M1034

Novel 5-plex Panel for the Detection of Acute Kidney Injury with Improved Analytical Performance

Candace M Adamo[1], Timothy H Carlson[1], Eibhlin M McCole[2], Marie McGarvey[2], Ciaran Richardson[2], Peter Fitzgerald[3], John Lamont[3] and Amar A Sethi[1]

[1]Pacific Biomarkers, Seattle, WA, United States; [2]Randox Teoranta, Dungloe, Co. Donegal, Ireland and [3]Randox Laboratories, Crumlin, Co. Antrim, United Kingdom

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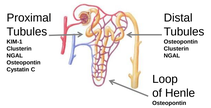
PURPOSE

Acute kidney injury (AKI) is currently classified by the KDIGO guidelines based on serum creatinine and urine output. However, since creatinine is a lagging index of impending AKI, a novel set of more sensitive AKI biomarkers has been qualified in rodents. Studies are now underway to qualify a similar set of human biomarkers, but no high-throughput multiplex for these biomarkers is currently available to support an earlier and more robust classification of AKI.

OBJECTIVE

This work describes the development and optimization of a novel multiplex immunoassay panel comparing its performance with predicate ELISA methods for KIM-1, NGAL, cystatin C, clusterin, and osteopontin.



METHODS

Randox Biochip technology was used to develop a multiplex immunoassay panel for the urinary biomarkers KIM-1, cystatin C, osteopontin, clusterin and NGAL, using proprietary and commercially available antibodies. The ideal standard curve range was derived using data extracted from testing each analyte by ELISA in urine from over 1000 subjects with normal kidney function or potential AKI.

The functional sensitivities and upper limit of quantification were assessed by the precision (%CV) of the lowest and highest non-zero standard (n=6 each).

Method comparison evaluated the analytical performance of this panel by comparing against the following singlicate ELISA methods using about 30 normal subjects; KIM-1, cystatin C, osteopontin (OPN), clusterin (R&D Systems) and NGAL (BioPorto).

Cross reactivity was tested for each analyte at the midpoint of the standard curve when spiked with x10 concentration of the highest standard of the other panel antigens.

Cross reactivity from various related, but non-panel proteins that may be present in urine were also tested for cystatin C, clusterin and KIM-1. Cross-reactivity of non-panel proteins for NGAL and osteopontin are pending.

Interference of analytes known to be present in urine were tested for each panel analyte. The concentration of interferent was titred to determine a non-interfering concentration (within +/- 10%).

RESULTS

Figure 1: Evidence Investigator™ AKI Multiplex Panel

The multiplex biochip contains immobilized antibodies for each of the five biomarkers. A sandwich chemiluminescent immunoassay is used to detect an increased signal of each of the five biomarkers from one specimen.

Table 1: Measurable Range Comparison

Calibrator	KIM-1 (pg/mL)		NGAL (ng/mL)		Cystatin C (ng/mL)		Osteopontin (ng/mL)		Clusterin (ng/mL)	
	Multiplex	ELISA	Multiplex	ELISA	Multiplex	ELISA	Multiplex	ELISA	Multiplex	ELISA
L1	0	0	0	0	0	0	0	0	0	0
L2	25	31.25	0.78	1	1.41	3.13	62.5	125	7.81	12.5
L3	50	62.5	1.56	2.5	2.81	6.25	125	250	15.6	25.0
L4	100	125	3.13	5	5.63	12.5	250	500	31.3	50.0
L5	250	250	6.25	10	11.3	25.0	500	1000	62.5	100
L6	500	500	12.5	25	22.5	50	1000	2000	125	200
L7	1000	1000	25	50	45	100	2000	4000	250	400
L8	2000	2000	50	100	90	-	4000	8000	500	800
L9	4000		100		180		8000		1000	

Table 2: Sensitivity

Biomarker	Sensitivity	% Coefficient of Variation (% CV)
KIM-1	24.38 pg/mL	13.97%
NGAL	0.89 ng/mL	3.32%
Cystatin C	1.22 ng/mL	8.84%
Osteopontin	50.9 ng/mL	8.50%
Clusterin	7.02 ng/mL	13.56%

Table 3: Upper Limit of Quantification

Biomarker	Upper Limit of Quantification	% Coefficient of Variation (% CV)
KIM-1	4067.37 pg/mL	3.76%
NGAL	97.79 ng/mL	3.91%
Cystatin C	184.72 ng/mL	8.02%
Osteopontin	8028.68 ng/mL	9.94%
Clusterin	1021.04 ng/mL	2.88%

Figure 2: Method Comparison of Panel against predicate ELISA methods

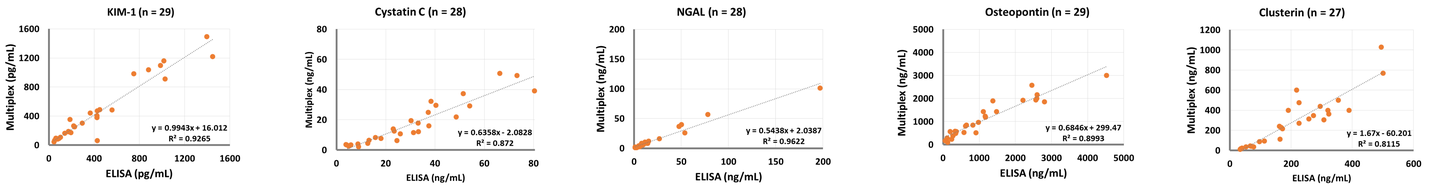


Table 4: Method Comparison Summary

Biomarker	R^2 correlation	Slope
KIM-1	0.9265	0.9943
NGAL	0.9622	0.5438
Cystatin C	0.8720	0.6358
Osteopontin	0.8993	0.6846
Clusterin	0.8115	1.67

Table 5: Cross Reactivity with Panel Cross-Reactants

Biomarker	KIM-1	NGAL	Cystatin C	Osteopontin	Clusterin
KIM-1	-	9.42%	-7.89%	-2.17%	-9.66%
NGAL	-7.92%	-	0.00%	0.00%	0.00%
Cystatin C	-2.11%	-0.01%	-	0.00%	0.00%
Osteopontin	-2.81%	-0.45%	-0.09%	-	-0.70%
Clusterin	-0.09%	0.71%	-0.03%	0.01%	-

Green Bold <1% Cross Reactivity; Black Font <10% Interference

Table 6: Interference Cutoff Concentrations

Interferent	NGAL	KIM-1	Clusterin	Cystatin C	Osteopontin
Bilirubin	> 1 mg/mL	> 0.5 mg/mL	> 0.2 mg/mL		
Glucose	> 30 mg/mL				
Sodium	> 60 mg/mL			> 20 mg/mL	
Creatinine	> 5 mg/mL				> 3 mg/mL
pH	<4.5 and >8.5				< 5.5 and > 8.5

Table 7: Cross Reactivity with Non-Panel Cross-Reactants

Biomarker	TIM-3	TIM-4
KIM-1	-3.88%	-5.08%

Biomarker	Complement C4	Cathepsin L	Cathepsin B	Cathepsin D	Cathepsin S	MMP-9	Cystatin D	Fetuin A	Fetuin B	HPRG	Kininogen
Cystatin C	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.01%	0.01%	0.00%

Biomarker	ApoA1	ApoA2	ApoB	ApoB100	ApoC1	ApoC2	ApoD	ApoE3	ApoE4	ApoH	ApoM
Clusterin	0.00%	0.01%	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Green Bold <1% Cross Reactivity; Black Font <10% Interference

NOTE: Testing of NGAL and OPN cross-reactants are pending.

Analytical Performance Summary of the Randox Evidence Investigator™ AKI Multiplex

- Expanded measurable range
- No cross reactivity with panel or tested non-panel proteins
- No interference with common urine analytes
- Comparable sensitivity and correlation to predicate methods
- Low sample volume required
- Fast turn-around time

CONCLUSIONS

The Randox Evidence Investigator™ AKI multiplex panel simultaneously detects KIM-1, NGAL, cystatin C, clusterin and osteopontin with improved performance compared to the predicate ELISA methods. The AKI multiplex panel provides a robust and cost-effective solution for AKI detection during clinical trials and has great potential for future diagnostic use.