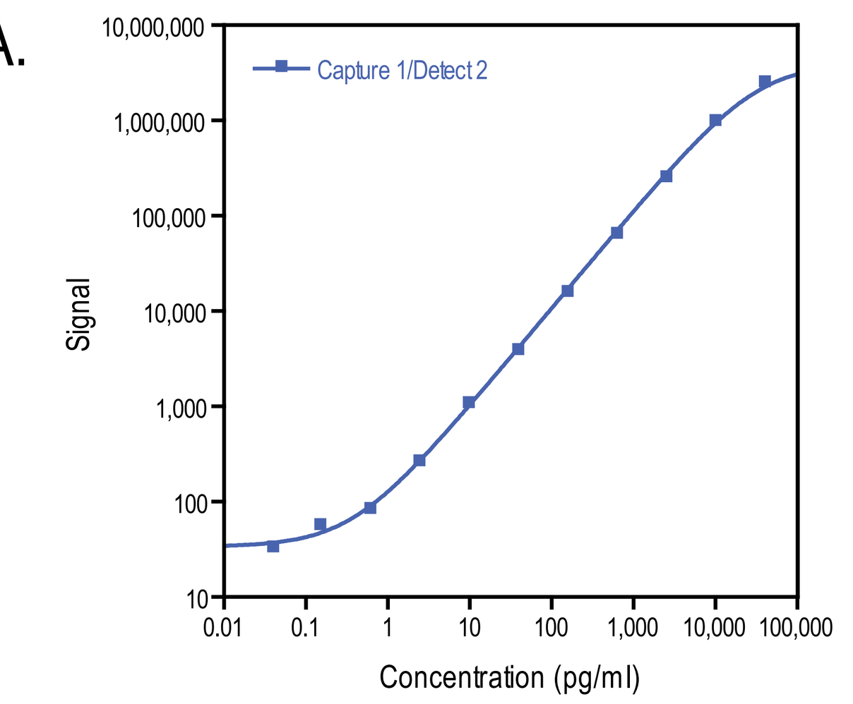
7 Matrix Tolerance


Dilution Linearity
A.
Capture 1/Detect 2
Capture 2/Detect 2
Capture 3/Detect 2
% Recovery
Normalized to 1-Fold Dilution
Fold Dilution
Spike and Recovery
B.
Serum
EDTA Plasma
Diluent
AVG % Recovery
Capture 1/Detect 2
Capture 2/Detect 2
Capture 3/Detect 2

Three antibody pairs from the original nine were selected to evaluate matrix tolerance on the U-PLEX platform. A) To assess dilution linearity, normal human serum (n=3) and EDTA plasma samples (n=3) and diluent (n=3) were spiked with recombinant calibrator and diluted 2-, 4-, 8-. and 16-fold before testing. The average percent recovery is based on samples that measured within the quantitative range of each assay. All three antibody pairs were found to recover within 20% of the targeted range, suggesting that the samples dilute linearly 2- to 16-fold. B) Spike recovery was evaluated by spiking normal human serum (n=3) and EDTA plasma (n=3) from a commercial source or diluent with calibrators at three different levels (high, mid, and low). All three antibody pairs were found to recover within 20% of the targeted range. Overall, the data suggested that the three selected antibody pairs were not sensitive to matrix effects. Of these three viable antibody pairs, Capture 1/Detect 2 and Capture 2/Detect 2 were selected for optimization.

8 Assay Range

A.

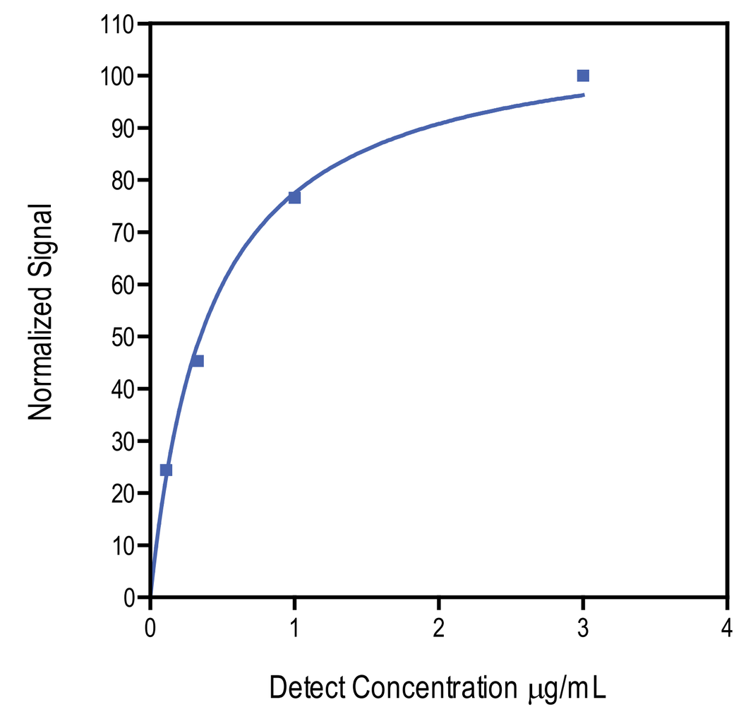


B.

Capture	Capture 1		
Detection	Detect 2		
	Calibrator Concentration (pg/mL)	Average Signal	%CV
Cal-1	40,000	2,577,580	4.9
Cal-2	10,000	1,013,735	3.5
Cal-3	2,500	260,250	0.1
Cal-4	625	66,355	1.4
Cal-5	156	16,232	4.1
Cal-6	39.1	4,006	0.5
Cal-7	9.77	1,113	12.1
Cal-8	2.44	272	2.3
Cal-9	0.61	86	20.2
Cal-10	0.15	58	35.5
Cal-11	0.04	34	37.2
Cal-12	0.00	37	4.9
		Hillslope	LLOD
		1.02	0.79

Characterization of assay range for one of selected antibody pairs (Capture 1/Detect 2). A twelve point standard curve was prepared with an initial top of curve concentration of 40,000 pg/mL. A) Standard curve for proposed primary antibody pair. Pair shows 3- to 4-logs of dynamic range. B) Signals and %CVs of each point in the proposed primary assay standard curve.

9 Detection Antibody Titration



Detection antibody titration optimization on the U-PLEX platform. Detection antibody titration was performed with antibody pair (Capture 1/Detect 2) selected from screening. Resultant data was used to select an optimal detection antibody concentration, which in this case was 1 µg/mL.

10 Assay Specificity

	Assay Specificity						
	Calibrator						
Capture	1	2	3	4	5	6	7
Assay-1	100%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%
Assay-2	0.1%	100%	0.2%	0.1%	0.1%	0.1%	0.0%
Assay-3	0.1%	0.3%	100%	0.1%	0.1%	0.3%	0.1%
Assay-4	0.1%	0.2%	0.1%	100%	0.0%	0.1%	0.0%
Assay-5	0.1%	0.4%	0.2%	0.1%	100%	0.2%	0.2%
Assay-6	0.1%	0.1%	0.2%	0.1%	0.1%	100%	0.0%
Assay-7	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	100%

Assay specificity testing on the U-PLEX platform. The selected assay pair (Capture 1/Detect 2) was multiplexed with six additional assays to evaluate calibrator/antibody specificity. In this study, the captured antibody for each assay was linked to individual spots in wells on the U-PLEX plate and tested with individual calibrators and a blended detection antibody solution. The data demonstrated that non-specific binding (non-specific binding signal/specific signal X 100) was less than 0.5% for all of the assays. Overall, the data indicated that the new selected antibody pair can be successfully multiplexed with six additional assays to form a potential U-PLEX panel.

11 Conclusion

We demonstrated the utility of the MSD U-PLEX platform for the rapid screening and selection of viable antibody pairs from over 3,000 potential antibody combinations. The platform was subsequently used to select and optimize multiple antibody pairs based on parameters such as dynamic range, sensitivity, sample recognition, matrix tolerance, and non-specific binding. These studies demonstrated that the U-PLEX platform can be adapted to facilitate both large and small immunoassay development programs.

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