Supporting Information

A PCR-free electrochemical point of care test for clinical detection of Methicillin Resistant *Staphylococcus Aureus* (MRSA).

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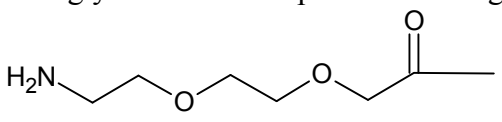
Online EIS experiments – electrode preparation and measurement information

For online detection screen printed gold electrodes (Working electrode diameter 1.6 mm) were purchased from DropSens (Oviedo, Spain). Each electrode was pre-cleaned by cyclic voltammetry in 0.1 M H_2SO_4. Electrode potential was scanned between 0 and 1.6 V for 20 cycles with care being taken to remove any bubbles forming on the surface with a pipette. A second round of cleaning in 0.1 M H_2SO_4 was then carried out where cyclic voltammetry was again performed, this time electrodes were scanned between potentials of 0 and 1.3 V for 20 cycles. Finally, the electrodes were thoroughly rinsed with deionised water and dried under a stream of nitrogen. After cleaning, screen printed electrodes were incubated with a solution of 1.5 µM thiol-modified PNA solution + 30 µM mercaptohexanol in 50 % (v/v) DMSO for 16 h at room temperature in a humidity chamber. In order to block the surface, electrodes were rinsed in 50 % (v/v) DMSO and incubated in 1 mM mercaptohexanol in 50 % (v/v) DMSO for 1 h at room temperature in a humidity chamber. Finally, the electrodes were washed with 50 % (v/v) DMSO and the EIS measurement buffer (0.1 mM $K_4[Fe(CN)_6]$ + 0.1 mM $K_3[Fe(CN)_6]$ + pH 7.0 10 mM phosphate buffer. Online EIS measurements were performed with a screen printed electrode (WE – Au, CE – Pt, RE – Ag) connected to an Autolab potentiostat. EIS measurements were performed at a DC potential of 0.03 V with an amplitude of 10 mV rms using a frequency range between 100 000 Hz – 0.1 Hz (15 frequencies) in 0.1 mM $K_4[Fe(CN)_6]$ + 0.1 mM $K_3[Fe(CN)_6]$ + pH 7.0 10 mM phosphate buffer.

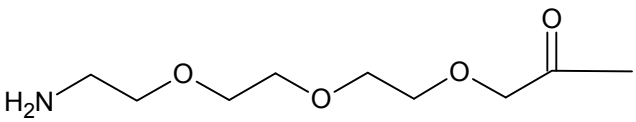
The online assay was performed by recording continuous multisine EIS measurements of 1 min in duration with a gold screen printed electrode (DropSens, Oviedo, Spain). A single well from a Schott Nexterion 16-well self-adhesive superstructure (Stafford, UK) was cut out and fitted around the electrode to contain 50 µL of EIS measurement buffer. The well was sealed with an adhesive lid from the Schott Nexterion 16-well self-adhesive superstructure kit (Stafford, UK). 45 µL of sample was mixed with 5 µL of 10x EIS measurement buffer and pretreated by heating at 95°C for 5 mins, storing on ice for 2 mins and heating at 30°C for 5 mins. Once the sample was prepared the EIS measurement buffer was removed from the electrode surface and replaced with the 50 µL sample + measurement buffer solution. The adhesive lid was resealed and EIS measurements taken as a function of time.

Chemical Structures of Spacer Molecules

Spacer molecules were incorporated into the PNA probe in order to improve hybridisation efficiency at the electrode surface. The chemical structures of the AEEA (Probe 01) and AEEEA (Probe 02) ethylene glycol linkers are presented in Figure S1.



AEEA linker – 1.3 nm & 9 atoms



AEEEA linker – 1.8 nm & 12 atoms

Figure S1. Chemical structures of the AEEA and AEEEA linkers.

qPCR and quantification of gDNA samples

Samples of genomic DNA were prepared in two ways.

1) gDNA was extracted from a sample of MRSA at 10^8 cells/mL (1 McFarland standard) spiked into wound fluid. The obtained DNA was then serially 1:10 diluted to give a range of concentrations equivalent to10^8 to 10^2 cell.smL.
2) MRSA was cultivated at 10^8 cells/mL in wound fluid and then 1:10 serially diluted in wound fluid to give a range of preparations ranging from 10^8 to 10^2 cells/mL. The DNA extraction process was performed on each concentration of MRSA.

qPCR was then performed on the samples prepared using the two methods and it was found that the cycle threshold was lower and showed a greater degree of linearity from samples prepared using method 1. This meant that dilution of gDNA extracted from a culture of 10^8 cells/mL produced more reliable dilution series than by diluting cultures of MRSA and then performing a DNA extraction (See Figure S2).

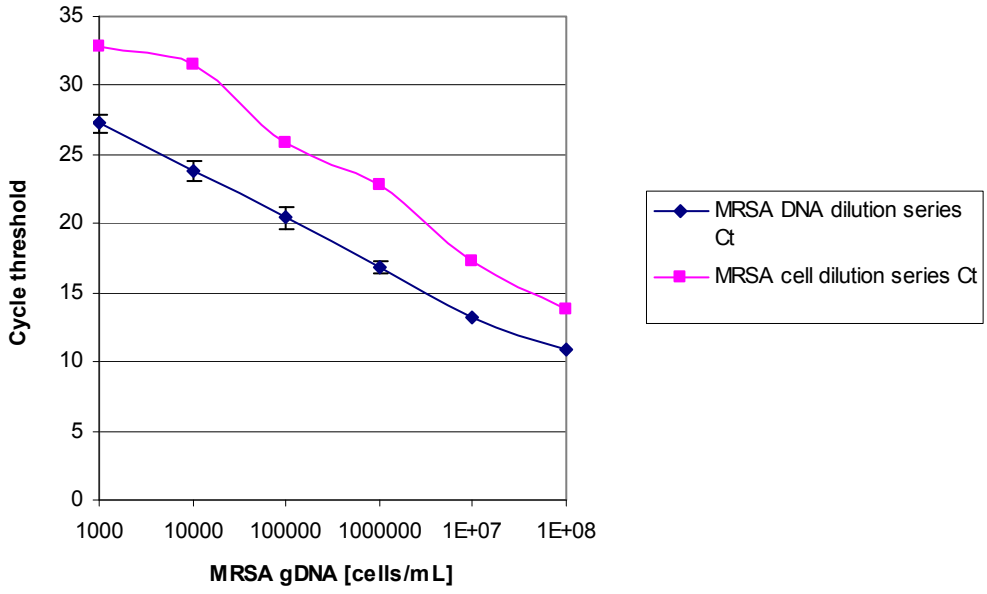


Figure S2. qPCR results from tests conducted with a DNA template recovered from MRSA. The plot shows cell/mL concentrations from WF vs cycle threshold.

To better understand any variation observed in EIS data, extracts of genomic DNA were quantified using a NanoDrop spectrophotometer (see Table S1). It can be seen that yields of MRSA genomic DNA showed considerable variation along with levels of recovered DNA from human wound fluid alone. The heterogenous nature of the DNA extraction process is likely to contribute to variation observed in the EIS data. Efforts were made to ensure good reproducibility of data such as experiments being conducted by one person however as shown in Figure S3 whilst reproducibility was good from a single batch of wound fluid, batch to batch variation was high and this can be attributed to the variable nature of human wound fluid and other experimental factors such as aggregation of MRSA and variability inherent in the process of enzymatically digesting the MRSA cell wall with lysotaphin.

Table S1: MRSA total DNA quantification using the NanoDrop

MRSA gDNA Quantification (Nanodrop) [ng/µL]								
	1. 06/01	2. 06/01	3. 06/01	4. 06/01	1. 18/01	2. 18/01	3. 18/01	4. 18/01
10^8	1164.5	434.2	350.0	1164.5	340.8	263.3	701.4	529.0
10^7	47.5	22.1	35.1	47.5	63.1	69.5	19.0	179.7
WF neat	79.0	47.2	81.2	79.0	188.4	378.6	99.3	-
WF 10^{-1}	6.5	4.3	71.3	6.5	16.3	16.7	26.3	-
MRSA qPCR [Ct]								
	1. 06/01	2. 06/01	3. 06/01	4. 06/01	1. 18/01	2. 18/01	3. 18/01	4. 18/01
10^8	10.93	12.10	11.63	-	10.7	9.7	10.09	9.96
10^7	13.23	13.81	15.26	-	12.53	13.53	13.29	13.16
WF neat	-	-	-	-	-	-	-	-
WF 10^{-1}	-	-	-	-	-	-	-	-

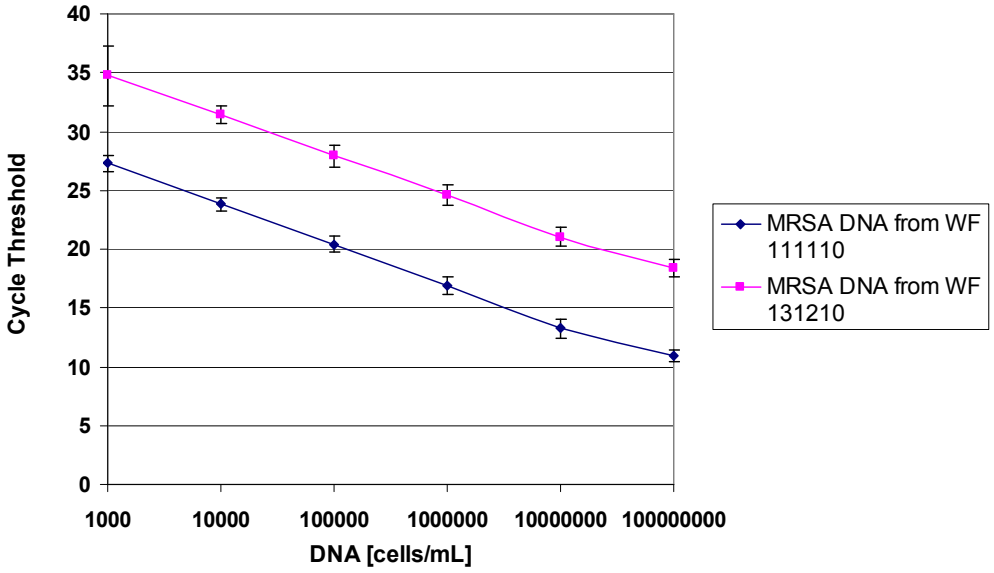


Figure S3. qPCR data demonstrating variation in gDNA yield upon extraction from 10^8 cells/mL MRSA in wound fluid.

Prototype potentiostat for point of care testing

With point of care testing in mind a prototype potentiostat was designed and assembled. The potentiostat was assembled with parts totalling less than US$100 and was able to measure phase and magnitude changes over a frequency range of 100,000 to 0.1 Hz. The system was initially evaluated using a fully complementary short artificial target and it was found that increases in charge transfer resistance following target addition were observable.

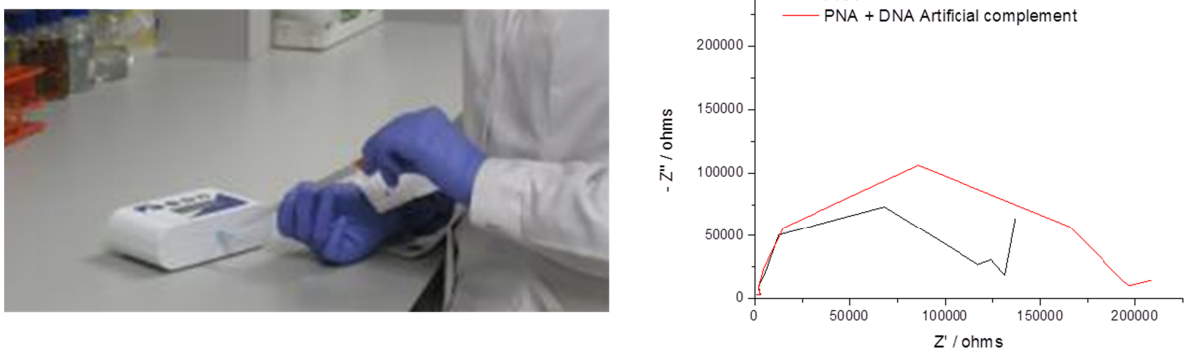


Figure S4. (A) Image of the prototype potentiostat. (B) Nyquist plot pre and 10 minutes post introduction of a 23 bp fully complementary oligonucleotide (1 µM).

DNA fragmentation

Figure S5A, B & C show Bioanalyser electropherograms of MRSA gDNA following heat treatment at 95^{o}C for 0, 1 and 5 mins respectively. It can be seen that heat denaturation time coincided with the production of smaller fragments of DNA. Similar samples were also analysed by gel electrophoresis (Figure S6) and whilst DNA fragmentation was observed from heat treated fragments sizing was not possible due to smearing of the sample.

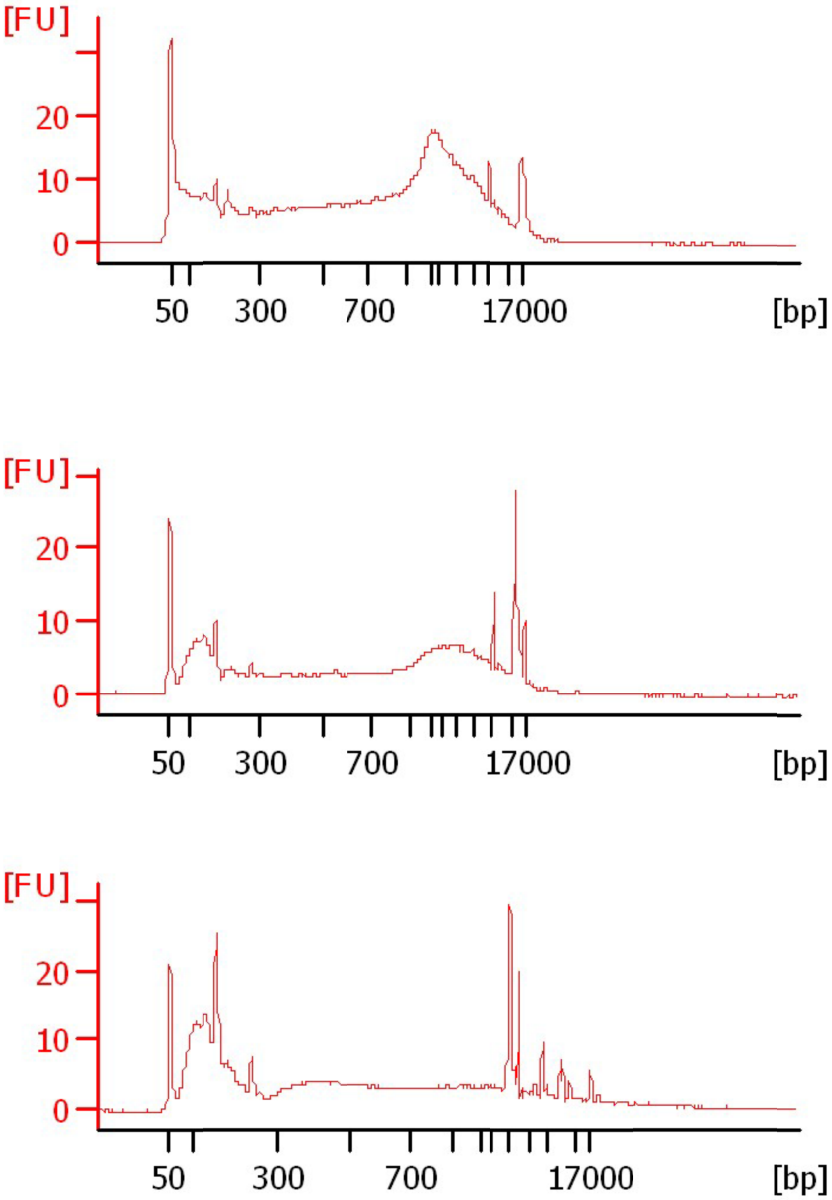


Figure S5 (A,B & C). Bioanalyser data from samples of MRSA gDNA heated for 0, 1 and 5 mins respectively.

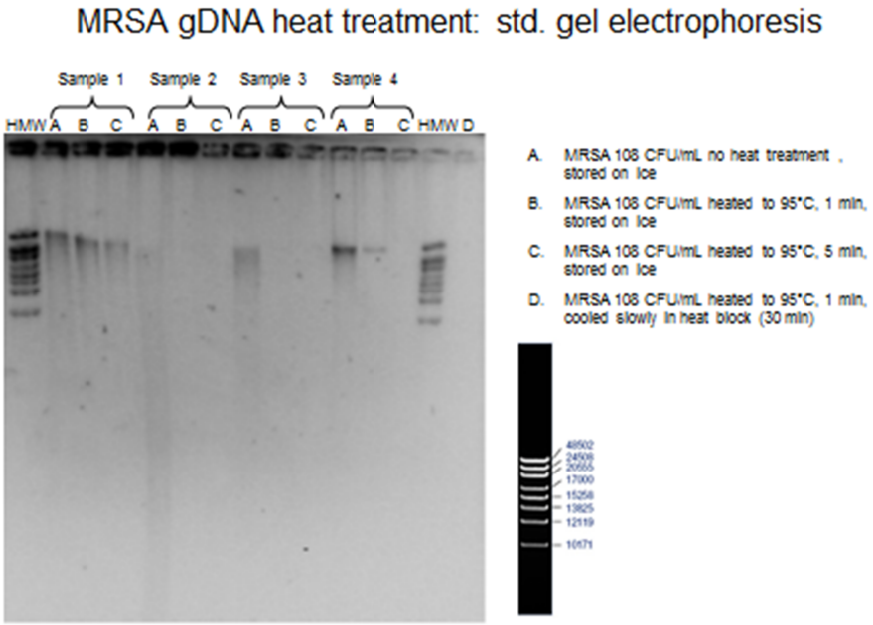


Figure S6. Agaraose gel electrophoresis of MRSA samples following no heat treatment, 1 min at 95°C and 5 mins at 95°C.