

Evaluating Urinary CXCL10 as a Biomarker Associated with Kidney Transplant Immune Activity

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Introduction

Elevated urine chemokine ligand 10 (CXCL10), also known as IP 10, is a potential biomarker for researching early renal allograft rejection. To research an affordable, user-friendly, and quick response test for evaluating urine CXCL10, we explored the utility of a bead-based CXCL10 product and studied the functional characteristics of this assay.

Materials and methods

CXCL10 present in urine samples or standards bind to CXCL10 capture beads, incubated first with a biotin labeled CXCL10 detection antibody, followed by the secondary streptavidin R-phycoerythrin conjugate (SAPE), and then the plate is read on a LABScan3D™ instrument. The standard curve and CXCL10 concentrations are calculated with 4 parameter logistic (4PL) regression using MyAssays software (www.myassays.com).

Results

The overview of the experimental urine CXCL10 bead-based test is shown in Figure 1A. The assay flow is less than 5 hours and less than 2 hours analysis (Figure 1B). A representative standard curve is shown in Figure 2, with R² >0.99.

The spike recovery was evaluated by spiking 6 levels of recombinant CXCL10 into 6 diluted (1:4) healthy urine samples (3.125 -100 pg/ml). Recoveries were determined in 3 independent experiments with duplicates each sample. The recovery ranges from 72% to 115% with an overall mean recovery of 98%. Data was shown in Table 1.

The detection limit of CXCL10 was below 6.25 pg/ml with intraassay coefficient variation (CV) < 20%. Although the CV at 3.125pg/ml was over 20%, the absolute difference at 3.125 pg/ml was less than 1 pg/ml.

Results

Figure 1A. Overview of screening process for urine CXCL10 bead-based assay.

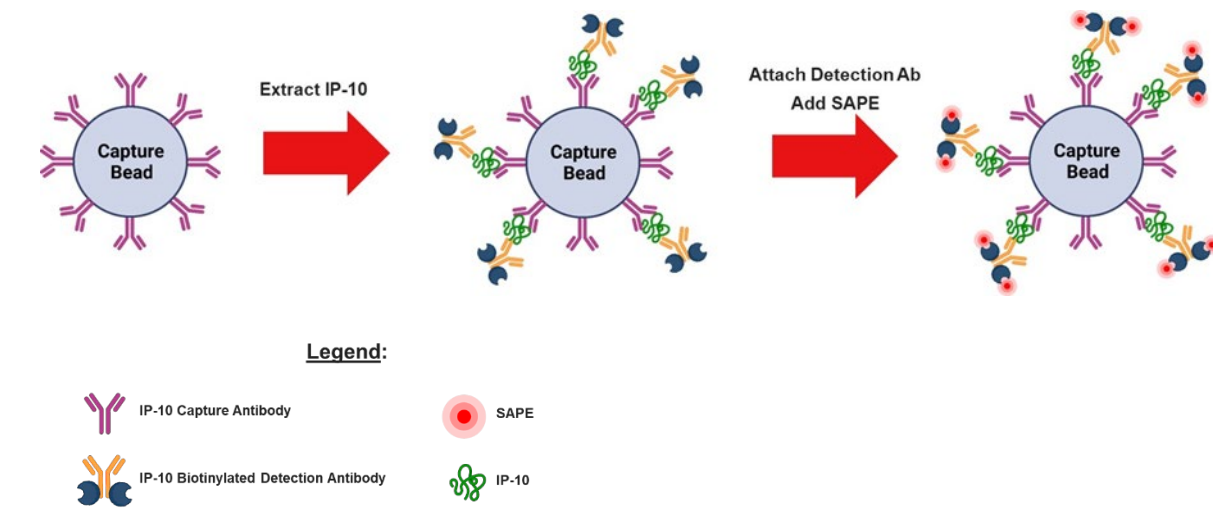


Figure 1B. The experimental urine CXCL10 assay flow.

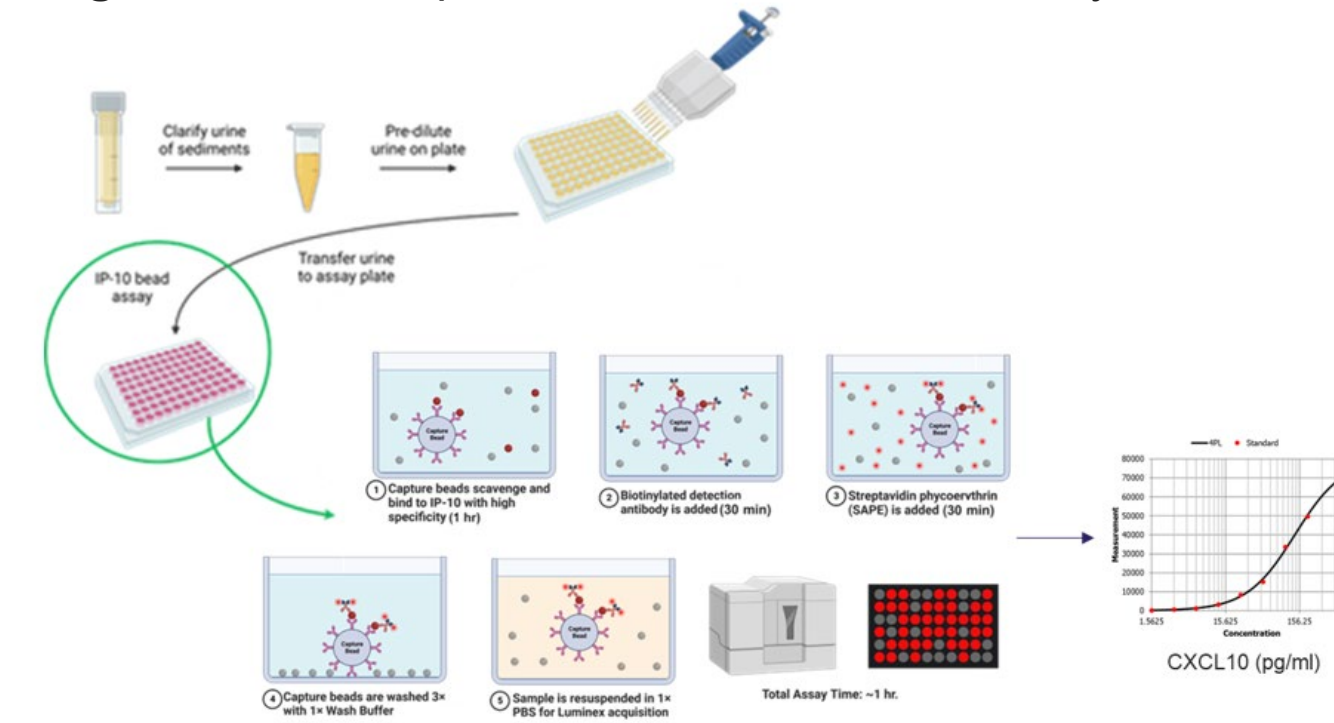


Figure 2. Representative standard curve for urine CXCL10 bead-based test with 4PL regression using MyAssays™ software. A standard curve must be run for each plate assayed.

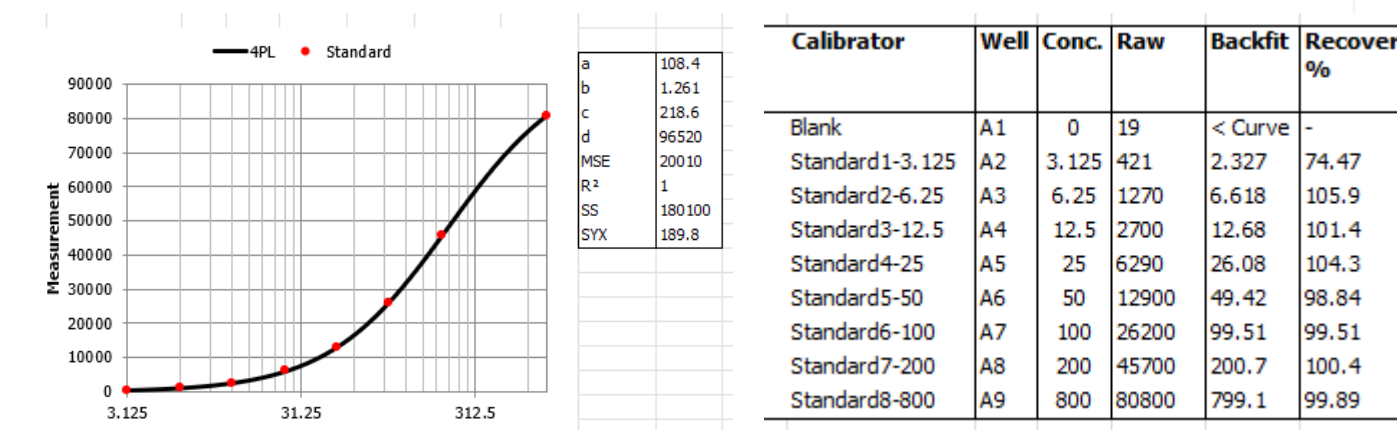


Table 1. The spike recovery was evaluated by spiking 6 levels of recombinant CXCL10 into 6 diluted (1:4) healthy urine samples (3.125 -100 pg/ml). The recovery ranges from 72% to 115% with an overall mean recovery of 98%. The inter assay CV for internal controls and for the urine control samples with spiked CXCL10 from 6.25-100 pg/ml ranges from 2%-16% with an overall mean CV% of 7%. Although the CV at 3.125pg/ml was over 20%, the absolute difference between the expected and observed concentration is within 1 standard deviation of the expected concentration.

Scientist	IP10 (pg/ml)	Scientist 1			Scientist 2			Scientist 3			Mean	SDev	CV%	Recovery %	Absolute difference (pg/ml)	meansD	
		Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3	Lot 1	Lot 2	Lot 3							
Internal Control -1	25	22.0	19.3	22.8	outlier	24.1	24.8	25.0	26.7	22.6	23.5	2.3	10%	94%	-1.47	21.3	25.8
Internal Control -2	50	49.7	34.0	43.5	outlier	43.8	51.0	44.9	50.0	47.6	45.3	5.5	12%	91%	-4.74	39.8	50.7
F1	3.125	1.2	4.1	2.7	1.2	1.9	3.5	< Curve	< Curve	4.7	2.4	1.4	57%	78%	-0.70	1.0	3.8
	6.25	6.1	7.4	5.5	6.4	6.7	6.6	4.2	8.5	6.7	6.4	1.2	19%	102%	0.16	5.2	7.6
	12.5	13.2	14.4	11.2	14.6	14.7	14.3	12.0	15.2	11.9	13.7	1.5	11%	110%	1.20	12.2	15.2
	25	25.3	23.6	25.2	24.7	27.9	23.6	23.8	26.3	22.3	25.0	1.7	7%	100%	0.04	23.4	26.7
	50	48.7	52.7	50.2	55.8	60.8	55.5	45.7	41.0	43.0	51.3	6.5	13%	103%	1.31	44.8	57.8
	100	98.5	105.7	100.3	105.1	107.8	109.4	94.7	112.7	113.2	104.3	6.4	6%	104%	4.27	97.9	110.6
M1	3.125	1.1	3.7	3.1	3.4	2.0	4.2	1.2	< Curve	4.8	2.7	1.4	51%	86%	-0.45	1.3	4.0
	6.25	7.0	7.7	6.0	8.2	5.5	8.8	3.5	3.6	8.2	6.3	2.0	32%	100%	0.03	4.3	8.3
	12.5	14.9	14.8	12.9	15.9	12.6	17.1	11.6	14.5	13.6	14.3	1.7	12%	114%	1.78	12.6	16.0
	25	27.4	28.5	27.6	30.4	30.6	30.8	25.7	28.6	25.2	28.7	2.0	7%	115%	3.70	26.7	30.7
	50	52.3	61.2	56.3	53.7	48.0	63.5	47.4	51.6	45.9	54.2	6.1	11%	108%	4.24	48.1	60.3
	100	96.2	104.1	106.4	107.0	76.0	86.2	85.2	100.7	106.7	95.2	11.4	12%	95%	-4.77	83.8	106.6
F2	3.125	< Curve	3.3	2.6	1.1	1.6	3.5	< Curve	< Curve	5.6	2.4	1.6	65%	78%	-0.69	0.9	4.0
	6.25	6.3	6.7	5.3	5.5	6.5	7.1	4.5	4.9	6.5	5.9	0.9	15%	94%	-0.40	5.0	6.7
	12.5	12.4	13.7	11.9	14.5	15.2	13.8	11.6	14.5	11.7	13.4	1.4	10%	108%	0.94	12.1	14.8
	25	25.0	24.7	24.8	27.7	29.3	28.2	22.7	25.4	23.5	26.0	2.2	9%	104%	0.97	23.7	28.2
	50	47.0	53.4	50.2	53.6	55.0	50.8	48.6	53.4	46.8	51.5	3.0	6%	103%	1.51	48.5	54.5
	100	92.7	97.7	99.2	77.0	113.2	95.8	83.6	98.4	86.8	94.7	10.5	11%	95%	-5.31	84.2	105.2
M2	3.125	0.7	3.9	2.8	2.5	1.1	3.8	< Curve	< Curve	4.5	2.5	1.4	59%	78%	-0.67	1.0	3.9
	6.25	5.7	6.8	5.2	7.0	5.4	6.1	5.2	5.4	6.9	5.9	0.8	13%	94%	-0.39	5.1	6.6
	12.5	10.9	15.0	9.9	15.1	12.9	12.5	11.6	9.9	11.8	12.2	1.9	16%	98%	-0.27	10.3	14.1
	25	25.6	25.9	22.8	29.7	26.5	23.6	35.2	26.1	21.8	26.9	4.1	15%	108%	1.92	22.9	31.0
	50	50.1	52.1	45.1	51.4	54.0	54.7	42.4	46.6	41.8	49.5	4.9	10%	99%	-0.47	44.7	54.4
	100	79.4	96.6	98.1	99.3	97.3	103.8	74.1	98.1	85.0	93.3	10.3	11%	93%	-6.66	83.1	103.6
F3	3.125	0.6	3.5	2.6	2.0	1.9	4.0	1.1	< Curve	4.8	2.3	1.5	64%	72%	-0.87	0.8	3.7
	6.25	6.1	6.5	5.3	6.6	7.1	7.0	6.6	2.9	7.5	6.0	1.4	23%	96%	-0.24	4.6	7.4
	12.5	13.0	12.6	11.9	15.9	15.7	14.1	12.7	10.9	12.6	13.3	1.7	13%	107%	0.84	11.7	15.0
	25	27.4	23.8	24.1	29.9	30.0	27.8	28.5	25.3	24.1	27.1	2.5	9%	108%	2.10	24.6	29.6
	50	50.6	51.3	54.5	55.1	57.1	60.1	48.1	51.2	47.8	53.5	4.1	8%	107%	3.48	49.3	57.6
	100	95.0	99.9	102.8	110.1	113.3	99.5	92.8	103.6	96.6	102.1	6.8	7%	102%	2.13	95.3	108.9
M3	3.125	3.5	3.8	3.5	3.4	2.4	4.0	1.2	< Curve	4.6	3.1	1.0	34%	100%	-0.02	2.1	4.2
	6.25	8.8	7.3	5.8	7.7	5.4	7.5	6.3	4.5	6.2	6.7	1.3	20%	106%	0.40	5.3	8.0
	12.5	15.0	13.7	11.0	15.3	13.9	13.1	12.3	13.0	10.8	13.4	1.6	12%	107%	0.91	11.8	15.0
	25	28.0	22.8	22.3	29.4	24.3	26.7	27.0	26.8	20.3	25.9	3.0	12%	104%	0.90	22.9	28.9
	50	52.8	44.4	45.1	49.9	45.3	49.9	45.7	47.3	41.4	47.5	3.5	7%	95%	-2.46	44.1	51.0
	100	85.5	90.0	92.7	92.5	81.2	95.3	89.7	86.9	80.4	89.2	5.2	6%	89%	-10.76	84.1	94.4

Conclusions

This experimental urine CXCL10 bead-based test is a quick test that may provide a cost-effective method to evaluate urinary CXCL10 for research purposes.

References

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- Julie Ho, Atul Sharma, Kristine Kroeker, Robert Carroll, Sacha De Serres, Ian W Gibson, et al. Multicenter randomized controlled trial protocol of urine CXCL10 monitoring strategy in kidney transplant recipients. *BMJ Open* 2019 Apr 11;9(4):e024908.

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