Supplementary Data for research article manuscript entitled:

Tunable Detection Sensitivity of Opiates in Urine via a Label-Free Porous Silicon Competitive Inhibition Immunosensor

Lisa M. Bonanno, Lisa A. DeLouise*

Department of Dermatology and Department of Biomedical Engineering University of Rochester

Medical Center 601 Elmwood Avenue, Box 697, Rochester, NY 14642 (USA)

E-mail: lisa_delouise@urmc.rochester.edu

	Hair (pg ml ⁻¹)	Oral Fluid (ng ml ⁻¹)	Sweat (ng per patch)	Urine ^a (ng ml ⁻¹)
Cannabinoids	1	4	4	50
Cocaine metabolites	500	20	25	150
Opiates ^b	200	40	25	300 ^c
Phencyclinidine	300	10	20	25
Amphetamines	500	50	25	500 ^d

Table S1: Cutoff Values for DOA Screening Used in Medical Testing

^a Urine is the most common and only matrix that guidelines set by the Department of Health and Human Services, Substance Abuse Mental Health Services Administration (SAMHSA) apply to at this time.

^b Initial test specific to 6-AM only permitted at cutoffs of 200 pg ml⁻¹ (hair), 4 ng ml⁻¹ (oral fluid), 25 ng per patch (sweat), and 10 ng ml⁻¹ (urine).

^c For forensic (non-medical) testing the initial cutoff has been raised to 2000 ng ml⁻¹ which is sufficiently higher than what has been seen following ingestion of contaminated poppy seeds.^[REF Kwong]

^d For forensic (non-medical) testing the initial cutoff for amphetamines is 1,000 ng ml⁻¹.

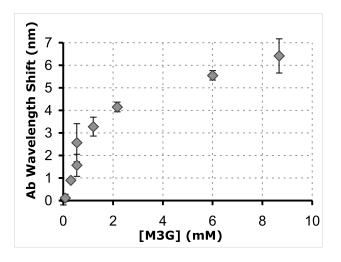


Figure S1: Anti-Morphine Ab (α -M Ab concentration fixed at 1.83 μ M) Binding with Increasing M3G Applied in EDC Coupling Chemistry.

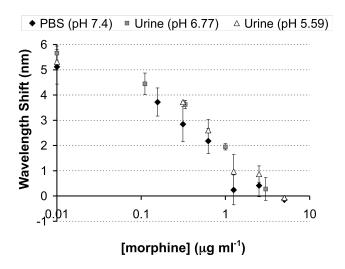


Figure S2: No Effect of Urine pH vs. PBS on Morphine Response Curve. Error bars represent interday assay variability for n=4 for PBS curve vs. n=3 interassay variability for two different urine specimens with different pH (6.77, 5.59) on different days.

DOA type	R ²	Chi ² × DoF ⁻¹	Initial Plateau, A1 (nm)	Final Plateau, A2 (nm)	EC50, x0 (μM)	Curve Steepness, (nm µM ⁻¹)
morphine	phine 0.9811 0.0043		0.986 <u>+</u> 0.033	0.001 <u>+</u> 0.037	1.336 <u>+</u> 0.145	1.936 <u>+</u> 0.382
M3G	0.9905	0.0022	0.993 <u>+</u> 0.044	-0.019 <u>+</u> 0.043	0.606 <u>+</u> 0.101	1.036 <u>+</u> 0.170
oxycodone	0.9829	0.0040	1.035 <u>+</u> 0.063	-0.135 <u>+</u> 0.120	1.432 <u>+</u> 0.483	0.762 <u>+</u> 0.193
6-AM	0.9935	0.0014	1.021 <u>+</u> 0.037	-0.023 <u>+</u> 0.044	0.961 <u>+</u> 0.146	0.935 <u>+</u> 0.131

Table S2: Logistic Fit Parameters to PSi Sensor Response to Various Opiates Spiked into Urine

Table S3: Logistic Fit parameters to ELISA Response to Various Opiates Spiked into Urine

DOA type	R ²	$\begin{array}{c c} Chi^2 \times \\ DoF^{-1} \end{array} \begin{array}{c} Initial Plateau, \\ A1 \\ (nm) \end{array} \begin{array}{c} Final Plateau, \\ A2 \\ (nm) \end{array}$		EC50, x0 (μM)	Curve Steepness, (nm µM ⁻¹)	
morphine	phine 0.9964 0.0007		1.015 <u>+</u> 0.021	0.113 <u>+</u> 0.019	0.109 <u>+</u> 0.013	0.910 <u>+</u> 0.092
M3G	0.9903	0.0018	0.945 <u>+</u> 0.029	0.125 <u>+</u> 0.025	0.058 <u>+</u> 0.010	1.114 <u>+</u> 0.189
oxycodone	0.9942	0.0012	1.009 <u>+</u> 0.022	0.129 <u>+</u> 0.024	0.156 <u>+</u> 0.021	1.063 <u>+</u> 0.139
6-AM	0.9976	0.0005	0.997 <u>+</u> 0.018	0.145 <u>+</u> 0.012	0.038 <u>+</u> 0.003	1.042 <u>+</u> 0.086

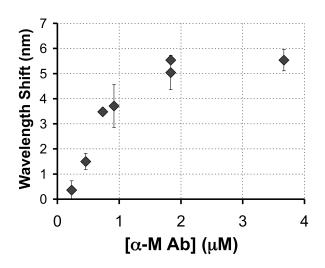


Figure S3: α -M Ab Binding with Increasing α -M Ab Applied to Sensor that was derivatized with EDC-M3G Analogue Attachment Chemistry (M3G concentration fixed at 8.67 mM).

_								
	[α-M Ab] (μM)	R ²	Chi ² × DoF ⁻¹	Initial Plateau, A1 (nm)	Final Plateau, A2 (nm)	EC50, x0 (μM)	Curve Steepness, (nm µM ⁻¹)	Detection Range (µM)
ĺ	0.73	0.9834	0.0405	3.516 <u>+</u> 0.137	-0.348 <u>+</u> 0.252	0.534 <u>+</u> 0.112	0.921 <u>+</u> 0.170	0.126 - 1.762
	1.83	0.9832	0.1201	5.521 <u>+</u> 0.188	-0.209 <u>+</u> 0.218	1.356 <u>+</u> 0.149	1.682 <u>+</u> 0.299	0.359 - 4.023

Table S4: Logistic Fit parameters to Morphine Response Curve with Varying Antibody Concentration