

## Supporting Information

### **Low-Cost printed circuit board (PCB) electrochemical biosensors for rapid and label-free detection of *Streptococcus pneumoniae***

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## **S1. Electrochemical set-up for gold plating and cleaning**

All boards used the same electrochemical cell setup for the plating process. The cell consisted of a Pt counter electrode (CE), Ag/AgCl reference electrode (RE) and the electrodes of the PCB are connected to a ten-pin connector that was electrically bridged across all contacts including working electrodes (WE) and submerged in plating solution. The bridge allowed for current to flow to all electrodes so they could all be plated simultaneously (**Figure S1**). For electrochemical cleaning methods PCBs P1, P2 and P3 are used their built-in gold counter and reference electrodes. A MUX8-R2 multiplexer (Alvatek) in combination with PalmSens4 and connector box (Metrohm Runcorn, UK) that connects the multiplexer cables to a ten-pin connector into which the PCBs can be inserted was used. PCB P4's configuration consisted of a Pt counter electrode, Ag-AgCl reference electrode and the electrode was connected to the multiplexer via a custom Peripheral Component Interconnect (PCI) connector (**Figure S1**). The measurement buffer was aliquoted over all electrodes and the surface tension of the buffer-maintained electrode coverage.

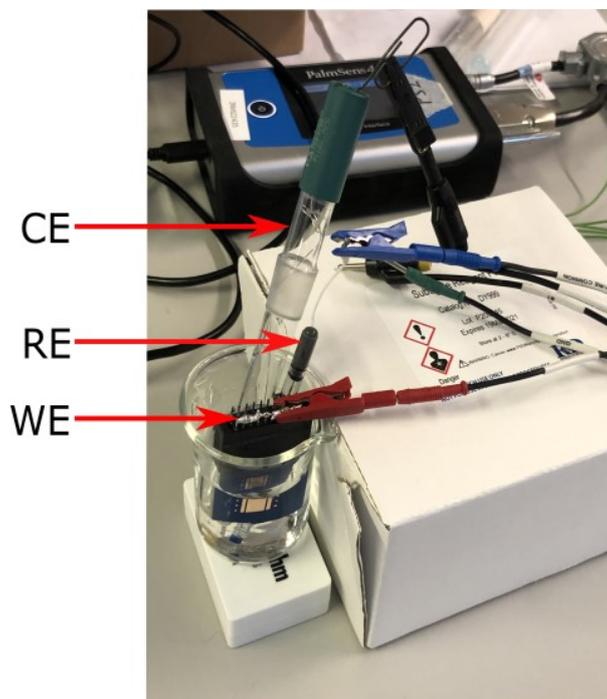
## **S2. Gold plating on PCB electrodes**

Since PCBs P1 and P3 were bare copper sensors they had to be plated in the lab to achieve a gold surface layer for making sepsis biosensor. A combination of electro and electroless plating was employed in forming gold surfaces on the PCBs. PCBs P1 and P3 were gold plated in the lab using optimized combinations of plating solutions, plating times (10 min) and plating currents. For PCB P1, the PCBs were submerged in bright electroless solution at 50°C for 10 min for the best plating results. For PCB P3, just electroless gold plating alone was not sufficient, therefore, after the initial 10 min electroless plating, it was undergone for electroplated for another 10 min, using an optimized applied current of  $-2.50 \mu\text{A}$ . PCB P2 was plated by the manufacturer, which consisted of a hard gold plating, i.e. a thin nickel layer plated onto the copper before the gold layer was added. PCB P4 came with gold finish, so gold plating was not performed.

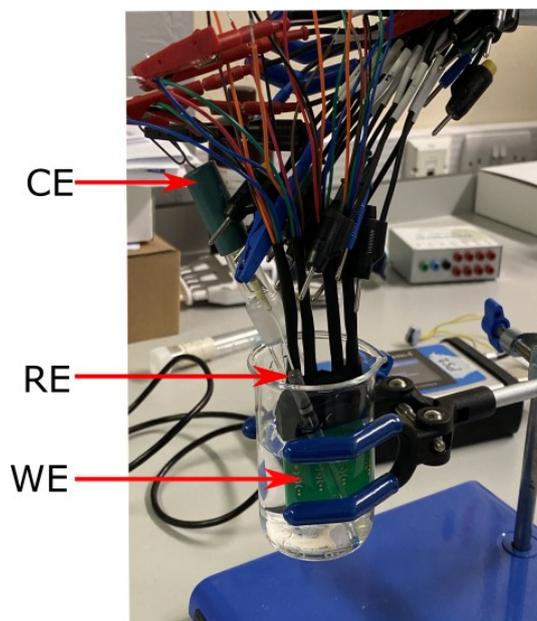
## **S3. Cleaning of gold PCB electrodes**

Once each board had obtained a gold surface finish it was essential to clean the gold surface to remove any impurities and contaminants. The PCBs were subjected to various cleaning methods to determine the effect of each cleaning method and aiming to discover the

optimal cleaning protocol. Cleaning methods performed included mechanical, chemical and electrochemical cleaning using solvents, acids and bases shown.

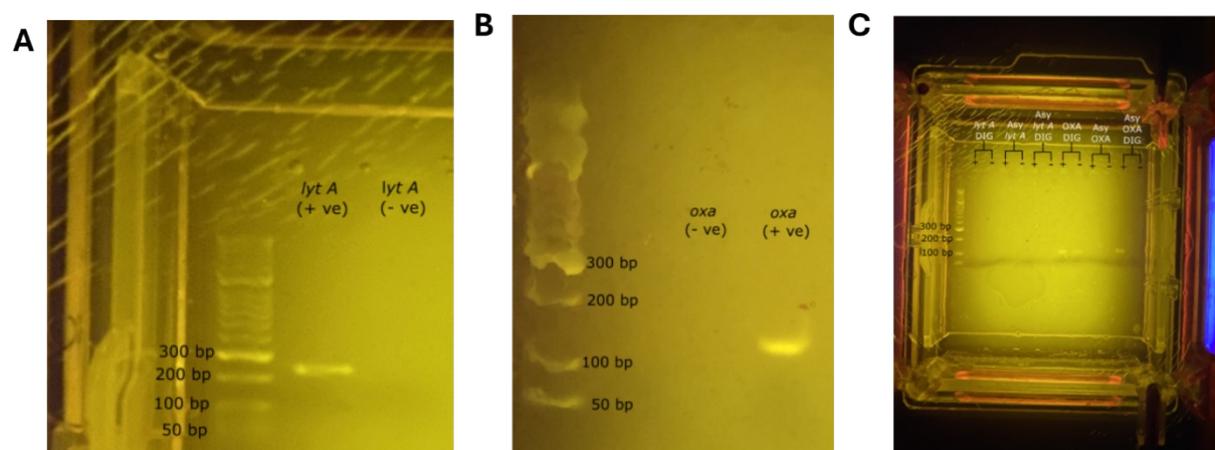


**Figure S1.** Electrochemical cell configuration for gold plating PCB boards using Pt counter electrode, Ag/AgCl reference electrode and bridged working electrodes on PCB.

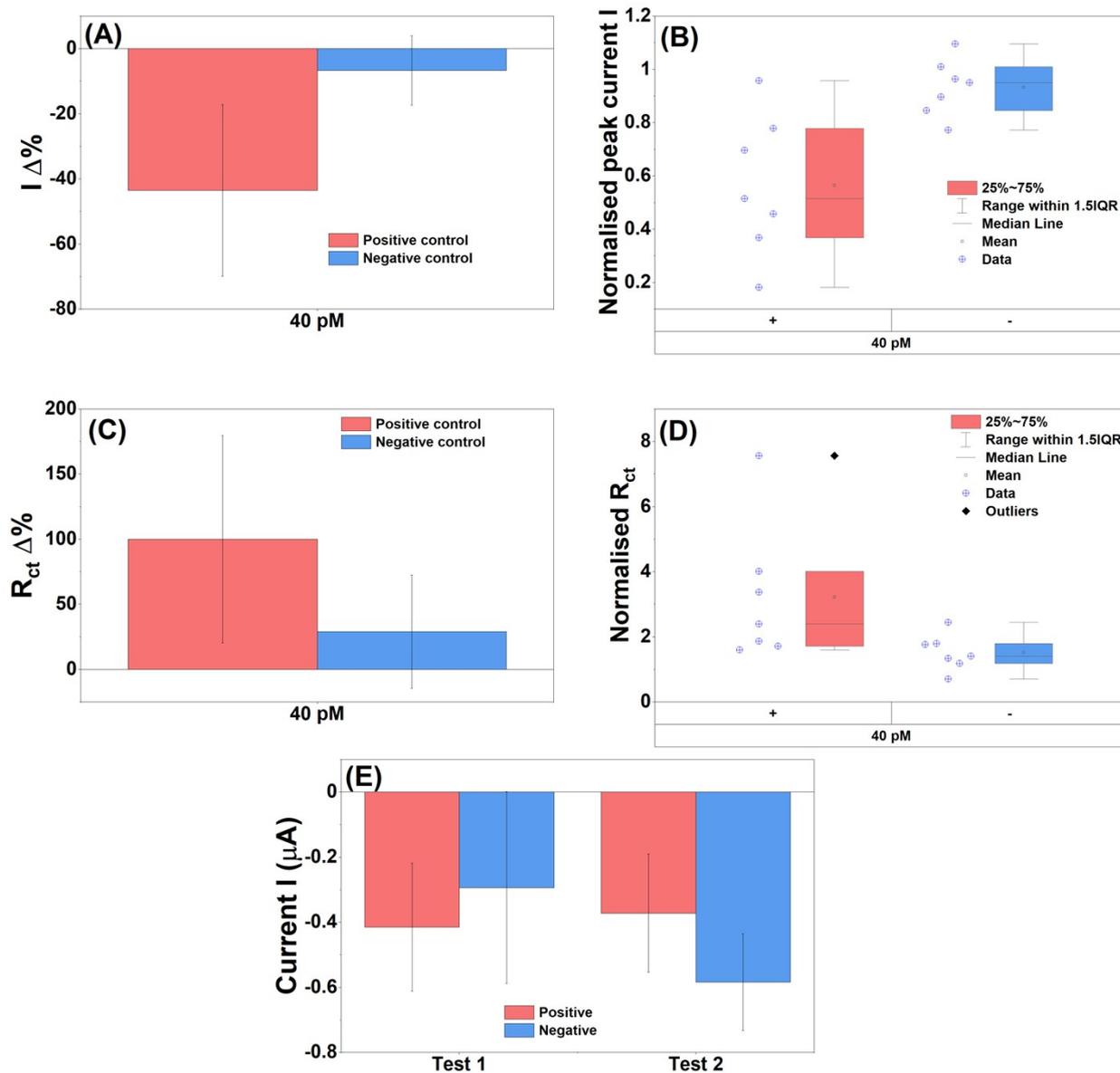


**Figure S2:** Electrochemical cell configuration for cleaning PCB P4 boards, Pt counter electrode, Ag/AgCl reference electrode and multiplexed working electrodes on PCB connected

to custom PCI connector. Optimized electrochemical cleaning conditions: 15 CV cycles in 50 mM KOH, potential window:  $-1.20$  to  $0.60$  V vs. Ag/AgCl, and scan rate:  $0.1$  V/s.



**Figure S3.** (A) Electrophoresis gel of *lytA* amplicons. The positive sample displays a band between the 300 bp and 200 bp ladder reference, consistent with the expected 235 bp amplicon. No visible band was observed in the negative sample. Distortion in the image is due to condensation on the plastic lid. (B) Electrophoresis gel of OXA amplicons. The positive sample shows a band between the 200 bp and 100 bp ladder reference, consistent with the expected 115 bp amplicon. No band was visible in the negative control. (C) Electrophoresis gel of asymmetric and DIG-labelled amplicons. No bands were observed for either the positive or negative *lytA* PCR samples. For OXA, DIG-labelled samples showed bands in both positive and negative lanes, while asymmetric DIG-labelled OXA showed a band in the positive lane only.



**Figure S4.** Asymmetric DIG-labelled amplicons in PBS (*lytA* 235 bp vs OXA 115 bp). (A) Mean DPV peak current percentage change in response to 40 pM. (B) Corresponding DPV raw data. (C) Mean  $R_{ct}$  percentage change in response to 40 pM. (D) Corresponding EIS raw data. (E) Chronoamperometry response to 20 min of TMB incubation (test 1), followed by a PBS rewash and re-application of TMB for 20 min. 2.0 mM redox buffer used for measurements.  $n = 8$ .

**Table S1.** Specifications of the PCB electrodes, including type, dimensions, manufacturer, and size. Diameter is given for circular electrodes, area is given for annulus electrodes, and width and height are given for rectangular electrodes. PCB P2-P3 used a polymer solder resist to insulate the tracks. PCB P1 used a manually applied nitrocellulose layer to insulate tracks.

<b>Type of PCB</b>	<b>Electrode size</b>	<b>Supplier details</b>
PCB P1	WE 1 = 0.20 mm	Custom-made in our lab.
	WE 2 = 0.457 mm	
	WE 3 = 0.711 mm	
	WE 4 = 0.965 mm	
	WE 5 = 1.016 mm	
	WE 6 = 1.0668 mm	
	WE 7 = 1.1118 mm	
	WE 8 = 1.168 mm	
	CE = 175.93 mm <sup>2</sup>	
	RE = 6.0 mm	
PCB P2	WE = 1.0 mm	P & M services (Rochdale) Ltd
	CE = 8.0 x 15 mm	
	RE = 2.0 x 15 mm	
PCB P3	WE = 1.0 mm	Quick-teck Electronics Ltd (Royston, UK)
	CE = 8.0 x 15 mm	
	RE = 2.0 x 15 mm	
PCB P4	WE = 1.0 mm	BIOTIP biodevice technology IP
	CE = 2.0 mm	
	RE = 3.365 mm <sup>2</sup>	

**Table S2.** optimized chemical cleaning procedure and electrochemical cleaning procedure. The procedure provided by the manufacturer was used for PCB P4.

<b>PCBs</b>	<b>Optimized chemical cleaning procedure</b>	<b>Optimized electrochemical cleaning</b>
PCB P1	None	CV: 20 cycles in 0.10 M H <sub>2</sub> O <sub>2</sub> . Potential window: -0.40 V to 1.20 V vs. Au
PCB P2	Acid piranha (10 s), followed by base piranha (10 s), with a final DI water rinse.	CV: 20 cycles in 0.10 M H <sub>2</sub> O <sub>2</sub> . Potential window: -0.40 V to 1.20 V vs. Au
PCB P3	Acid piranha (10 s), followed by base piranha (10 s), with a final DI water rinse.	CV: 20 cycles in 0.10 M H <sub>2</sub> O <sub>2</sub> . Potential window: -0.40 V to 1.20 V vs. Au
PCB P4	Submerged in 50 mM KOH + 35% H <sub>2</sub> O <sub>2</sub> , followed by a DI water rinse	15 CV cycles in 50 mM KOH, potential window: -1.20 V to 0.60 V vs. Ag/AgCl, and scan rate: 0.1 V/s