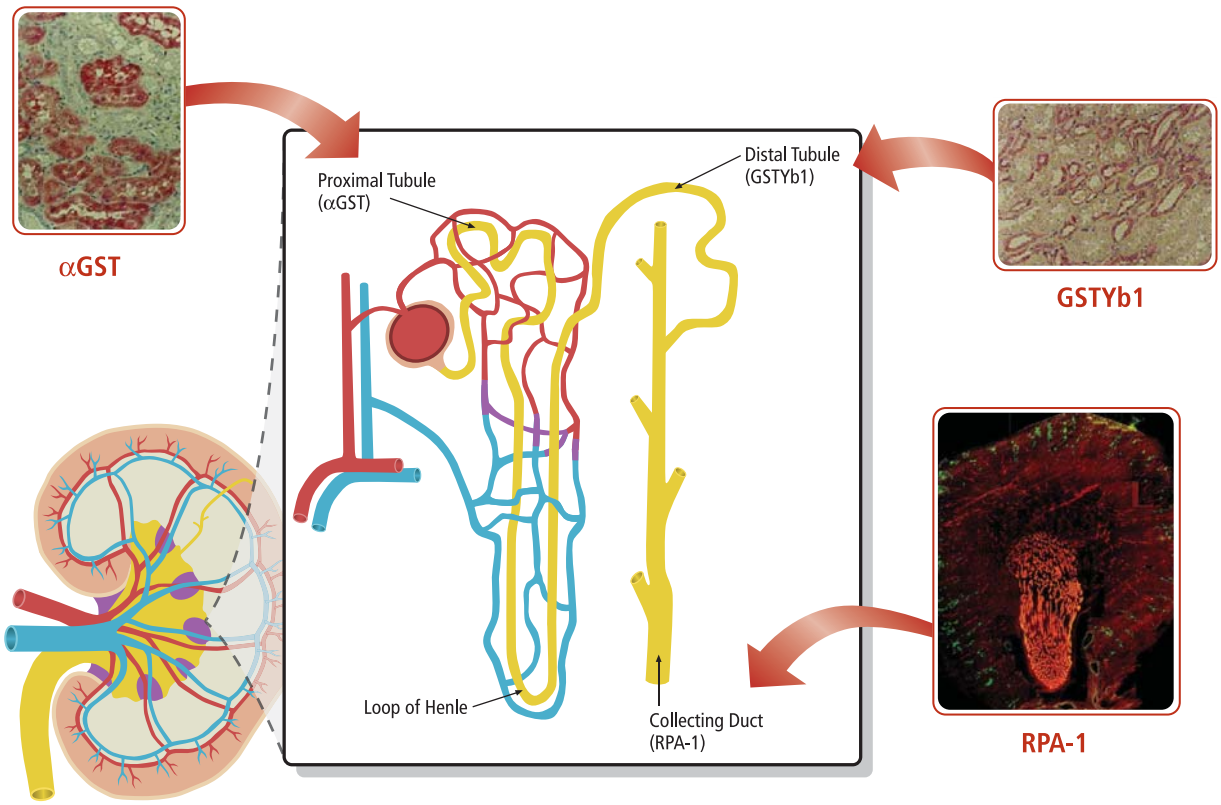
Qualification of MSD's Argutus AKI Test® for Preclinical Studies

Traditional clinical chemistries such as BUN and serum creatinine are not sensitive enough to detect subtle drug induced kidney injury and often do not correlate to histochemically measured injury. The volume of sample required for these traditional assays is often larger than is feasible for rodent models in preclinical studies. This poster describes a multiplex panel of emerging biomarkers from Argutus' acute kidney injury test (AKI Test®). Our Argutus AKI Test includes αGST, GSTYb1, and RPA-1. These novel biomarkers have been validated by the HESI consortium as injury markers for specific cells in the kidney. αGST and GSTYb1 are found in luminar cells of the proximal and distal tubule, respectively. The combination of these biomarkers allows researchers to stratify acute kidney injury between specific cell types and to pinpoint the site of injury. RPA-1 is a biomarker for injury to the luminal epithelial cell of the collecting duct. These panels have advantages that are typical of assays from Meso Scale Discovery (MSD): greater sensitivity, reduced sample volume, a greater dynamic range (both endogenous and elevated levels can be measured at a single dilution factor) and improved throughput.

Description of Markers

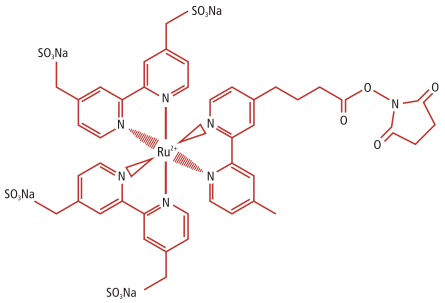
Glutathione S-transferases (GSTs) are proteins found in high concentrations (2% of soluble protein) in the luminar cells of the proximal and distal tubules. Different isoforms are found in different parts of the nephron, therefore, by measuring urinary alpha GST (αGST) one can study the proximal tubule and by measuring GSTYb1 in rats, one can study the distal tubule. Renal tubular injury can thus be precisely localised. These proteins are not released in the healthy rat and as such act as a very sensitive indicator of site specific injury.

Renal Papillary Antigen 1 (RPA-1) is the first urinary biomarker for the collecting duct. Injury to the collecting ducts in the renal papilla can lead to Renal Papillary Necrosis (RPN), a serious condition for which urinary biomarkers are currently lacking.

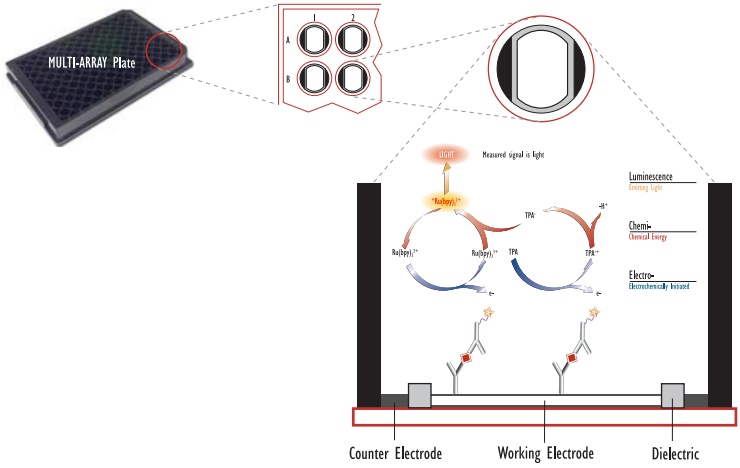


The MSD® Platform

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.



Ruthenium (II) tris-bipyridine-(4-methylsulfonate)
NHS ester (MSD SULFO-TAG™ label)



Electrochemiluminescence Features:

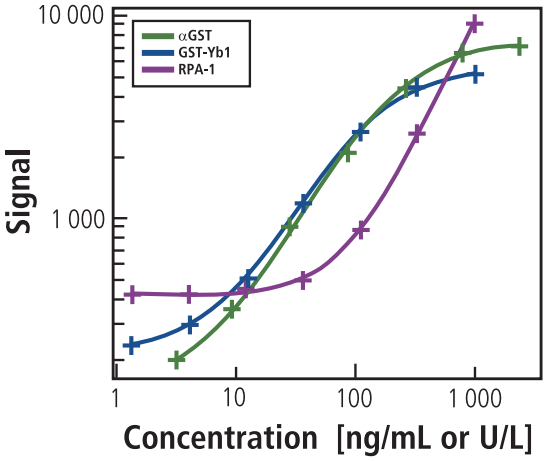
- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity

Argutus AKI Test®

MSD's Argutus AKI Test measures αGST, GSTYb1, and RPA-1 in rat urine. The analytes have been validated as markers of acute kidney injury by others. We qualified this panel according to typical practices for pre-clinical biomarkers. The qualification procedure involved multi-day controls, establishment of limits of quantititation, spike recovery, dilutional linearity, and measurement of control and treated samples.

Standard Curve

Representative standard curves from a typical run are shown below. The assays are quantitative over a 100- to 1000-fold range, enabling measurement of both normal and elevated levels of biomarkers. The lower limit of detection (LLOD) was determined by calculating 2.5 standard deviations above the average background (no analyte) signal. The lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) were assigned following a multi-day study. We assigned the LLOQ (or ULOQ) as the lowest (or highest) concentration where the %CV of the calculated concentration was less than 20% and the percent recovery of the concentration was between 80% and 120%.

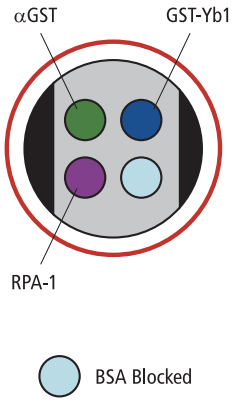


	αGST (ng/mL)	GSTYb1 (ng/mL)	RPA-1 (U/L)
LLOD	0.6	1.6	17.3
LLOQ	8.0	12.4	50
ULOQ	237	333	1000

αGST		
Concentration (ng/mL)	Average Counts	% CV
0	77	11.5
3.25	201	3.3
9.75	353	8.8
29.3	900	4.7
87.8	2098	9.3
263	4316	1.7
790	6395	1.8
2 370	6944	3.8

GSTYb1		
Concentration (ng/mL)	Average Counts	% CV
0	206	17.8
1.37	237	11.2
4.12	296	6.4
12.3	508	5.7
37.0	1174	1.3
111	2644	3.0
333	4370	2.1
1 000	5025	4.1

RPA-1		
Concentration (U/L)	Average Counts	% CV
0	407	6.1
1.37	420	4.0
4.12	426	1.0
12.3	452	1.1
37.0	501	3.0
111	874	2.3
333	2603	7.8
1 000	8979	5.1



Protocol:

1 Add 25 µL Assay Diluent GF1, incubate 30 min at RT.

2 Add 25 µL of standard/sample, incubate 2 hours at RT. Wash.

3 Add 25 µL of detection antibody, incubate 2 hours at RT. Wash.

4 Add 150 µL of Read Buffer T, read.

Argutus AKI Test®

Precision: Multi-Day Study

Control samples of high, mid, and low levels of each analyte were measured on each plate. Controls were made by spiking papilla medulla extract into rat urine.

	Control	Plates	Concentration	Average Concentration CV	Interday Concentration CV
αGST (ng/mL)	High	15	76.8	9.3	14.2
	Mid	15	60.9	6.2	14.4
GSTYb1 (ng/mL)	High	15	128	12.4	12.1
	Low	15	30.5	8.5	12.3
RPA-1 (U/L)	High	15	523	5.4	4.7
	Mid	15	134	6.1	6.5

Spike Recovery

Papilla medulla extract was spiked into 5X diluted rat urine and tested on the Argutus AKI Test. The concentrations of the spikes were distributed throughout the linear range of the assay. All of the spiked samples above the LLOQ had acceptable recoveries (between 80% and 120%).

	Spike Concentration	Concentration	Concentration CV	% Recovery
αGST (ng/mL)	125	132	7.0	100
	41.7	46.7	1.0	94
	13.9	22.8	8.6	105
	0	7.8	1.5	
GSTYb1 (ng/mL)	250	239	4.4	95
	83.3	90.2	22.2	105
	27.8	28.8	8.6	94
	0	2.9	111	
RPA-1 (U/L)	250	345	6.2	95
	83.3	202	6.1	102
	27.8	138	7.2	98
	0	114	8.5	

Reference Samples

Rat urine samples from known injury inducing drugs were run at a 5-fold dilution. We confirmed high levels of RPA-1 in samples from animals treated with NPAA. Tenidap has been shown to induce elevated levels of Clusterin, another emerging biomarker of injury to the collecting duct. The MSD multiplex panel found that RPA-1 is also elevated upon treatment with Tenidap, supporting the expectation that RPA-1 is related to injury of the collecting duct. Our panel confirmed elevated levels of αGST in Cisplatin treated animals. The measurements made with our multiplex were in agreement with the concentrations determined from the Argutus EIA kits. Measurements in italics were below the assay LLOQ at a 5-fold dilution. Measurements in bold were made at a 20-fold dilution of the urine samples.

			αGST		GSTYb1		RPA-1	
Nephro-toxicant	Associated Biomarker	Sample ID	Concentration (ng/mL)	Concentration CV	Concentration (ng/mL)	Concentration CV	Concentration (U/L)	Concentration CV
Control	None	B671	11.7	2.9	*13.4*	*35.6*	714	1.4
		B672	26.8	16.0	*16.7*	*20.5*	1382	2.6
NPAA	RPA-1	B673	*7.1*	*3.1*	*29.0*	*13.1*	**8700**	**9.8**
		B674	*7.3*	*13.3*	*14.9*	*34.7*	**5482**	**5.5**
Tenidap	Clusterin	B675	20.3	7.6	*23.4*	*7.2*	4363	9.1
		B676	*7.8*	*7.8*	*19.5*	*37.6*	1657	5.2
Cisplatin	αGST	B680	69.2	3.9	*38.0*	*36.6*	757	6.3
		B681	176	2.6	*43.3*	*30.2*	704	4.4

Concentrations from Argutus EIA Kits.

			αGST	GSTYb1	RPA-1
Nephro-toxicant	Associated Biomarker	Sample ID	Concentration (ng/mL)	Concentration (ng/mL)	Concentration (U/L)
Control	None	B671	14.0	4.0	831
		B672	30.0	3.0	1429
NPAA	RPA-1	B673	35.0	7.0	5588
		B674	12.0	3.0	4499
Tenidap	Clusterin	B675	14.0	4.0	2939
		B676	12.0	6.0	1950
Cisplatin	αGST	B680	138	12.0	668
		B681	246	18.0	663

Conclusions

MSD has developed a high performance multiplex panel to measure biomarkers of acute kidney injury. Composed of emerging biomarkers, these panels can identify acute kidney injury and may help stratify damage from different kidney cell types. The combination of multiplexing, wide dynamic range and increased throughput enables studies that measure many analytes from small pre-clinical samples. The analytes presented here have been studied by others to verify a positive correlation between the results of the MSD assays and results from traditional immunohistopathology.