T2095

Development and Validation of Multiplexed Human Kidney Biomarker Assay Panels

Pu Liu, Claire Lu, Nicolas Sammons, Pankaj Oberoi, and Jacob N. Wohlstadter

1 Abstract

Purpose: Measurement of protein biomarkers as indicators of drug-induced kidney toxicity shows promise in improving drug safety and accelerating development timelines. Selection of the appropriate combination of protein biomarkers and development of assays to accurately measure them presents a challenge. Currently, over 20 biomarkers of kidney damage have been proposed; 7 have been qualified in rat toxicology studies.

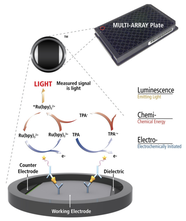
Methods: MESO SCALE DISCOVERY® (MSD) developed three panels of multiplexed assays according to fit-for-purpose assay development and validation principles. The panels were tested for sensitivity, precision, specificity, interferences, and matrix tolerance. Samples of normal human urine and serum were measured to establish a normal range. Urine and serum from multiple human patients suffering from kidney disease were then assayed to quantify biomarker modulation.

Results: The panels were assembled based on the abundance of the biomarkers in human urine: low abundance – αGST, calbindin, clusterin, osteoactivin, TFF3, KIM-1, and VEGF; mid abundance – πGST and RBP4; and high abundance – albumin, beta-2 microglobulin (B2M), cystatin C, epidermal growth factor (EGF), NGAL, osteopontin (OPN), and uromodulin (UMOD). The panels had minimum required dilutions (MRD) for human urine of 10-, 50-, and 500-fold, respectively. Matrix-based controls demonstrated recovery rates between 75% and 125% for all assays with most assays recovering between 85% and 115% over a multi-day, multi-plate precision study. Spike recovery in matrix results were between 80% and 120% for all assays with the exception of osteopontin, which is known to bind to calcium crystals in urine. All biomarkers were detectable in urine at their respective MRDs. The abundance of biomarkers in serum was significantly different than in urine for many assays. Cystatin-C, clusterin, albumin, and B2M were more than 50-fold higher in serum than in urine, whereas EGF and osteopontin are lower. Measured analyte levels varied significantly between normal and kidney disease samples.

Conclusions: These 3 panels will be useful tools for researchers studying kidney damage. Multiplexed assays offer simpler protocols and faster results with reduced sample volume requirements. The assays have been validated for both serum and urine matrices; however, custom panels for serum studies may be necessary to match biomarker abundance levels.

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.



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 well.
- Surface coatings can be customized.

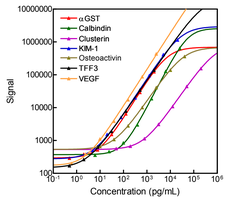
Protocol

1. Add 150 µL MSD® Blocker A per well. Incubate for 30 minutes at room temperature.
2. Wash with PBS-T. Add 50 µL of standard or diluted sample. Incubate for 2 hours at room temperature.
3. Wash with PBS-T. Add 25 µL of detection antibody. Incubate for 2 hours at room temperature.
4. Wash with PBS-T. Add 150 µL of Read Buffer T. Read on MSD SECTOR® Imager.

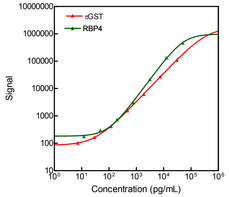
*MSD recommends 10-, 50-, or 500-fold dilution for Kidney Injury Panel 3 (KIP3), Kidney Injury Panel 4 (KIP4), and Kidney Injury Panel 5 (KIP5), respectively. Thus, actual sample volume needed is less than 10 µl.

3 Standard Curves for Kidney Injury Panels

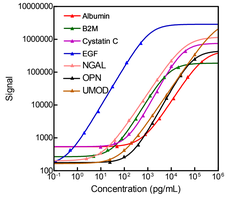
The following standard curves illustrate the sensitivity and dynamic range of the assays. The graphs display representative data from a single run. Reproducibility across runs is shown in the tables to the right of the graphs. Reproducibility was assessed across at least 3 days.



	Control	Runs	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV
αGST	Control-1	6	1019	5.3	19.0
	Control-2	6	119	2.5	19.0
	Control-3	6	11.0	5.5	18.2
Calbindin	Control-1	6	10302	4.9	4.5
	Control-2	6	1394	4.5	4.5
	Control-3	6	172	3.5	3.2
Clusterin	Control-1	6	28946	5.4	5.2
	Control-2	6	5782	12.4	11.4
	Control-3	6	771	8.7	16.2
KIM-1	Control-1	6	16656	8.0	7.9
	Control-2	6	2360	3.6	3.3
	Control-3	6	107	2.5	3.9
Osteoactivin	Control-1	6	46191	10.3	9.2
	Control-2	6	2687	5.6	6.0
	Control-3	6	230	5.0	5.6
TFF3	Control-1	6	1019	6.7	6.6
	Control-2	6	173	5.2	5.6
	Control-3	6	29.1	4.1	4.2
VEGF	Control-1	6	1045	3.5	4.2
	Control-2	6	119	3.6	5.0
	Control-3	6	10.4	6.0	5.6



	Controls	Runs	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV
πGST	Control-1	13	808	6.4	8.4
	Control-2	13	651	5.7	10.2
	Control-3	13	196	4.7	9.5
RBP4	Control-1	13	2112	2.8	9.8
	Control-2	13	1165	4.4	10.8
	Control-3	13	219	4.1	9.4



	Control	Runs	Average Conc. (pg/mL)	Average Intra-plate %CV	Inter-plate %CV
Albumin	Control-1	11	82714	7.0	10.4
	Control-2	11	15052	6.8	13.9
	Control-3	11	2688	3.4	9.5
B2M	Control-1	11	3728	6.5	13.8
	Control-2	11	563	3.4	8.6
	Control-3	11	119	4.7	10.1
Cystatin C	Control-1	11	32816	6.2	23.6
	Control-2	11	4364	4.3	13.9
	Control-3	11	823	4.1	14.7
EGF	Control-1	11	134	8.2	14.1
	Control-2	11	39.7	7.3	14.5
	Control-3	11	2.40	6.1	12.2
NGAL	Control-1	11	3105	5.2	9.7
	Control-2	11	2328	3.4	10.9
	Control-3	11	40.5	6.2	16.6
OPN	Control-1	11	9380	6.5	18.4
	Control-2	11	3157	7.6	16.0
	Control-3	11	349.2	7.1	19.9
UMOD	Control-1	11	28985	4.9	14.3
	Control-2	11	5660	4.1	13.4
	Control-3	11	1254	3.1	10.5

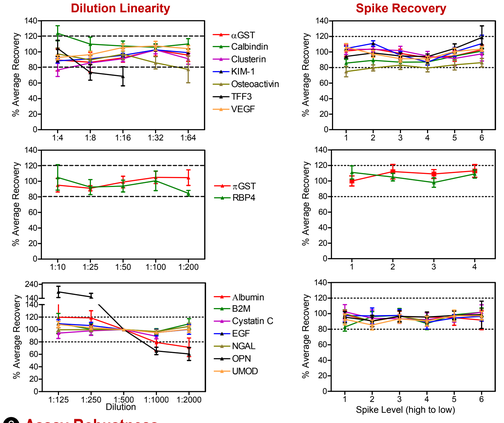
4 Assay Sensitivity and Dynamic Range

The lower limit of detection (LLOD) is a calculated concentration based on a signal that is 2.5 standard deviations over the blank (N=24). The values presented here are the average LLOD over at least 6 runs. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were established using kits from a single lot. Multi-plate, multi-day runs (N=6) were conducted to establish the LLOQ and ULOQ with acceptable precision (≤25%) and accuracy (between 75% and 125% of expected concentrations). Since urine and serum contain endogenous analyte levels that are too high to establish an LLOQ, a concentration range of calibrator spiked into diluent was used to assess the LLOQ and ULOQ.

	αGST	Calbindin	Clusterin	KIM-1	Osteoactivin	TFF3	VEGF	πGST	RBP4	Albumin	B2M	Cystatin C	EGF	NGAL	OPN	UMOD
LLOD (pg/mL)	1.76	11.0	54.4	0.784	5.57	1.14	0.670	1.76	11.0	107	5.72	26.5	0.116	1.75	150	15.0
LLOQ (pg/mL)	8.00	100	80.0	20.0	40.0	4.00	2.50	8.00	100	600	60.0	120	0.750	200	200	60.0
ULOQ (pg/mL)	1800	22500	180000	18000	36000	1800	2250	1800	22500	190000	17000	30000	475	8500	95000	95000

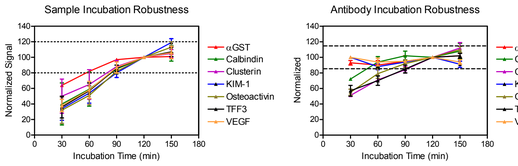
5 Matrix Tolerance

Eight individual human urine samples were tested for dilution linearity (left) and spike recovery (right). Error bars represent the standard deviation across the 8 samples tested. Most analytes show dilution linearity at the recommended dilution and spike recovery between 80% and 120%.

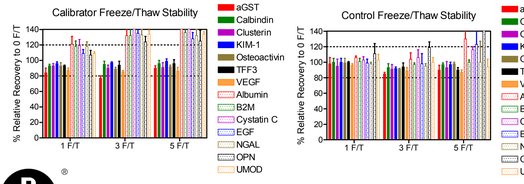


6 Assay Robustness

The protocol was optimized for sample and detection antibody incubation time. Representative data from Kidney Injury Panel 3 is shown below. The data shows that as long as assay incubation time varied by no more than 30 minutes, signals were within 20% of the 2-hour incubation signals. The other assays performed similarly. The signals are normalized to the 2-hour incubation time points.

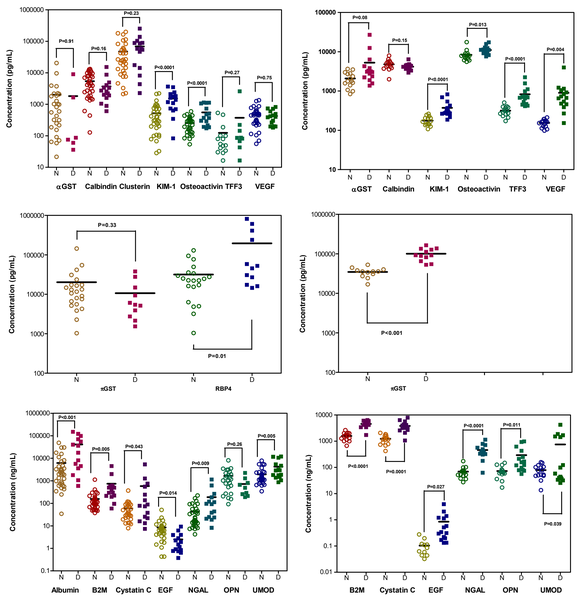


Freeze/thaw (F/T) stability for calibrators and matrix-based controls were conducted for 1, 3, and 5 F/T cycles. The calibrators were not stable beyond 1 freeze thaw. Most analytes in matrix are stable to 3 freeze thaw cycles.



7 Sample Testing for Kidney Injury Panels

Human urine (left) and serum (right) samples from normal (N) and kidney disease (D) patients were obtained from Bioreclamation, Inc. Kidney disease samples were from a variety of patients with kidney damage or injury. Specific clinical information associated with these samples was not available. Albumin, RBP4, and clusterin are known to be highly abundant in serum; thus they require higher dilution in serum relative to the other analytes.



8 Conclusions

MSD Kidney Injury Panels (human) can measure important kidney biomarkers. Validated bioanalytical testing has demonstrated assay accuracy, precision, sensitivity, reproducibility, and stability. The assays use a common protocol requiring a minimal amount of sample (<20 µl) to measure all 16 analytes. The organization of these panels was established based on the abundance of the biomarkers in urine. When running serum, different dilutions may be required and different custom assay configurations may be needed depending on the biomarkers of interest. Multiplexing allows rapid screening of samples to identify which biomarkers are important for the disease being studied. Using both urine and serum, we have provided proof-of-concept screening that demonstrates biomarker modulation. These panels can be useful tools for researchers studying kidney toxicity, inflammation-derived kidney damage, or immune-mediated complexes that may be caused by drug-induced kidney injury.

References

Lee JW, et al. Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res.* 2006;23(2):312-28.

Dieterle F, et al. Renal biomarker qualification submission: a dialog between the FDA-EMEA and Predictive Safety Testing Consortium. *Nat Biotechnol.* 2010;28(5):455-62.

Meso Scale Discovery®
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com®