

Novel Rat Kidney Injury Biomarker Assays Using a Gyros Nanotechnology Platform

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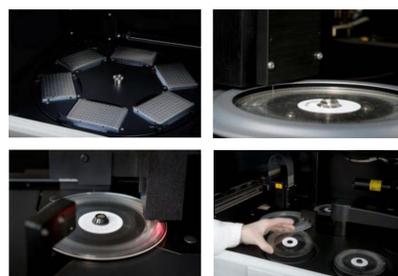
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Abstract

A leading cause of failure in any drug development program is organ toxicity, such as in the kidney. Traditional nephrotoxicity biomarkers may show insufficient tissue specificity and often cannot be detected until damage is irreversible. New markers, such as KIM-1 and Clusterin, help detect damage to the kidneys and offer clues to specific tissue injury in a more timely manner. We have developed rat KIM-1 and Clusterin GyroMark™ HT Biomarker assays, the first two commercially available toxicity assay kits for the Gyrolab® xP Workstation platform. This automated compact disc-based microfluidic nanotechnology for sandwich ELISA analysis uses a biotinylated capture antibody and a fluorescently labeled detection antibody. Gyros technology provides many advantages, including small sample size (200–1000 nL), high sensitivity, broad dynamic range, and short assay time (1 hour per assay). For the KIM-1 assay, we determined dynamic range, sensitivity, spike recovery, dilution linearity, intra-, and inter-assay variations of 0.015–60 ng/mL, 0.015 ng/mL, 100%, 107%, < 10% and < 15%, respectively for 1:2 diluted rat urine samples. The Clusterin assay had a dynamic range, sensitivity, spike recovery, dilution linearity, intra-, and inter-assay variations of 0.7–3000 ng/mL, 0.36 ng/mL, 93%, 108%, < 15% and < 15%, respectively for 1:10 diluted rat urine samples. We first evaluated rats treated with no, low, and high doses of gentamicin, a known nephrotoxicant. Urine samples (n=36) were collected on days 0, 3, 7, and 14. A significant increase in these markers was observed at day 3, tapering off as animals recovered towards day 14. These GyroMark™ HT assays were compared to a leading commercial ELISA platform in the analysis of vancomycin-treated rat urine samples (n=36); correlation was $r = 0.97$ (KIM-1) and 0.87 (Clusterin). These data demonstrate that our GyroMark™ HT toxicity assays, rat KIM-1 and Clusterin, are robust and reproducible, using 1000 nL samples, and can be useful for detecting kidney damage in rat urine samples for drug development and basic research.

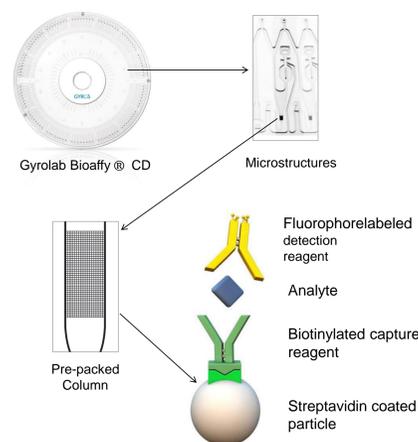
Methods

Gyrolab® xP Workstation



GyroMark™ HT Assay Format

- Capture antibody
 - Biotin-labeled antibody in buffer binds to streptavidin coated particles of Gyrolab Bioaffy® CD
- Standard curve / Samples
 - Serial dilutions of standard prepared in buffer and diluted samples bind to capture antibody
- Detection reagent
 - Dye-labeled detection antibody diluted in buffer attaches to bound standard/samples
- Quantification
 - Detected and quantitated by fully automated wash system
- Wash Buffer 1
 - PBS-0.01% Tween® 20
- Wash Buffer 2
 - Gyros pH 11
- Design run using Bioaffy® 1000 3-step C-A-D wash station wizard v2

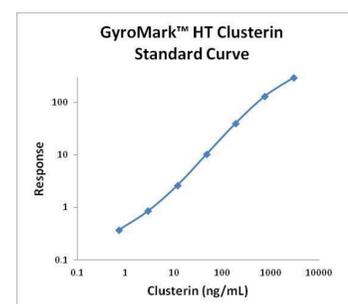
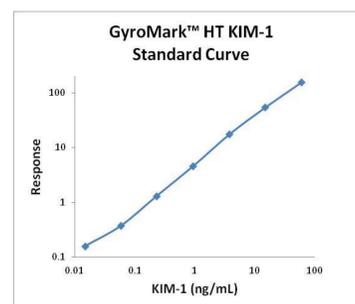


GyroMark™ HT Assay Results

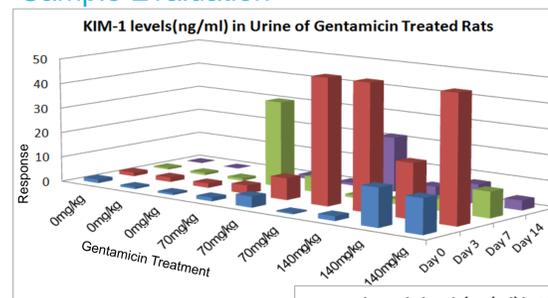
Assay Characteristics

	KIM-1	Clusterin (uses 1:10 diluted sample)
Standard Curve Range (ng/mL)	0.015-60	0.7-3000
LLOQ (ng/mL)	0.015	0.36
Spike Recovery	100%	93%
Dilution Linearity	107%	108%
Intra-Assay Precision (16 replicates)	<10%	<10%
Inter-Assay Precision (8 runs)	<15%	<15%

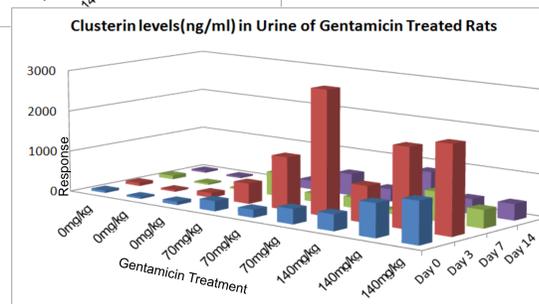
Standard Curves



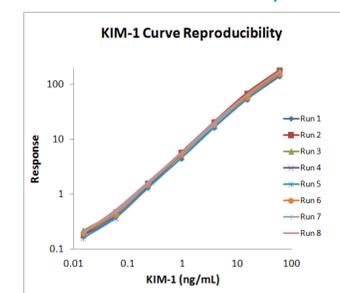
Sample Evaluation



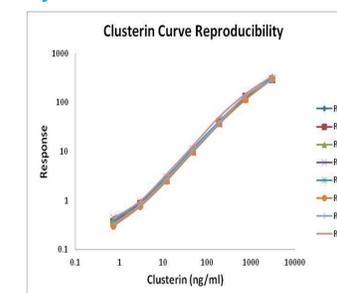
GyroMark™ HT kit detects changes in KIM-1 and Clusterin levels in urine of rats collected on day 0, 3, 7 and 14 after treatment with 0 mg/kg, 70 mg/kg, or 140 mg/kg gentamicin.



Standard Curve Reproducibility



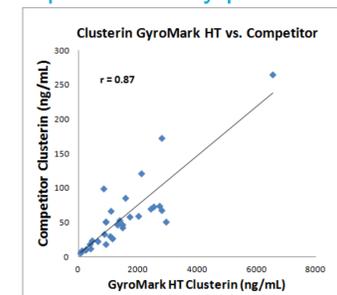
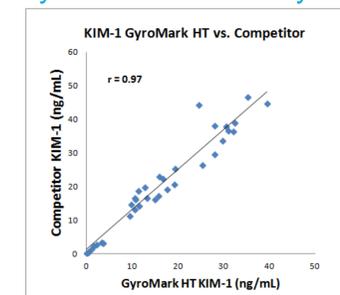
Response	Response	Response
Mean of 8 runs	Std Dev of 8 runs	% CVs
0.11	0.02	16.45
0.19	0.02	10.02
0.44	0.06	12.60
1.49	0.13	8.61
5.29	0.51	9.59
18.86	1.62	8.61
60.69	5.75	9.47
164.06	14.05	8.56
Mean % CVs of 8 runs =		10.49



Mean	Std Dev	% CVs
0.21	0.03	16.69
0.38	0.05	12.75
0.87	0.06	7.43
2.85	0.27	9.48
10.95	1.05	9.59
40.16	4.41	10.99
129.50	10.72	8.28
306.50	19.07	6.22
Mean % CVs of 8 runs =		10.18

The standard curves of eight separate runs are very reproducible.

GyroMark™ HT assay vs. competitor assay platform



Excellent correlation for urine samples from 32 rats treated with varying levels of vancomycin was achieved when comparing the newly developed Merck Millipore GyroMark™ HT assays developed for the Gyros Gyrolab® xP Workstation using a 1000 nL sample volume to a leading competitor ELISA-based platform.

Conclusions

- We have partnered with Gyros AB to develop the GyroMark™ HT portfolio of immunoassay kits, including the Rat KIM-1 and Rat Clusterin assays, that will be available commercially for use on the Gyros Gyrolab® xP Workstation.
- The kits have been fully validated and have been compared to a leading competitor immunoassay. Our results demonstrated that the assays have many desirable features such as small sample volume, improved sensitivity and wider dynamic range, while being highly robust and reproducible.
- These kits will provide customers with new tools needed to perform nephrotoxicity screening during drug development and toxicity research in one of the quickest immunoassay platforms available for high throughput screening.