supplementary material

Label Free, Lateral Flow Prostaglandin E2 Electrochemical Immunosensor for Urinary Tract Infection Diagnosis

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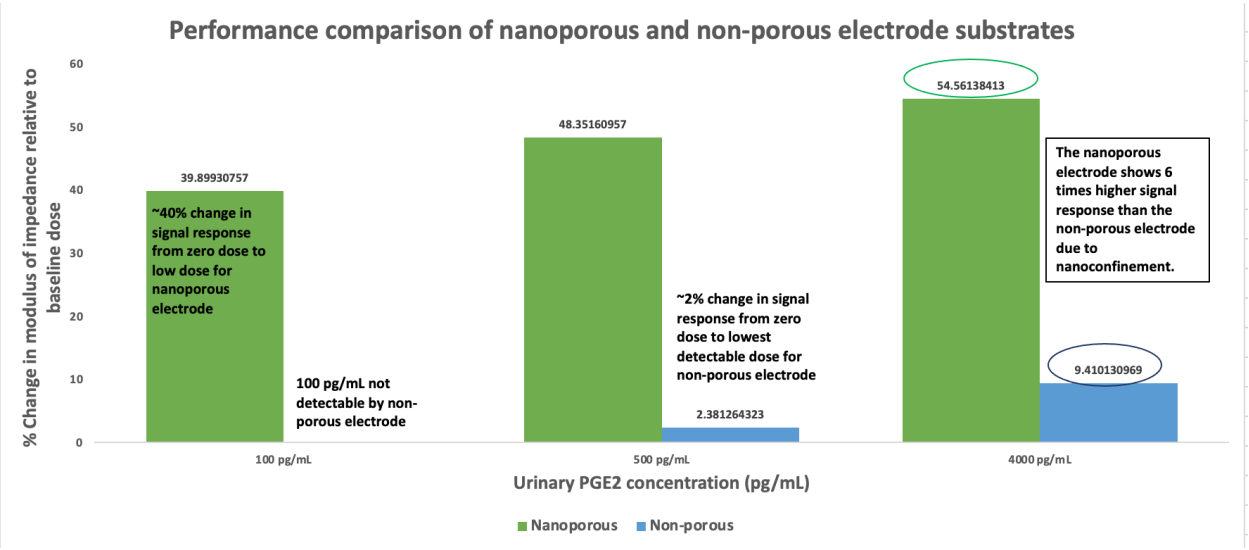


Figure S1: Performance comparison of nanoporous and non-porous electrode substrates.

The impact of signal enhancement was studied by comparing the impedance response of the developed nanoporous lateral flow electrochemical biosensor with a commercially available non-porous three electrode system (Metrohm USA) for the same electrode dimensions and urine volume and same assay protocol. The above figure shows the change in signal response from blank to low dose and compares the output response range. For the nanoporous membrane (shown in green), a mean signal change of 39.9% was observed from the 0 to 100 pg/mL PGE2 dose whereas 2.4% was observed for 0 to 500 pg/mL for that of the commercial electrode (shown in blue). The non-porous membrane has a limit of detection of 500 pg/mL and was unable to detect 100 pg/mL, unlike the nanoporous electrode system. Further, the output response for the highest physiological level of PGE2 was increased by 6 times for the proposed nanoporous system versus the non-porous (commercial) electrode system. In this way, biomolecular confinement was achieved in the nanoporous system to achieve enhanced sensor performance for <100 µL of neat, undiluted urine samples.

The surface wettability of the membrane was evaluated using a Ramé-Hart goniometer:

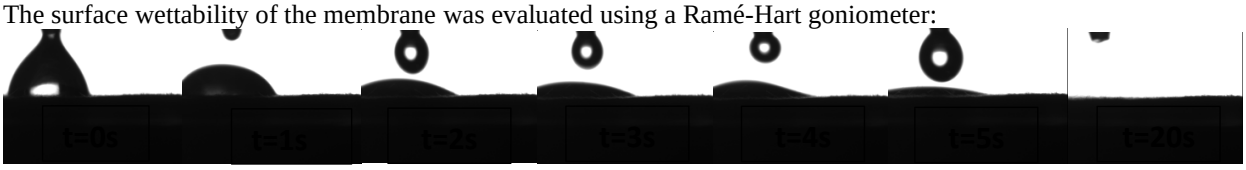


Figure S2: Results for Goniometer experiments.

Clearly, the substrate (lateral flow membrane) is hydrophilic (contact angle~0 at t=20s). It takes less than 20 s for the urine sample to completely wick and cover the electrodes which is well within the device turn-around time of 5 minutes. Thus, it was concluded that the substrate is highly wettable and is suitable for urine based biosensing.

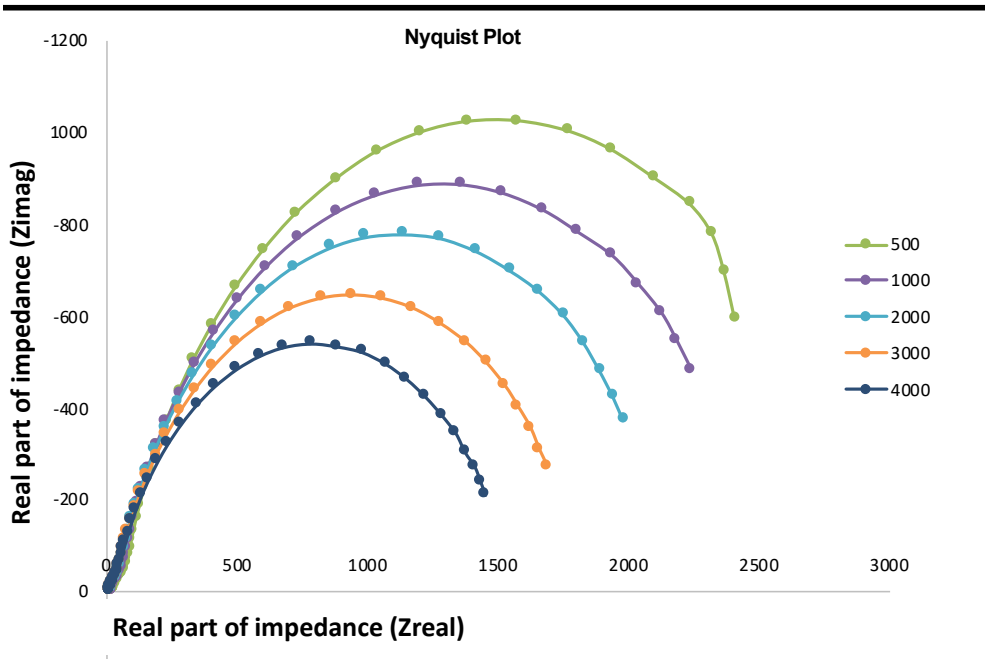


Figure S3: Nyquist plot of EIS response in pooled human urine samples.

The above figure shows the Nyquist plot of the EIS data for sensor response in pooled human urine for PGE2 dosing. The Nyquist plot represents the imaginary part of impedance plotted on the y-axis versus the real part of the impedance on the x-axis. A typical semi-circle Nyquist plot is characteristic of non-faradaic mode of operation. A dose-dependent change in the diameter of the semi-circle is observed as a function of dosing which validates that the dielectric properties at the electrical double layer interface are modulated due to the affinity capture of the PGE2 antigen in the urine by the highly specific monoclonal PGE2 antibody.

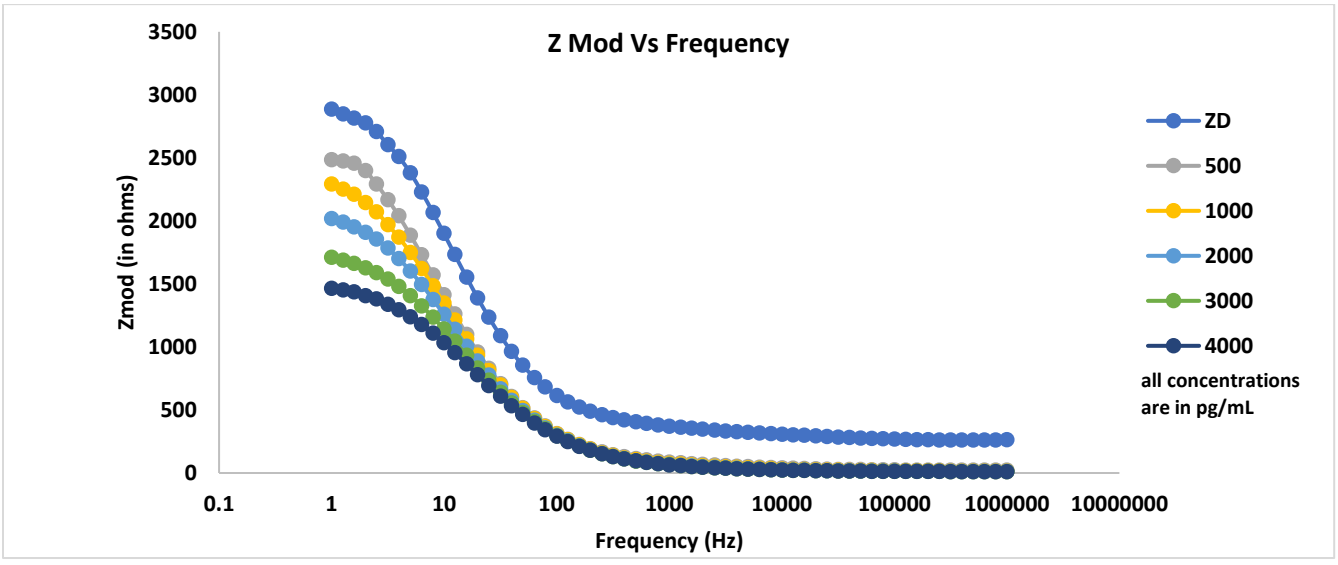


Figure S4: Bode magnitude plot of EIS response in pooled human urine samples.

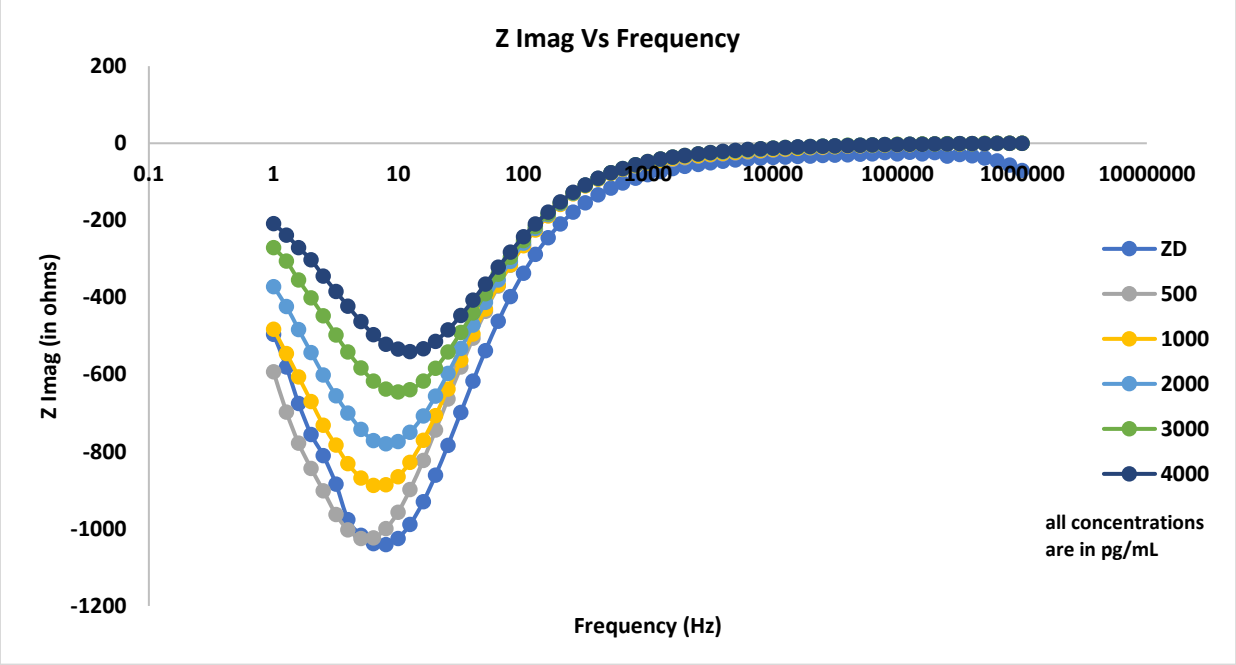


Figure S5: Imaginary part of impedance vs frequency plot of EIS response in pooled human urine samples.

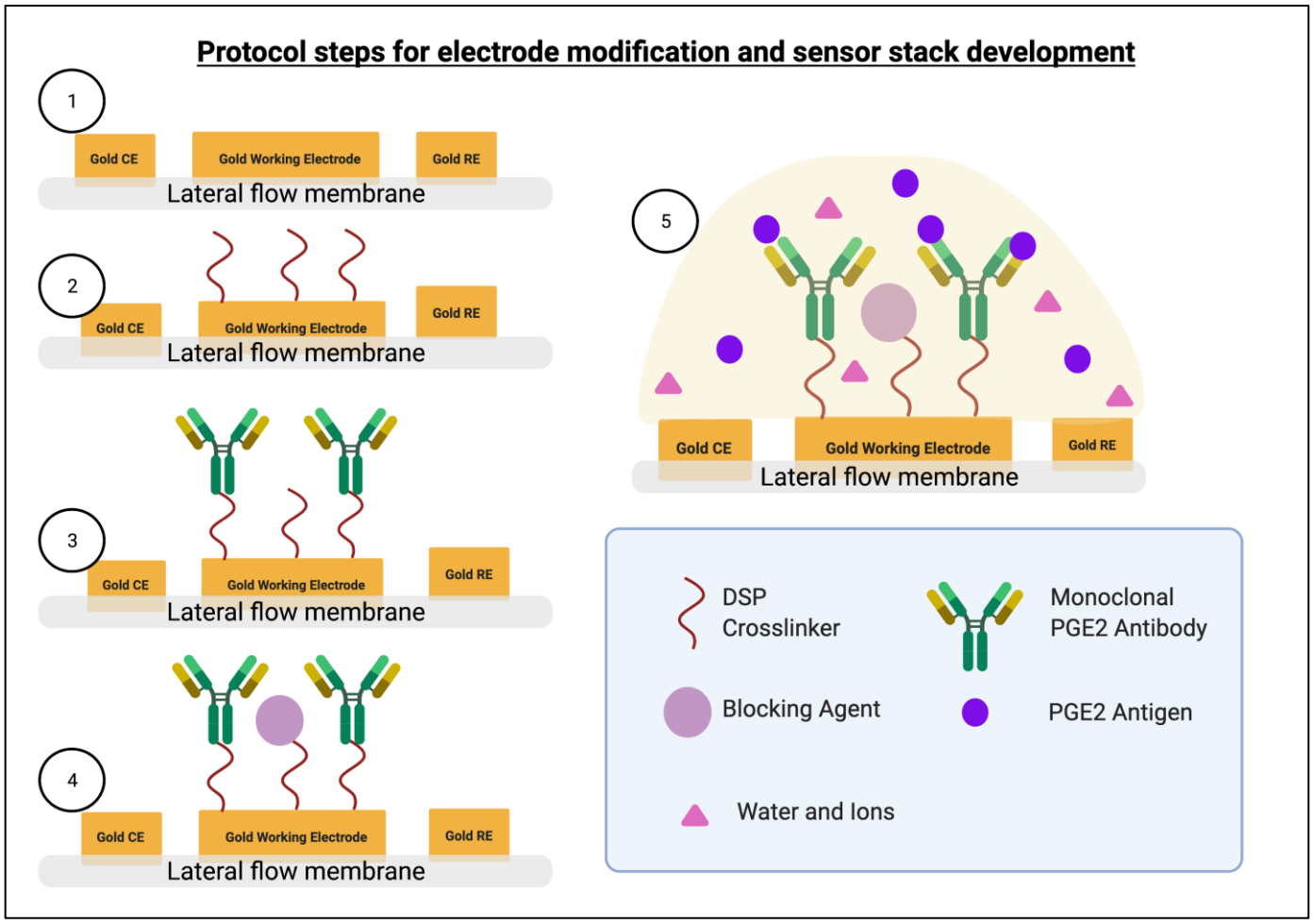
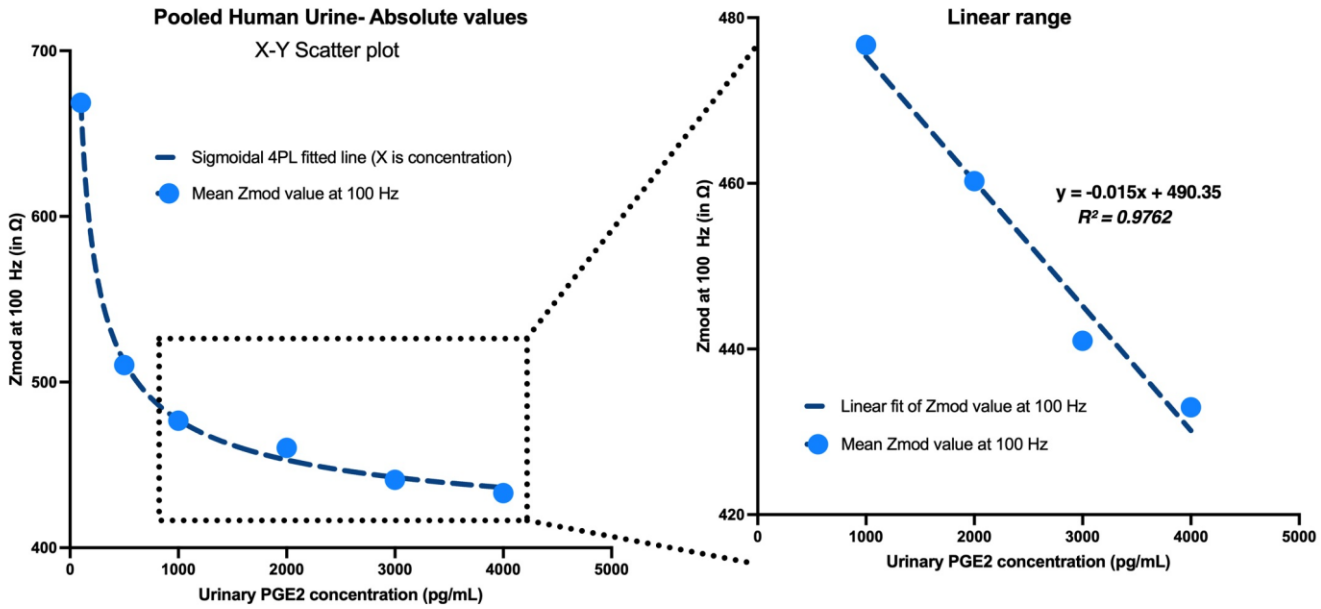


Figure S6: Protocol steps for electrode modification and sensor stack development.



Parameters of sigmoidal 4PL fitting

	Zmod at 100 Hz
Best-fit values	
Bottom	399.8
Top	77750
IC50	0.002875
HillSlope	-0.5412
logIC50	-2.541
Span	77350

Figure S7: Calibration dose response 4PL sigmoidal fitting and linear fitting.

The linear portion of the graph was chosen as the operable region for analysis. The linear region corresponding to the higher doses was chosen for further analysis as it is more prone to effects of sensor saturation and hence its linear operability needs to be analyzed.

The inset figure zooms into the 1000-4000 pg/mL range as shown in the figure above. A highly linear dose dependent response was observed with R^2=0.9762. The R^2 value and the fitted linear equation have been highlighted in the updated figure. Further, the fit parameters of the 4PL analysis have also been included.

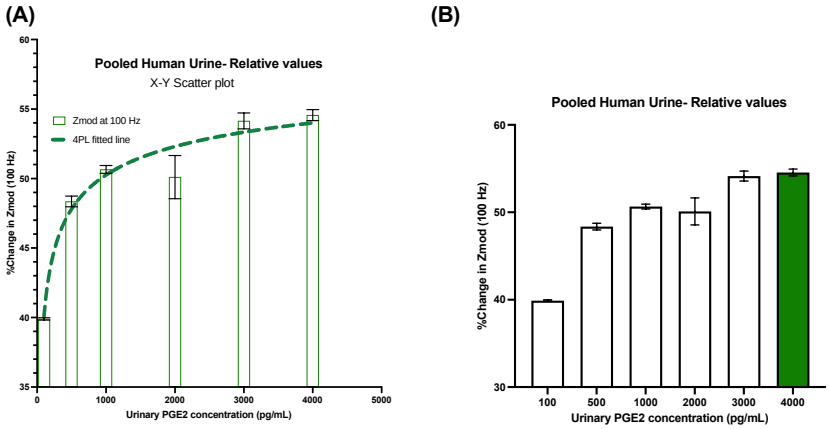


Figure S8: Calibration dose response 4PL sigmoidal fitting for relative impedance values.

The response is represented as the mean of the intra (n=3) and inter sensor (n=3) replicates. The slight variation from 4PL fit line at 2000 pg/mL is due to the presence of an outlier in one of the intra-sensor replicates these replicates because of possible glitch in measuring equipment. However, this effect is negligible when looking at the absolute change graphs, since the output range is in kilo-ohms and does not affect the UTI state classification (UTI positive and negative) and diagnosis. Figure 4 (B) has been updated to include the R2 value in the linear operable range of the PGE2 sensor (absolute impedance values). To determine the precision for the proposed biosensor, coefficient of variation (CV%) was evaluated. The CV% averaged over inter-sensor replicates for the UTI positive and UTI negative results were found to be 4.57% and 12.16% respectively will fall well within the acceptable range of clinical standard practice (< 20%) as per the Clinical laboratory Standards Institute (CLSI) guidelines.

Table S1: Observed and expected peaks of FTIR spectra.

Description	**Expected peak position (cm^{-1})**	**Observed peak position (cm^{-1})**
Stretching of CH alkane chain	2640–3000	2994
Breakage of CO-NHS bond of DSP due to NHS binding of DSP linker to PGE2 mAb	1745	1735
CH_2 bending	1465	1436
CH_3 bending	1375	1317
1°Amide bond	1600–1700	1646
2° Amide bond	1510–1580	1558