Bladder cancer risk stratification with the Oncuria 10 plex bead-based urinalysis assay using three different Luminex xMAP instrumentation platforms

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Summary

Introduction and Objectives

Oncuria® is a bead-based multiplex fluorescence immunoassay that coordinately measures 10 protein biomarkers in urine samples. The current study compared assay performance and output when urine samples were evaluated with the Oncuria assay using three different fluorescence-analyzing instruments commonly used in diagnostic laboratories worldwide.

Methods

We compared the performance of the clinically validated Oncuria bladder cancer (BC) multiplex immunoassay when data output was generated on three different analyzer systems. Voided urine samples from 36 subjects (18 with BC and 18 Controls) were reacted with Oncuria test reagents in three 96 well microtiter plates, and consecutively evaluated on the LED/image based MagPix, and laser/flow based Luminex 200 and FlexMap 3D (all xMAP instruments from Luminex Corp., Austin, TX). The BC assay uses magnetic bead based fluorescence technology (xMAP, Multi-analyte profiling; Luminex) to simultaneously quantify 10 protein analytes in urine specimens [i.e., angiogenin (ANG), apolipoprotein E (ApoE), carbonic anhydrase IX (CA9), CXCL8/interleukin-8 (IL-8), matrix metalloproteinase-9 (MMP-9), matrix metalloproteinase-10 (MMP-10), serpin A1/alpha-1 anti-trypsin (A1AT), serpin E1/plasminogen activator inhibitor-1 (PAI-1), CD138/syndecan-1 (SDC1), and vascular endothelial growth factor-A (VEGF-A)].

Results

All three platforms categorized all 10 analytes in identical samples at nearly identical concentrations, with variance across systems typically <5%. While the most contemporary instrument, the FlexMap 3D, output higher raw fluorescence values than the two comparator systems, standard curve slopes and analyte concentrations determined in urine samples were concordant across all three units. Fifty percent of BC samples registered ≥1 analyte above the highest standard concentration, i.e., A1AT (n=8/18), IL-8 (n=5), and/or ANG (n=2). In Controls, A1AT was higher in one sample.

Conclusions

In conclusion, the Oncuria BC assay performed similarly well across three different flow analysis platforms for all 10 analytes simultaneously evaluated in urine samples. This agreement across instruments indicates that the test is amenable to standardized performance in laboratories using existing xMAP, without requiring costly outlays for new equipment.

Materials and methods

Oncuria®

- a multiplex immunoassay that quantitatively measures 10 biomarkers associated with bladder cancer (MMP-9, VEGF-A, CA9, SDC1, PAI-1, IL-8, ApoE, A1AT, ANG and MMP-10)

- is in clinical trials to support FDA approval as an in vitro diagnostic test
  - ➢ for predicting BCG response in patients with BC (Oncuria-Predict)
  - ➢ for detecting de novo BC in patients with hematuria (Oncuria-Detect)
  - ➢ for detecting recurrent BC in patients with a history of BC (Oncuria-Monitor)

Oncuria®

Oncuria® Monitor
- Evaluation of patients with a history of bladder cancer on surveillance
- Unique algorithm

Oncuria® Detect
- Evaluation of patients with hematuria
- Unique algorithm including age, gender, and race

Oncuria® Predict
- Evaluation of patients for BCG response
- Unique algorithm including tumor grade and stage

Diagnostic performance of Oncuria assay in identifying high-grade/low-grade and high-stage/low-stage BC

Tumor Grade	Number of BC cases predicted by biomarker assay	AUC	Sensitivity(%)	Specificity(%)	NPV(%)	PPV(%)
Overall	42/45	0.95	0.93	0.93	0.99	0.65
Low-grade tumors	8/9	0.94	0.89	0.93	1.00	0.26
High-grade tumors	34/36	0.95	0.94	0.93	1.00	0.60
NMIBC	25/27	0.93	0.93	0.93	0.99	0.52
MIBC	15/16	0.97	0.94	0.93	1.00	0.39

xMAP instrumentation

	MAGPIX® System	Luminex® 200 System	FLEXMAP 3D
			
Read time (96-well plate)	< 60 min	< 45 min	< 20 min
Analytes	50	100	500
Features	compact	fast	high-sensitivity, high-speed

Results

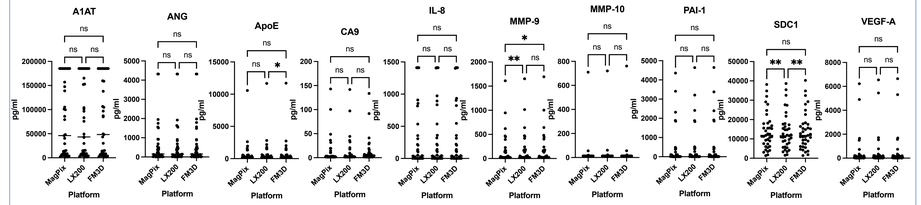
Subject characteristics

		Controls N = 18	Bladder cancer N = 18			Controls	Bladder cancer
Age		53.7 (19-79)	65.4 (20-87)	Stage	NMIBC	N/A	9
Male : Female		18:0	16:2		MIBC	N/A	9
Race	White	8	14	Grade	Low	N/A	2
	Other	6	3		High	N/A	16
	Unknown	4	1				

Biomarker protein concentrations in urine samples compared across three analyzers (pg/mL)

Sample ID	Instrument	A1AT	ANG	ApoE	CA9	IL-8	MMP-9	MMP-10	PAI-1	SDC1	VEGF-A
#0146	MagPix	185,250	1952	2618	11	855	161	13	1889	29,133	1496
Tumor	200	185,250	1922	2823	6	893	179	13	1832	27,662	1535
	FlexMap 3D	185,250	1978	2728	10	903	170	13	1914	29,256	1567
#0010	MagPix	3570	3570	204	1	2	21	13	8	3099	25
Control	200	3872	3872	204	4	2	21	13	8	3306	29
	FlexMap 3D	3581	3581	204	1	2	21	13	8	3155	28

- All three platforms categorized all 10 analytes in identical samples at nearly identical concentrations, with variance across systems typically <5%.



- In half of BC samples, A1AT, IL-8 and/or ANG were above the highest concentration of the calibration curve.

Raw fluorescence data outputs across three flow analyzers

Sample ID	Instrument	A1AT	ANG	ApoE	CA9	IL-8	MMP-9	MMP-10	PAI-1	SDC1	VEGF-A
#0146	MagPix	2889	1612	514	2	2876	150	3	1412	1663	962
Tumor	200	2991	1604	556	2	3090	185	4	1522	1782	1062
	FlexMap 3D	236,110	13,055	4246	17	23,851	1265	25	11,801	14,059	8080

- FlexMap 3D output higher raw fluorescence values than MagPix and 200.

Conclusions

Multiplex BC assays generate detailed molecular signatures useful for identifying BC, predicting treatment responsiveness, and tracking disease progression and recurrence. The similar performance of the Oncuria assay across three different analyzer systems supports test adaptation by clinical and research laboratories using existing xMAP platforms.