Supplementary information

"Non-invasive monitoring of circadian relevant biomarker from easily accessible body fluids using hybrid aqueous-ionic buffer interfaces on flexible substrates"

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S1: FTIR analysis for functionalized electrode system

Figure S1: FTIR spectrum for thiol linker and capture probe (Antibody)

The following table highlights the different peaks associated with the FTIR spectrum that confirm immobilization of thiol linker and antibody on the gold electrode surface:

Compound of interest	Description	Expected Peak position	Peak position obtained
	CH alkane chain stretch	3000 cm ⁻¹ - 2900 cm ⁻¹	3000 cm ⁻¹
THIOL LINKER	NHS group in DSP linker	1745 cm ⁻¹	1744 cm ⁻¹
	Amide-I bond	1500-1550 cm ⁻¹	1558,1540,1506 cm ⁻¹
CAPTURE PROBE	Amide-II bond	1652 cm ⁻¹	1652 cm ⁻¹



Figure S2: Nyquist plots for (A) sweat; (B) urine; (C) saliva

S2. Equations for regression analysis and SST:

1. Regression analysis was carried out to check for correlation between the dose response and the cortisol concentrations. A second-degree polynomial fit was used to fit the data and identify a trend. The following are the equations used to model the data:

- 1. Synthetic sweat pH 4: y = 4.3774x² 5.8823x + 23.278
- 2. Synthetic sweat pH 6: $y = -2.6125x^2 + 29.803x + 3.552$
- 3. Synthetic sweat pH 8: $y = -4.0385x^2 + 29.42x 2.076$
- 4. Synthetic urine pH 4.5: y = 0.9961x² 6.9566x + 38.365
- 5. Synthetic urine pH 6: y = 0.2472x² 2.4593x + 36.201
- 6. Synthetic urine pH 8: y = 0.1506x² 1.5882x + 38.179
- 7. Synthetic saliva pH 4: y = 1.9277x² 12.954x + 72.106
- 8. Synthetic saliva pH 6: y = -0.1713x² 1.9356x + 59.638
- 9. Synthetic saliva pH 8: y = 0.1502x² 2.4141x + 38.858
- 10. SST = 3 *($\sigma_{\text{zero dose}}$)

S2. Fitting of data using Z-view®



Figure S3: Modified Randle's equivalent circuit

Rs is solution resistance which is contributed from the bulk solution, CPEdI is the Constant phase element which was used to model the capacitive changes in the electrical double layer. Rct is the charge transfer resistance which is the resistance offered to transfer of charges when there are biomolecules like antibodies immobilized on the surface and CPEw is the Warburg impedance which is an artifact of diffusion.

The following table are the values for the fitting parameters for dose response in sweat, urine and saliva at physiologically stable pH, the highlighted values are the ones that are being modulated with the binding events.

Synthetic sweat					
Doses	Rs	Rct	Cedl		
Baseline	148.8	5.10E+06	5.13E-08		
10 ng/ml	224.6	6.67E+06	3.83E-08		
50 ng/ml	283.9	6.99E+06	3.17E-08		
100 ng/ml	279.7	7.27E+06	3.11E-08		
150 ng/ml	291.6	7.87E+06	3.10E-08		
200 ng/ml	310.7	8.37E+06	3.09E-08		

Synthetic urine

Doses	Rs	Rct	Cedl
Baseline	548.7	1.58E+06	4.85E-07
1 ng/ml	650.6	2.43E+06	5.90E-07
10 ng/ml	716.8	3.17E+06	6.01E-07
100 ng/ml	702.1	3.97E+06	6.10E-07
200 ng/ml	721.1	4.28E+06	5.94E-07
500 ng/ml	725.1	5.14E+06	5.67E-07

Synthetic saliva

Doses	Rs	Rct	Cedl	
Baseline	1104	3.56E+06	3.79E-07	
1 ng/ml	1472	4.05E+06	5.81E-07	
4 ng/ml	1332	4.55E+06	5.90E-07	
10 ng/ml	884.6	5.05E+06	6.07E-07	
40 ng/ml	1192	5.75E+06	6.15E-07	

Typical Chronoamperogram



Figure S4: Typical chronoamperogram from chronoamperometric analysis