Cross Reactivity and Interference for a Novel 5-plex Acute Kidney Injury Panel

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

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

Abstract

Background:
Acute kidney injury (AKI) is classified using serum-based creatinine (e.g., KDIGO) guidelines. However, since creatinine is a lagging index of impending AKI, studies are now underway to qualify a set of biomarkers for detecting drug-induced human kidney injury (DIKI) in clinical trials. Here, we present the performance of a previously reported multiplex panel composed of urine biomarkers of AKI after reagent optimization.

Methods:
The AKI multiplex was used to measure urine biomarkers from 16-37 subjects and compared to ELISAs; KIM-1, cystatin C, clusterin, OPN and NGAL. Cross-reactivity was tested for each analyte from non-panel related proteins. Interfering substances were titred to determine a non-interfering concentration (within ± 10%). Interferents included human serum albumin (HSA), hemoglobin, bilirubin, pH, glucose, sodium chloride and creatinine.

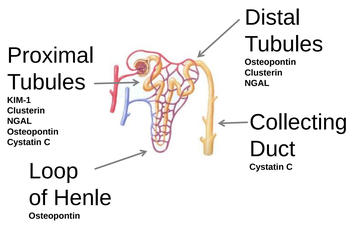
Results:
Method comparison between the two analytical methods provided correlations (r^2) of 0.8888, 0.9773, 0.9340, 0.7919, and 0.9311 for KIM-1, NGAL, cystatin C, clusterin and OPN, respectively. Slopes were 0.3828, 1.5828, 1.0651, 0.5542, and 0.2649, respectively. Cross reactivity was <1% for all tested non-panel, related proteins. HSA non-interfering concentration was <0.5 mg/mL for clusterin, <3 mg/mL for OPN and <5 mg/mL for cystatin C & NGAL. Hemoglobin was <0.5 mg/mL for cystatin C & NGAL, <1 mg/mL for OPN & KIM-1, and <62.5 µg/mL for clusterin. Bilirubin was <1 mg/mL for cystatin C and <2 mg/mL for remaining panel biomarkers. Glucose, sodium chloride and creatinine were <30 mg/mL, <60 mg/mL & <5 mg/mL, respectively, for all biomarkers.

Conclusion:
The AKI multiplex panel has been modified and its performance optimized with new reagents resulting in improved correlations when comparing the two methods. Interference for substances known to be present in urine was above physiological levels. Cross-reactivity results for non-panel proteins indicate no significant cross-reactivity. Previously reported data show this multiplex for urine biomarkers is sensitive, precise and has a wide dynamic range. These results and those from the current study demonstrating good selectivity that fills the need for an objective, robust and cost-effective solution for diagnosis and monitoring DIKI and AKI in other settings.

Introduction

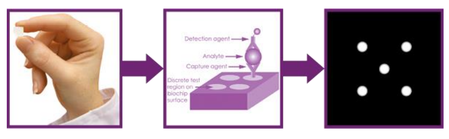
Recent efforts have identified several emerging urinary biomarkers that are more sensitive indicators of AKI than serum creatinine. These include urinary KIM-1, cystatin C, osteopontin, clusterin and NGAL, which are being studied at the Critical Path Institute and FNIH for qualification as biomarkers of drug-induced AKI.

Here, we developed a multiplexing biochip array that can simultaneously measure five urinary biomarkers from a single urine sample, and compared its performance with predicate ELISA methods.



Methods

Randox Biochip Array Technology (BAT) 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 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.



The target standard curve ranges were established after analyzing the span of biomarker concentrations in urine from over 1000 subjects with normal kidney function or potential AKI as determined by ELISA.

The functional sensitivities were assessed by serially diluting the lowest non-zero standard and determining the lowest concentration at which imprecision was ≤20% and the deviation from the target was ≤20% (n=6).

The upper limits of quantification were assessed by the precision (%CV) of the highest non-zero standard (n=6).

Stability of a reagent was determined by evaluating the % difference of each stress condition to a fresh control at which the % difference ≤20% for the concentration and ≤15% for the RLUs for each analyte. Stability was predicted based on accelerated stress conditions at 37°C.

Interference of substances 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%).

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

Method comparison evaluated the analytical performance of this panel by comparing against the following singlicate ELISA methods using up to 16-37 normal subjects; KIM-1, cystatin C, osteopontin, clusterin (R&D Systems) and NGAL (BioPorto).

Results

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	31.25	31.25	0.78	1	1.41	3.13	62.5	125	10.4	12.5
L3	62.5	62.5	1.56	2.5	2.81	6.25	125	250	15.6	25.0
L4	125	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	Functional Sensitivity	% Coefficient of Variation (% CV)
KIM-1	26.04 pg/mL	10.4%
NGAL	0.54 ng/mL	12.1%
Cystatin C	0.98 ng/mL	6.5%
Osteopontin	43.40 ng/mL	10.6%
Clusterin	5.42 ng/mL	14.3%

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%

Table 4: Reagent Stability

Kit Shelf Life	
4°C	1 year
Open Biochip	
Room Temperature	7 days
Calibrators	
4°C	2 years
Controls	
4°C	2 years
Reconstituted Calibrators	
4°C & 20°C	4 hours
Reconstituted Controls	
4°C & 20°C	4 hours

Table 5: Interference Cutoff Concentrations

Interferent	Osteopontin	Cystatin C	NGAL	KIM-1	Clusterin
Hemoglobin	> 1 mg/mL	> 0.5 mg/mL		> 1 mg/mL	> 62.5 µg/mL
Bilirubin	> 2 mg/mL	> 1 mg/mL	> 2 mg/mL		
Human Serum Albumin	> 3 mg/mL	> 5 mg/mL			> 0.5 mg/mL
pH	< 5.5 and > 8.5	< 4.5 and > 8.5			
Glucose	> 30 mg/mL				
Sodium Chloride	> 60 mg/mL				
Creatinine	> 5 mg/mL				

Table 6: Cross Reactivity with Non-Panel Cross-Reactants

Biomarker	Enterokinase	Thrombin	MMP-3
Osteopontin	-0.69%	0.21%	0.62%

Biomarker	α1 Acid Glyoprotein	α1 Microglobulin	HGF	MMP-2	MMP-8	MMP-9
NGAL	0.03%	0.01%	-0.03%	-0.02%	-0.01%	-0.08%

Biomarker	Complement C4	Cathepsin L	Cathepsin B	Cathepsin D	Cathepsin S	MMP-9	Cystatin D	Fetuin A	Fetuin B	HPRG	Kininogen
Cystatin C	-0.14%	-0.14%	-0.13%	-0.07%	-0.05%	0.03%	0.06%	0.03%	-0.05%	0.00%	-0.04%

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

Green Font: <1% Cross Reactivity
NOTE: Testing of KIM-1 cross reactants are pending.

Figure 1: Multiplex vs predicate ELISA Method Comparison

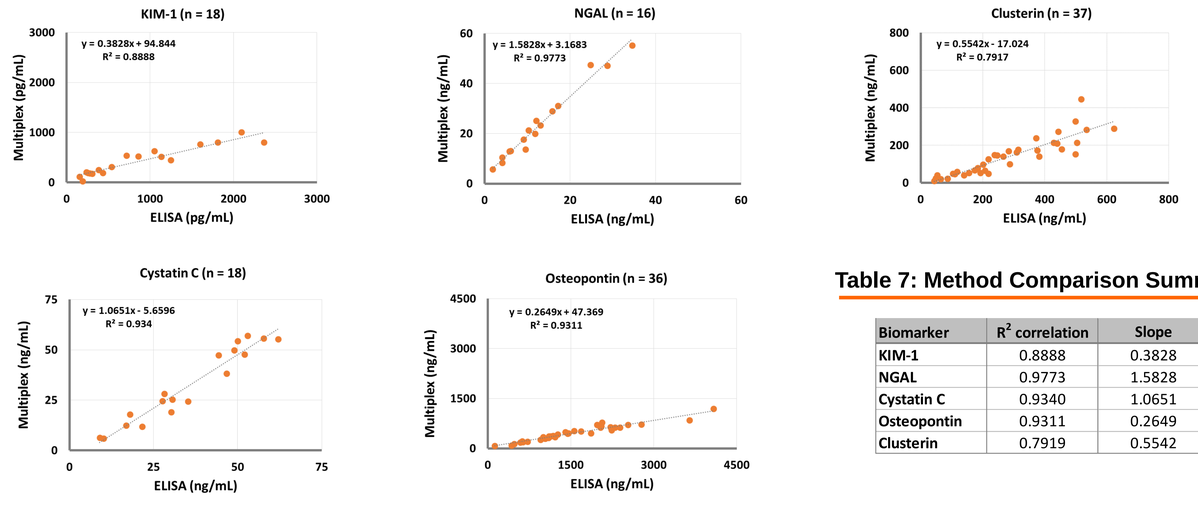


Table 7: Method Comparison Summary

Biomarker	R^2 correlation	Slope
KIM-1	0.8888	0.3828
NGAL	0.9773	1.5828
Cystatin C	0.9340	1.0651
Osteopontin	0.9311	0.2649
Clusterin	0.7919	0.5542

Analytical Performance Summary of the Randox Evidence Investigator™ AKI Multiplex

- Expanded measurable range
- Minimal cross reactivity with tested non-panel proteins
- Excellent reagent stability
- Comparable sensitivity and correlation to predicate methods
- Interference cutoffs for substances known to be present in urine are above physiological levels, however, caution should be taken in urine samples contaminated with blood.
- 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 comparable performance to the predicate ELISA methods. Interference for substances known to be present in urine is above physiological levels. Cross-reactivity results for non-panel proteins indicate no significant cross-reactivity. This AKI multiplex panel provides a robust and cost-effective solution for AKI detection during clinical trials and has great potential for future diagnostic use.