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Functionalisation data, materials, and method statements of a carbon electrode biosensor for detection of antimicrobial resistance

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Abbreviations

AMR	Antimicrobial Resistance
CE	Counter Electrode
CS	Commercial Sensor
CT	Complementary DNA Target
DNA	Deoxyribonucleic acid
EIS	Electrochemical Impedance Spectroscopy
HWS	Heriot-Watt Sensor
HWU	Heriot-Watt University
LTCC	Low-Temperature Co-fired Ceramic
NCT	non-complementary DNA Target
PNA	Peptide Nucleic Acid
R_{CT}	Charge Transfer Resistance
RE	Reference Electrode
SPCE	Screen-Printed Carbon Electrode
ssDNA	Single Strand DNA
WE	Working Electrode

Column heading

Explanations for ‘Functionalisation data for a carbon electrode biosensor for detection of antimicrobial resistance data in **Appendix 9**

Column heading	Explanation	Units	Notes
Code	Database serial number - unique ID	NA	
Identifier	unique label for file related to this data	NA	
Date	Date of experiment	NA	
Experiment Number	Experiment number		
Target Type	DNA target for control	NA	The type of Antimicrobial Resistance (AMR) DNA for positive control (Complementary Target (CT)) or Negative control (Non-complementary Target (NCT))
Target Concentration	Target Concentration	µ Molar	
Incubation time	Time the sensor incubated with target	second	
R_{CT} (MΩ)	Charge Transfer Resistance	Mega Ohm	

Introduction

Antimicrobial resistance (AMR) has been considered one of the greatest global threats to humanity's healthcare and economic well-being. Suppose AMR is not tackled by 2050, In that case, there will be a significant impact on society, and the economy would lead to 10 million human deaths annually (**Figure 1**) and up to $100 trillion losses in gross domestic product [1]. As a part of developing novel and inexpensive diagnostic AMR sensing platforms at the point of care, this document represents handover notes and results of work conducted at Heriot-Watt university to functionalize a carbon electrode electrochemical sensor. HWU successfully produced the low-cost carbon LTCC electrochemical sensors for use in the molecular detection of nucleic acids [2].

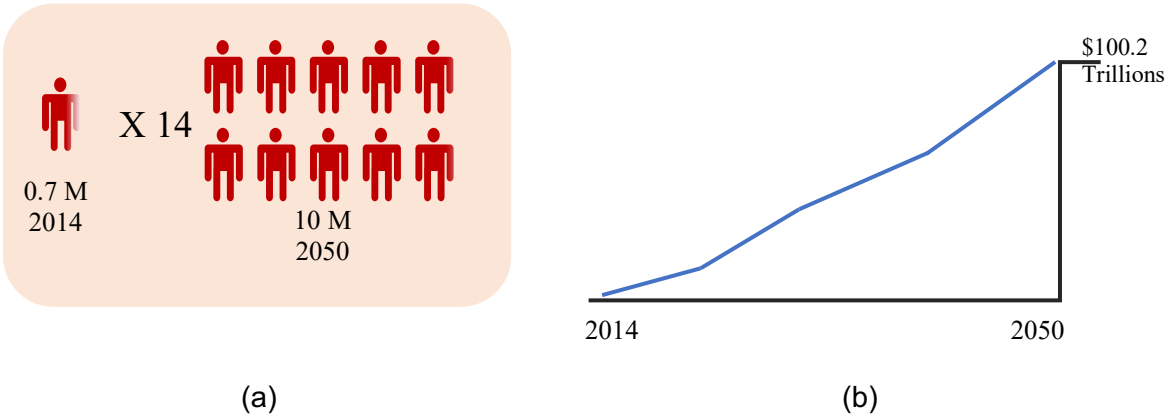


***Figure** 1 AMR impact in 2050. (a) Fourteen times compare to 2014, and (b) the increase in GDP losses.*

The idea is based on the concept of the electrochemical cell (**Figure 2**) when generating a chemical reaction via electrolysis by applying sinewave voltage in different frequencies to measure the Charge Transfer Resistance (R_{CT}).
R_{CT} is a measurement related to electrochemical reactions when the electron encounters difficulty to transfer from one phase (atom or compound) to another one.

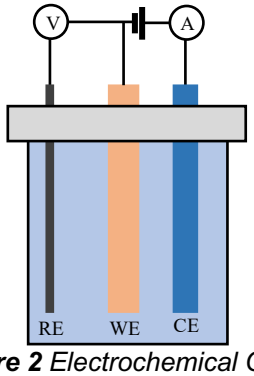


***Figure 2** Electrochemical Cell*

1- Materials and method statement

1.1 Sensor fabrication

It is an Electrochemical biosensor based on SPCEs on LTCC substrate for detection of AMR genes, **Figure3.**

The fabrication process involves:

- Laser cutting LTCC sheets.
- Screen printing the first layer of silver electrodes and curing under 115 ^{o}C.
- Stacking 6 layers of LTCC and align it in a mechanical jag and vacuum in a proper sealing bag.
- Hydrostatic pressurization for 10 min under 20MP pressure at 70 ^{o}C.
- Sintering the LTCC substrate.
- Screen printing and sintering of carbon and dielectric layers.
- Sensors dicing from the substrate at clean room

All fabrication steps are listed and detailed in **Appendix 1**.

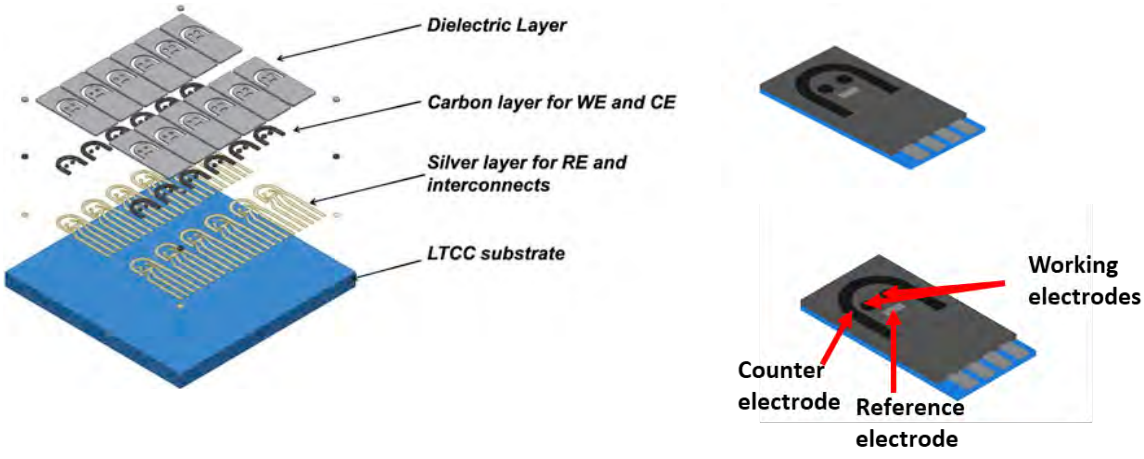


Figure 3 *HWU SPCE Sensor. (a) Exploded view and (b) the sensor and electrodes.*

1.2. Peptide Nucleic Acid (PNA)

The immobilization of PNA on carbon electrode has been developed to detect the OXA-1 gene. More details about the OXA-1 DNA sequence are in **Appendix 2**.

NH2-C27-AEEEA-AACAGAAGCATGGCTCGAAA

1.3. ssDNA:

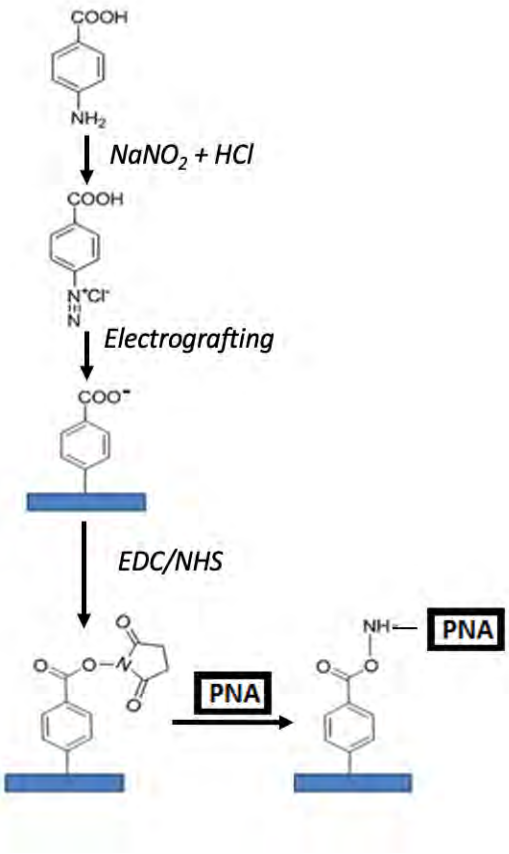
Two ssDNA were purchased to conduct positive and negative control tests for PNA and target DNA hybridization.

- OXA Oligo Target (Complementary): TTTCGAGCCATGCTTCTGTT
- tetA Oligo Target (Non-complementary): TGGCGGTCTTCTTCATCATGC

1.4. PNA Functionalisation

Functionalization processes A-Z (**Appendix 3**) takes approximately 3hrs and 20 minutes (**Appendix 4**), including the following main steps:

- Pre-treatment: Chronoamperometric cleaning of electrodes in a saturated Sodium Carbonate (Na_2CO_3) solution by applying a fixed 1.5V for 120 sec.
- Electrodeposition: preparing 20 mL In-situ salt (2mM Sodium Nitrite, 2mM 4-aminobenzoic acid, and 0.5 M hydrochloric acid): Electrochemical Modification of SPCEs by in situ generated diazonium cations. Then creating a 4-carboxyphenyl (AP) film on the electrode surface by reduction of the in situ generated 4-carboxyphenyl diazonium salt, using four cyclic voltammetry (CV) scans from +0.4 to -0.6 V at a scan rate of 100mVs^{-1}[3].

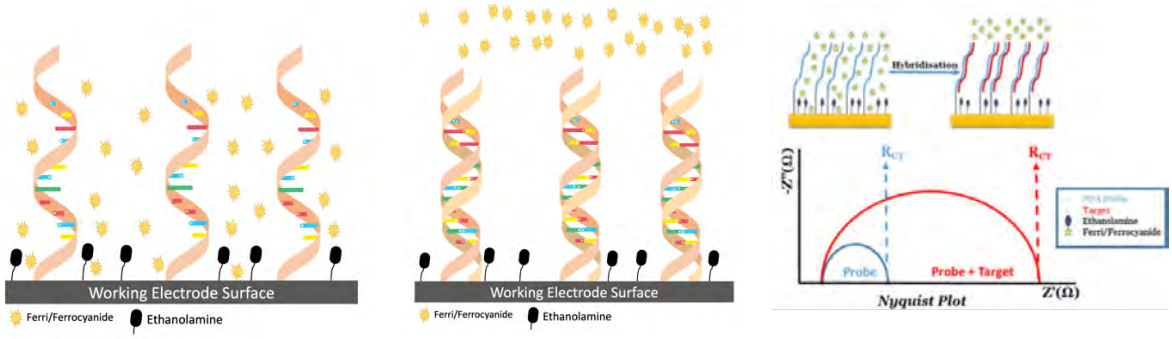


- Electrode activation: The AP film then activated via 60 min incubation in EDC (carbodiimide hydrochloride) and NHS (N-hydroxysuccinimide) in an MES (2-(Nmorpholino) ethanesulfonic acid), pH 5.0.
- PNA immobilization: Incubate the electrode in an amino-modified PNA probe droplet for 60 min at room temperature.
- Blocking: Formation of a blocking film by droplet deposition to the electrode of 1% v/v ethanolamine/PBS buffer and incubated for 30 min at room temperature.

1.5. Hybridization and EIS measurements:

The EIS measurement was conducted by Autolab PGSTAT12 potentiostat, controlled by Nova software from metrohm to measure Electrochemical Impedance Spectroscopy after activating the electrode surface and immobilizing the PNA in a water-saturated environment. 52 min incubation with complementary DNA target and electrochemical impedance spectroscopy (EIS) measurements (dR_{CT} 100kHz to 0.3Hz) in a 1mM KCl + 2mM Ferri/Ferrocyanide solution. ssDNA hybridizing to probs lead to an accumulation of negative charge on the electrode surface, causing an increase in the charge transfer resistance (R_{CT}) [2].

To compare R_{CT} before and after hybridization, it is recommended to record R_{CT} value one after incubating the electrode in EIS buffer and one after 52 min (**Figure 4**).



(a) (b) (c)

Figure 4 *PNA-DNA Hybridisation. (a) Immobilized PNA on the electrode surface, (b) ssDNA target hybridization with the PNA, and (c) The Nyquist Plot for RCT measurement before and after hybridization.*

R_{CT} can be measured by using Nyquist plot for real resistance (Z') and imaginary resistance (Z") (**Figure 3 (c)**), then indicating three points from the graph, and NOVA software will automatically graph the Nyquist plot and measure the $R_{CT,}$ which is

referred as R_p.p. at Nova software. The equivalent circuit for the electrochemical cell (sensor incubated in EIS) is represented in **Figure 5**.

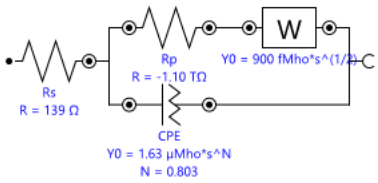


Figure 5 *Equivalent circuit of SPCE sensor in EIS buffer*

2. Results and Discussion

2.1 Label/filename format: A_B_C_D_E_.jpeg

All files related to these experiments are named, labelled as follows:

A Date	**B** Ex + Number (Experiment)	**C** DNA Type (CT/NCT)	**D** DNA concentration in µM	**E** Incubation time in Min

- A= date ddmmyy format. i.e. 150721 is July 15th 2021.
- B= Experiment No. i.e., EX01.
- C= CT (Complimentary Target) or NCT (Non-Complimentary Target).
- D= DNA concentration in micro molar.
- E= incubation time in seconds.

Example:

160721_EX02_CT_20_30

The interpretation of this label or file name is:

On July 16th , 2021, experiment number 02 with complementary DNA target at 20 µM concentration, EIS reading after 30 min of incubation.

2.2. Charge Transfer Resistance (R_{CT}) Measurement

Figure 6 demonstrates the change in R_{CT} after incubation of 0, 30 and 60 min. The more incubation time, the higher R_{CT} measured. (**Appendix 9**)

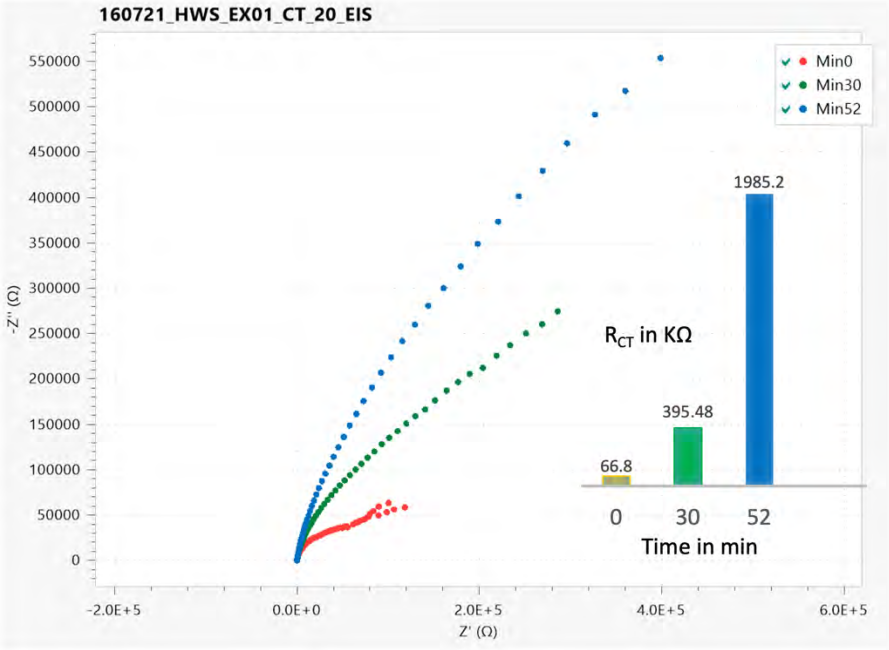


***Figure 6** The change of R_{CT} overtime with CT*

2.3. Complementary vs Non-Complementary Target

EIS measurements were taken after incubating the sensor in CT and NCT droplet in EIS buffer for 0,30, and 60 min to prove the theory (**Appendix 9**). For NCT, the change in signal is very small, unlike the change for CT (**Figure 7**)

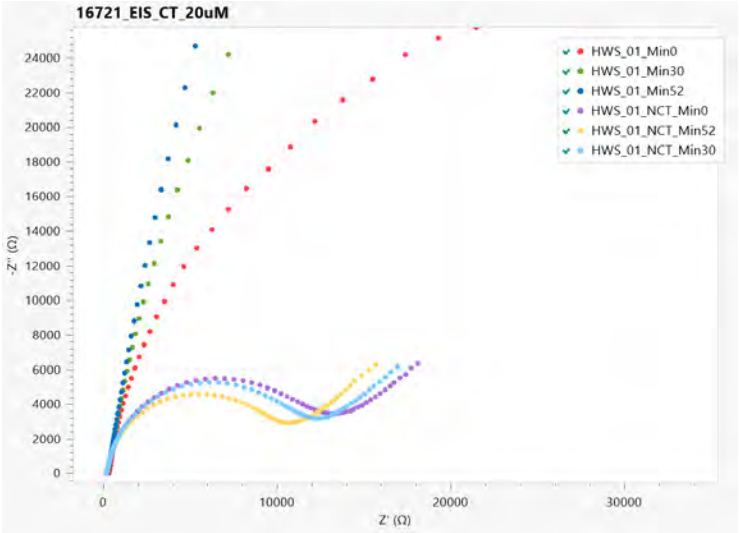
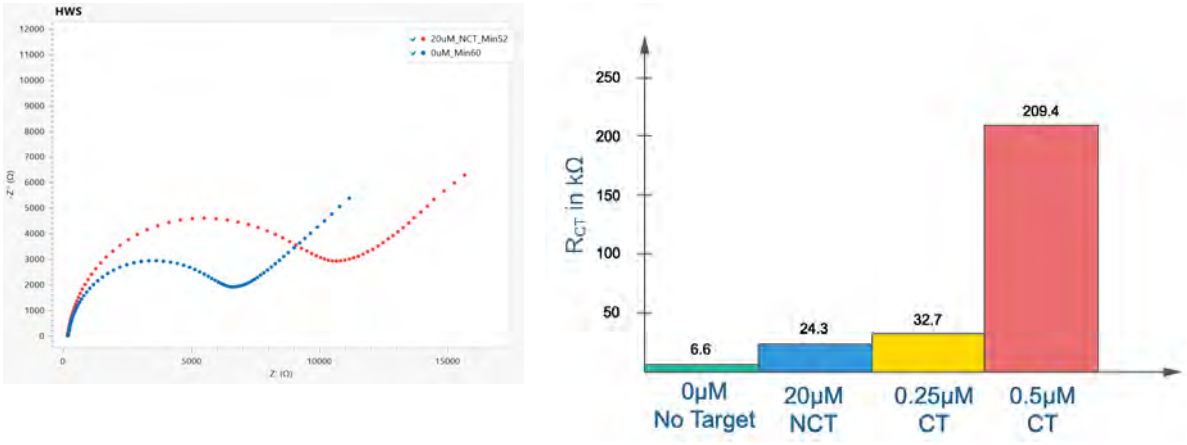


***Figure 7** Comparison between the R_{CT} in the presence of CT and NCT.*

Figure 8 shows R_{CT} changes after incubating the sensor in EIS buffer for 52 min with:

- 0 µM of DNA, No DNA target at all.
- 20 µM of NCT.
- 0.25 µM and 0.5 µM with CT.

There is a slight difference in resistance reading between R_{CT} values with no DNA and NCT as **Figure 8 (a),** in contrast, there is a rapid increase in the R_{CT} value starts at 0.5 µM, R_{CT} is very small when there is no DAN at the sample droplet (**Figure 8 (b)**).



(a) (b)

Figure 8 *R_{CT} in EIS buffer. (a) Compare change with 0 µM of DNA (No DNA) and 20 µM of NCT, (b) Rapid change at 0.5 µM of CT.*

2.4. R_{CT} vs Concentration of target ssDNA and LOD

The experiment was repeated to assess the relationship between R_{CT} and CT concentration, and to determine the limit of detection (LOD), after examining the relationship with Pearson's correlation by SPSS data analysis software, the result shows, as in **Table 1 and Appendix 9**, a significant strong positive correlation (0.810) between the concentration of target ssDNA and the value of the $R_{CT.}$

Table 1 *R_{CT} to CT concentration relationship*

		Cons	RCT_Min52
Cons	Pearson Correlation	1	.810**
	Sig. (2-tailed)		<.001
	N	19	19
RCT_Min52	Pearson Correlation	.810**	1
	Sig. (2-tailed)	<.001	
	N	19	19

Figure 9 and **Figure 10** illustrate that the higher concentration of Target DNA has a higher R_{CT} value.

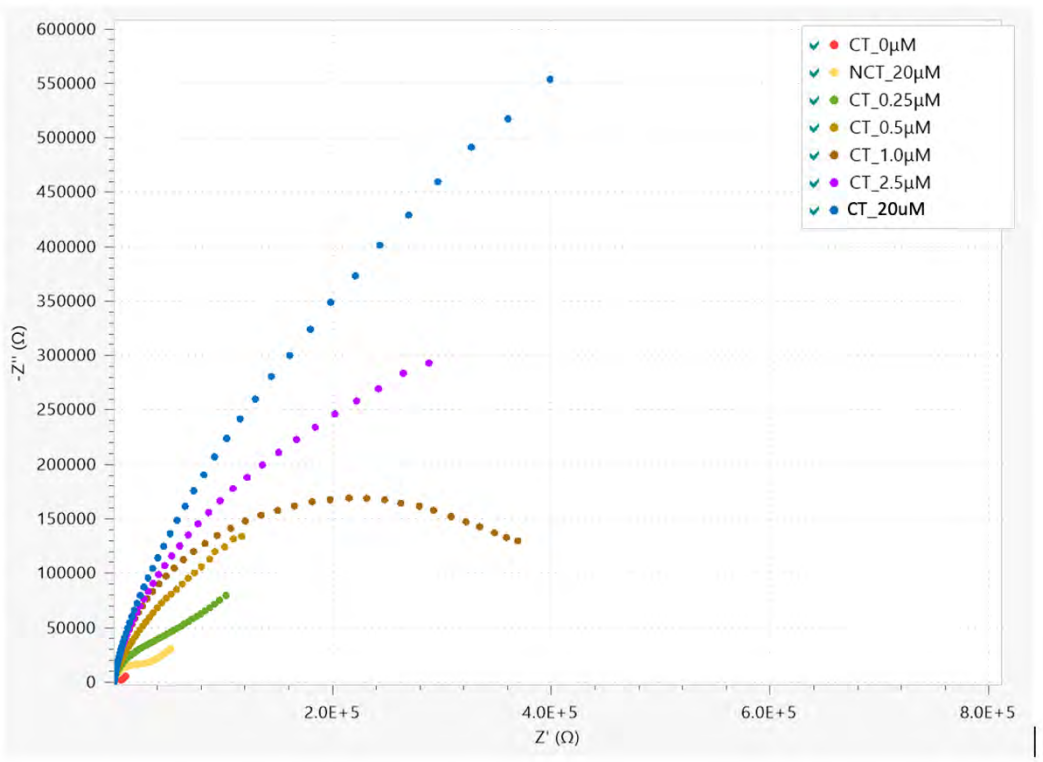


Figure 9 *Nyquist plot for EIS measurement with different CT concentration*

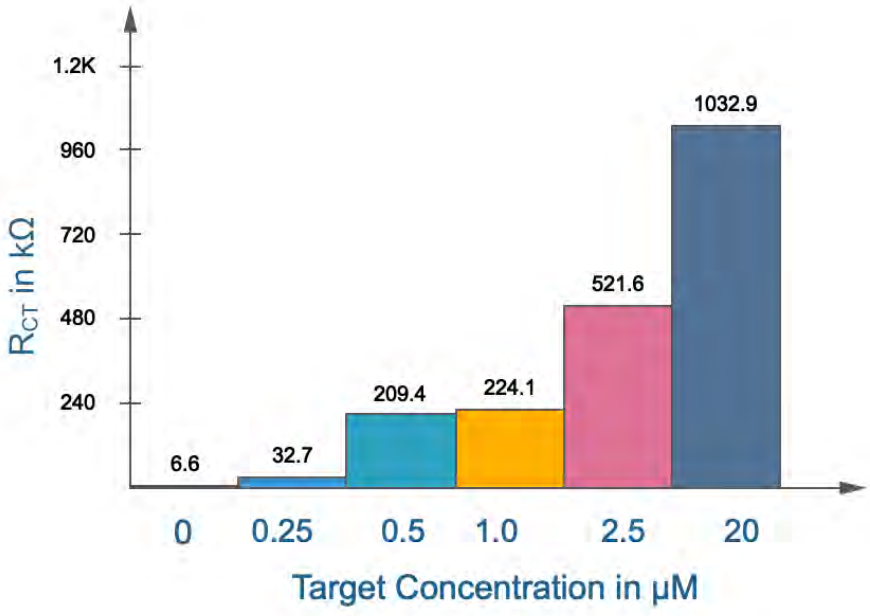
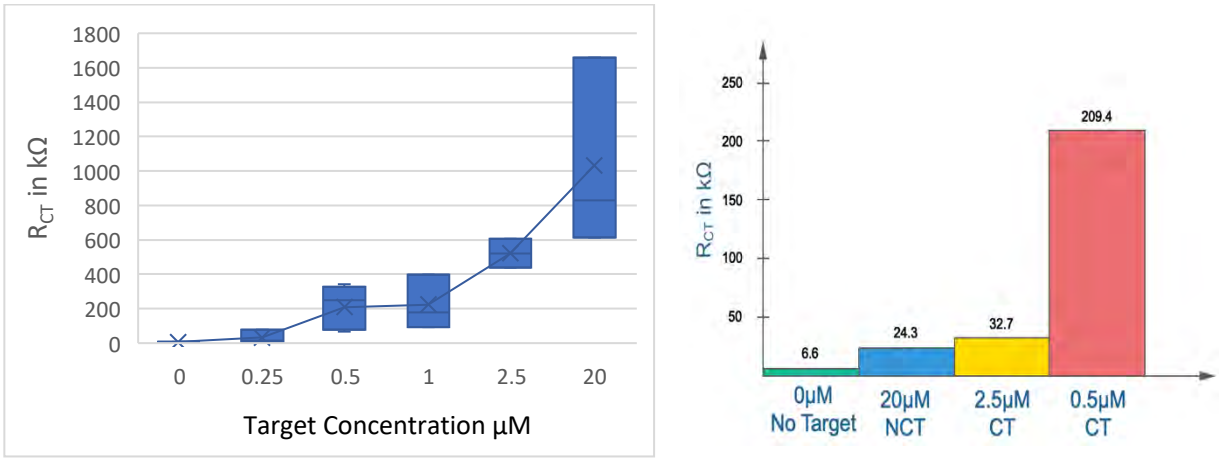


Figure 10 *R_{CT} values vs CT concentrations*

After measuring the R_{CT} with negative control of samples including NCT or no target, the LOD starts in the presence of **500 nM** of OXA-1 gene ssDNA, which causes a significant change in R_{CT} compared to lower concentrations that have an R_{CT} close to values of NCT as shown in **Figure 11**.



(a) *(b)*

Figure 11 *LOD for HW SPCE with OXA-1 gene CT*

3- Future Works

a. Complete the assembly of the readout multiplexing system.
b. Assess the readout system stability and accuracy.
c. Design a microfluidic multiplexer for multiple sensors reading.
d. Design and produce a new approach of multi-WEs sensor to reduce the readout system's size.

Reference

[1] J. O'neill, "Antimicrobial resistance: tackling a crisis for the future health and wealth of nations, the review on antimicrobial resistance.," 2014.

[2] E. A. Obaje, G. Cummins, H. Schulze, S. Mahmood, M. P. Y. Desmulliez, and T. T. Bachmann, "Carbon screen-printed electrodes on ceramic substrates for label-free molecular detection of antibiotic resistance: Carbon Sensors for Antibiotic Resistance Detection," *Journal of interdisciplinary nanomedicine,* vol. 1, no. 3, pp. 93-109, 2016, doi: 10.1002/jin2.16.

[3] S. Eissa and M. Zourob, "A graphene-based electrochemical competitive immunosensor for the sensitive detection of okadaic acid in shellfish," *Nanoscale,* vol. 4, no. 23, pp. 7593-7599, 2012, doi: 10.1039/c2nr32146g.

Appendix 1: Sensor fabrication process steps

All equipment to produce the HW SPCE sensor is set at room EM 2.23. it's allocated to the room as shown in **Figure AP 1** below.

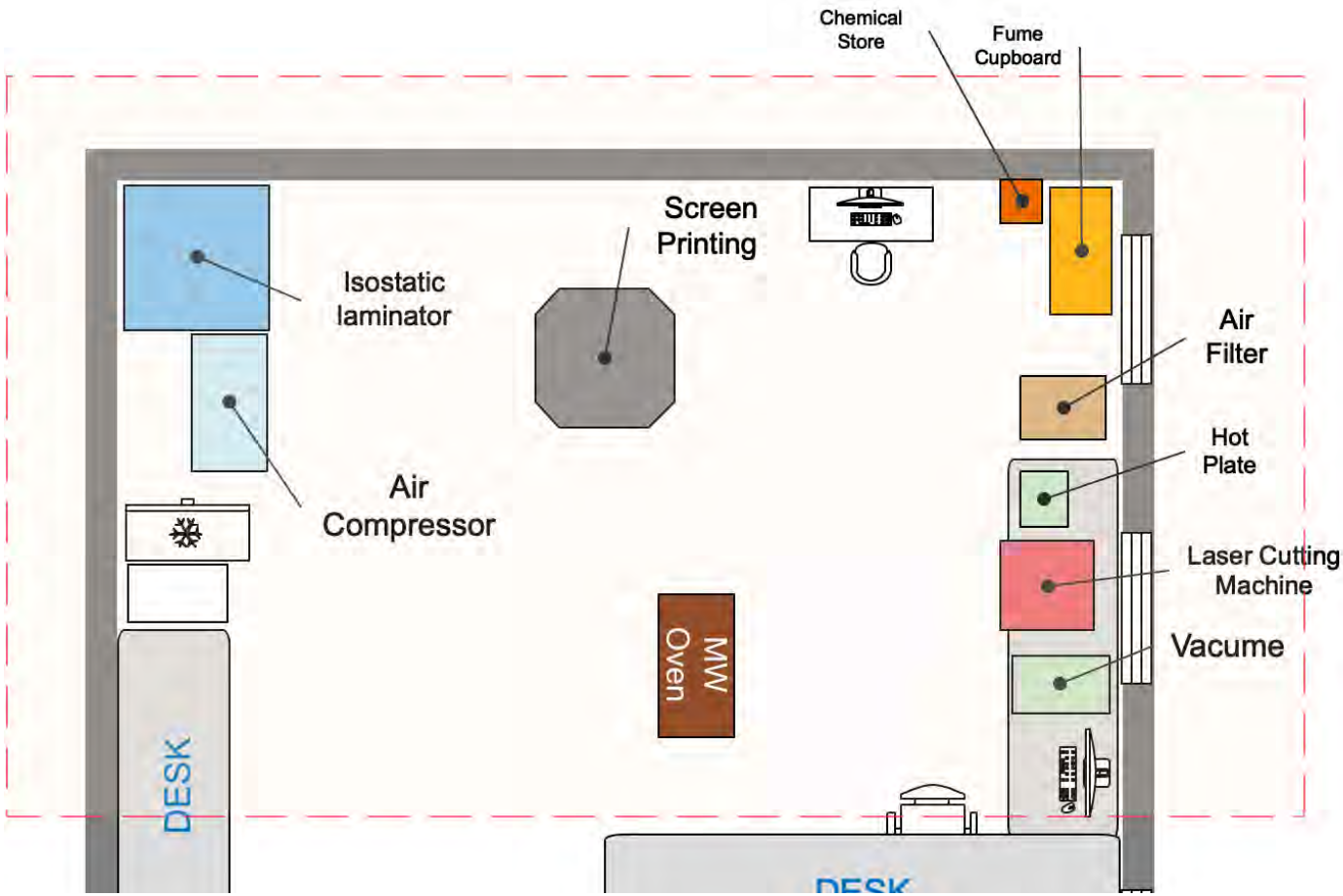


Figure AP 1 *EM2.23 SPCE production device's location plan*


EPILOG

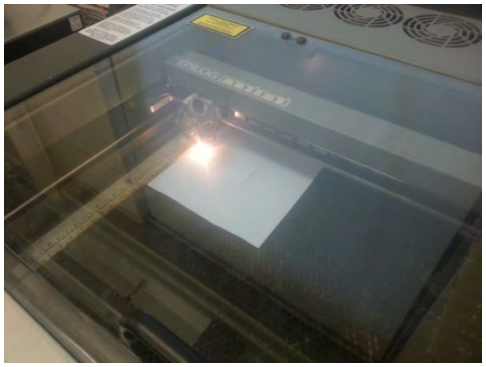

EPILOG

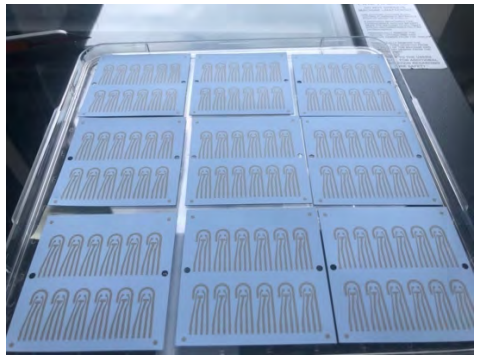

EPILOG

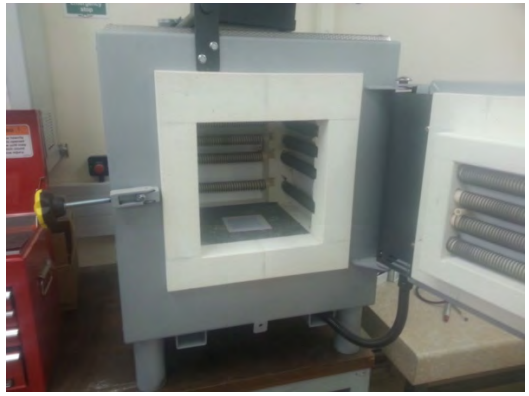
SPCE Sensor producing step by step

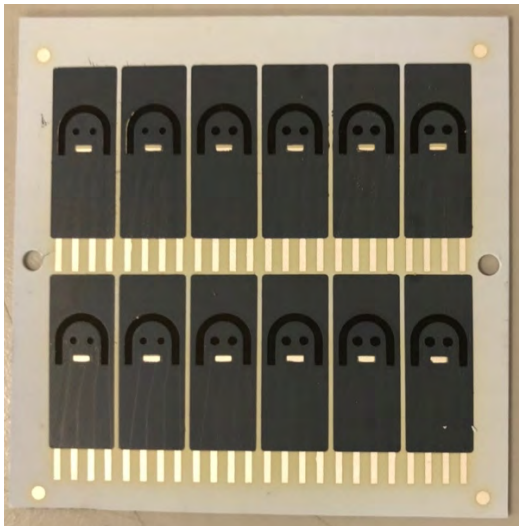
Step	Description	Equipment	Recipe/Parameters
1.	Cut LTCC sheets (6 per device)	Epilog Mini 18, 40W CO_2 laser	Vector cut, Line width – 0, Speed – 40%, Power-25%, Frequency -50kHz. Use Hereaus LH2000 LTCC.
2.	Screenprint Silver	Dek Horizon 03i Screenprinter	"Carbon Electrodes" program with Heraeus TC 7304 silver via paste. Clean system after use.
3.	Inspection	Workspace	Inspect printed silver to ensure pattern fidelity and continuous features
4.	Bake	Hotplate	115 degrees Celsius for 90 seconds at hotplate (Peel off mylar before placing on the hotplate)
5.	Mechanical Alignment	Jig	Peel off mylar at the back of LTCC sheets. Assemble sheets in the same orientation – matt side facing outwards. Finish with glossy mylar sheet between LTCC and aluminium jig.
6.	Vacuum packing	Vacuum packer	Vacuum dial between 8-9, S-dial at 4. Double bag to ensure no moisture ingress.
7.	Isostatic Press	Keko ILS-4 Isostatic Press	20 MP, 10 min, at 70 ºC
8.	Unseal bag	Sink	Remove from Isostatic press. Dry outside of the bag and cut open to release a mechanical jig over the sink. Bag will be hot straight from the press.
9.	Remove from Jig	Workspace	Carefully remove the compressed LTCC stack from the jig.
10.	Inspection	Workspace	Visually inspect LTCC for any signs of blistering or discolouration due to water.
11.	Sinter LTCC	Furnace (Room EM G.17)	Program 1. Ramp to 450 degC over 7 hours. Maintain at 450 deg C for 45 minutes. Ramp to 870 degC over 90 minutes. Maintain at 870 deg C for 30 minutes. Ramp down to room temp.
12.	Screenprint Carbon	Dek Horizon 03i Screenprinter	"Carbon Electrodes" program with BQ242 carbon paste. Clean system after use.
13.	Inspection	Workspace	Inspect printed carbon to ensure pattern fidelity and continuous features
14.	Bake	Hotplate	130 degrees Celsius for 15 minutes on a covered hotplate under an ambient atmosphere.

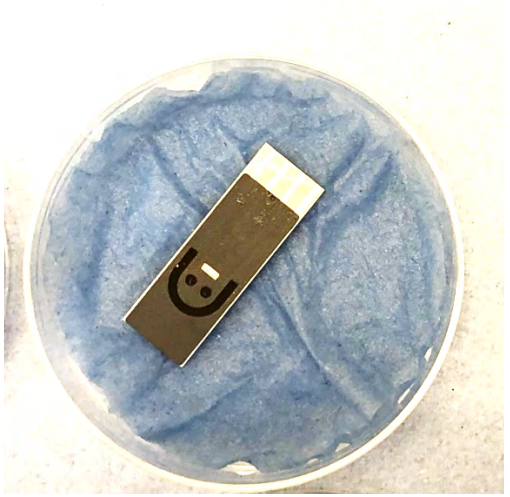
Step	Description	Equipment	Recipe/Parameters
15.	Screenprint Dielectric	Dek Horizon 03i Screenprinter	"Carbon Electrodes" program with Gwent D2070423P5 dielectric paste. Clean system after use. Sericol Universal screenwash may be needed to clean the system after use.
16.	Inspection	Workspace	Inspect printed dielectric to ensure pattern fidelity and continuous features
17.	Bake	Hotplate	130 degrees Celsius for 10 minutes on the covered hotplate.
18.	Dice	DISCO DAD-640 dicing saw (glass blade)	Contact the manager of the cleanroom for conducting the dicing of the sensors from the substrate.











Appendix 2: OXA-1 PNA and ssDNA details

OXA-1 DNA Sequences

A. Butterworth & D.K. Corrigan

Templates and Primers

- OXA-1 Template Sequence for PCR

TTAACAGAAGCATGGCTCGAAAGTAGCTTAAAAATTTCACCAGAAG
AACAAATTCAATTCCTGCGTAAAATTATTAATCACAATCTCCCAGTTA
AAAACTCAGCCATAGAAAACACCATA
120bp, primers provided produce a 116 bp amplicon, annealing temp 53°C.

- Forward Primer:
  AACAGAAGCATGGCTCGAAA

- Reverse Primer:
  TGGTGTTTTCTATGGCTGAGTT

- Primer BLAST Details:

Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
AACAGAAGCATGGCTCGAAA	Plus	20	58562	58581	58.10	45.00	4.00	0.00
TGGTGTTTTCTATGGCTGAGTT	Minus	22	58677	58656	57.90	40.91	3.00	0.00

Targets

- OXA Oligo Target:
  TTTCGAGCCATGCTTCTGTT

- tetA Oligo Target (Non-complementary):
  TGGCGGTCTTCTTCATCATGC

Probe

- OXA-1 Carbon Probe

NH2-C27-AEEEA-AACAGAAGCATGGCTCGAAA

Appendix 3: SPCE Functionalisation process protocol

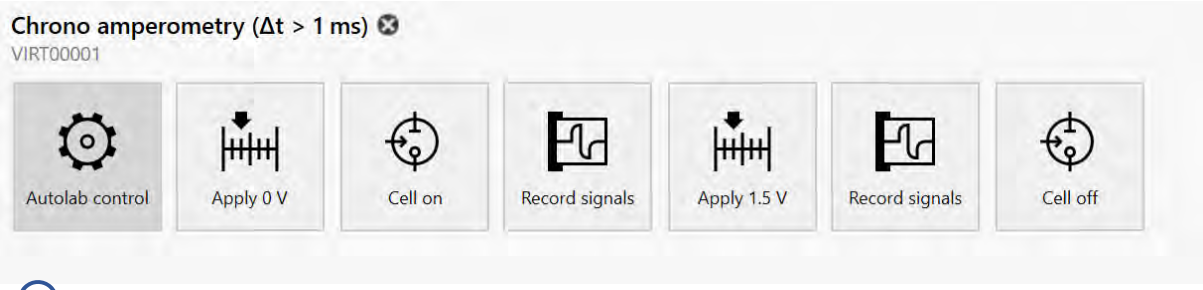
Before starting the functionalisation process, the Autolab instrument must be connected as per the sensor electrodes' arrangement. It is not necessary that the connector's labels have the same sensors' terminals—more details in **Appendix 6.** The check list in **Appendix 8** will be useful, too.

Step 1- Pre-treatment

Chronoamperometric cleaning of electrodes in saturated Sodium Carbonate (Na_2CO_3) solution by applying a fixed 1.5V for 120s

A. Preparation of saturated sodium carbonate solution:
   22g of Na_2CO_3 to 100ml of DI water; add until you see cloudiness (i.e., until there is a quantity laying in the bottom that doesn't dissolve anymore)

USE the "*step 1- chronoamperometry.nox*" process in the NOVA software of the Autolab PGSTAT204 equipment



Wash by DI Water

Step 2. In-situ diazonium salts formation

In-situ development of diazonium cations via the formation of a Sodium Nitrite (2mM), 4-aminobenzoic acid (2mM) and hydrochloric acid (0.5 M) 20ml solution:

2.1. Solution A preparation:

I. Prepare 5 mL of DI Water in an ice bath.

II. Dissolve 5.48 mg of 4-aminobenzoic acid in the DI water.

III. Stir the solution

IV. Gradually add 5mL of 2M HCl (5mL) to the stirred solution and keep stirring.

V. Cool the mixture to 0°C in the ice bath and leave to stir at this temperature for 10min minimum.

2.2. Solution B preparation:

I. Prepare 10 mL of DI water.

II. Dissolve 2.76 of Sodium Nitrite in double DI water.

2.3. Use a blast shield before starting the next process.

2.4. Slowly add dropwise solution B to the cooled solution A.

2.5. Ensuring the temperature of the reaction remains below 5°C.

2.6. Leave reaction to stir for 30min at 0° C.

A. Preparation of 2M HCl solution from 37% HCl:

I. Prepare 5 mL of DI water.

II. Slowly add 3.285 mL of HCL.

III. Adjust the final volume of solution to 20 mL with DI water.

Step 3. Electro-deposition

Electrochemical deposition of a 4-carboxyphenyl film on the carbon-based screen-printed electrode surface of electrochemical sensors via voltammetric reduction cycling (PGSTAT204/ FRA32M, Metrohm-Autolab) with sensor electrodes immersed in the developed solution in step 2

USE the "*step 2 – electrodeposition.nox*" process in the NOVA software of the Autolab PGSTAT204 equipment.

4 CVs: +0.4/-0.6V/100mV-1s

Followed by thorough DI water rinse

Step 4. Surface Activation

To activate the terminal carboxyl groups (AP film) at the electrode surface, incubation it for 60 min in (250mM, 4.79g) EDC (carbodiimide hydrochloride) and (50mM, 0.57g) NHS (N-hydroxysuccinimide) in a MES (2-(Nmorpholino) ethanesulfonic acid) (100mM (1.95g), pH 5.0) 100ml buffer.

4.1. Activation Buffer Preparation:

4.1.1. MES buffer preparation:

I. Prepare 100 mL DI water
II. Add 1.95 mg MES
III. Adjust the pH to 5.0 by adding 10 M NaOH.

4.1.2. Add 4.79 g of EDC to the solution

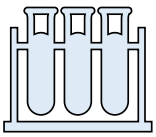
4.1.3. Add 0.75g of the NHS to the solution.

4.2. Incubate the surface in the buffer for 60 min.

Rinse by MES buffer

It is recommended to prepare 200 ml of MES buffer, 100 mL for incubation activation and 100 for rinse.

- 100 mL DI water.
- 3.9 g MES.
- Adjust the pH to 5.0 by 10 M NaOH.



pH is adjusted to the desired value by adding 10M NaOH and monitoring with a pH meter.

4.3.1. Preparing 10 M NaOH

I. Prepare a suitable container.
II. Add 40 g of NaOH.
III. Add 100 mL of DI water

Step 5. PNA functionalization

Surface functionalization of the sensors (Working Electrode) with an amino-modified PNA probe (10μM) dispersed in a droplet (50μL) of PBS buffer (10mM sodium phosphate, 150mM sodium chloride, pH 7.4) and 60min incubation at room temperature in a water-saturated atmosphere

5.1. Prepare 10 μM of PNA probe:

The PNA is received with a certificate including information like Molecular weight and mol.

Example: To prepare 10 μM of PNA in BPS from 50.1 nmol PNA

$$M = \frac{mol}{V}$$

$$V = \frac{50.1^{-9}}{10\ x\ 10^{-6}}$$

You need to add **5 mL** of BPS to the probe container to prepare **10 μM** of PNA in BPS.

5.2. Ensure to mix the solution very well using an ultrasonic vibrator and vortex.

5.3. Add 50 μL of the dispersed PNA in BPS (Step 5.1) on the working electrode.

5.4. Incubate the electrode for 60 min in a water-saturated atmosphere.

Step 6. Blocking process

Formation of a blocking film via droplet deposition (50μL) to the electrodes of a 1% v/v ethanolamine/PBS buffer (pH = 7.4) and 30min incubation in a water-saturated atmosphere.

6.1. Preparation 10 mL of Blocking buffer:

I. Prepare a suitable container.
II. Add 1 mL of Ethanolamine.
III. Add 99 mL of PBS buffer (pH 7.4).

6.2. Block the film (Electrode) by incubating it for 30 min in 50μL of blocking buffer in a water-saturated atmosphere.

Rinse after that with PBS and argon dry prior to use in the next step.

Step 7. Target

52 min incubation with a Polydeoxyadenylic acid sodium salt target (0-20μM) dispersed in EIS Buffer.

7.1. Dispense a 60 μL droplet on the sensor and retain for the 60min duration of the measurements,

7.2. Conduct measurements for different target concentrations and with negative control (i.e. other non-complementary targets and no target at all) to characterize sensors.

7.3. Sensor measurement via electrical impedance spectroscopy (EIS) equipment PGSTAT204/ FRA32M, Metrohm-Autolab. Measurement protocol:

USE the "*impedance measurement with ocp.nox*" process in the NOVA software of the Autolab PGSTAT204 equipment

Following the measurements, a Randles circuit can be fitted on the data to extract the transfer resistance (see as detailed in ("Carbon screen-printed electrodes on ceramic substrates for label-free molecular detection of antibiotic resistance, E. A. Obaje et al., Journal of Interdisciplinary Nanomedicine, 2016) as detailed in:

https://www.youtube.com/watch?v=zzP9vnBneP8&t=65s

7.3.1. One measurement immediately after addition of the target and,

7.3.2. One measurement after 52min incubation of the target on the sensor.

7.4. Preparation of EIS buffer:

I. Prepare 100 mL of BPS buffer (pH 7.4) in a suitable container.
II. Add 32.924 mg of K3 (Potassium hexacyanoferrate (III)) to the buffer (1mM K3).
III. Add 42.239 mg of K4 (Potassium hexacyanoferrate (II) trihydrate) to the solution (1 mM K4).
IV. Add 7.45513 mg of KCL to the solution (1 mM KCL).
V.

7.5. preparation of 0-20 μM ssDNA in EIS buffer:

Prepare max concentration (20 μM) in a suitable container:

Example: To prepare 20 µM of ssDNA in EIS buffer from 89.7 nmol DNA

$$M = \frac{mol}{V}$$

$$V = \frac{89.7^{-9}}{20\ x\ 10^{-6}}$$

You need to add 4.485 mL of BPS to the probe container to prepare 20 µM of it.

a. To prepare 10 µM:
   Take 1 mL of 20 µM stoke and dissolve it in 1 mL of EIS buffer in a suitable container.
b. To prepare 5 µM:
   Take 0.5 mL of 20 µM stoke and dissolve it in 1.5 mL of EIS buffer in a suitable container.
c. To prepare 2.5 µM:
   Take 1 mL of 5 µM stoke and dissolve it in 1 mL of EIS buffer.
d. To prepare 0 µM:
   Only EIS buffer.

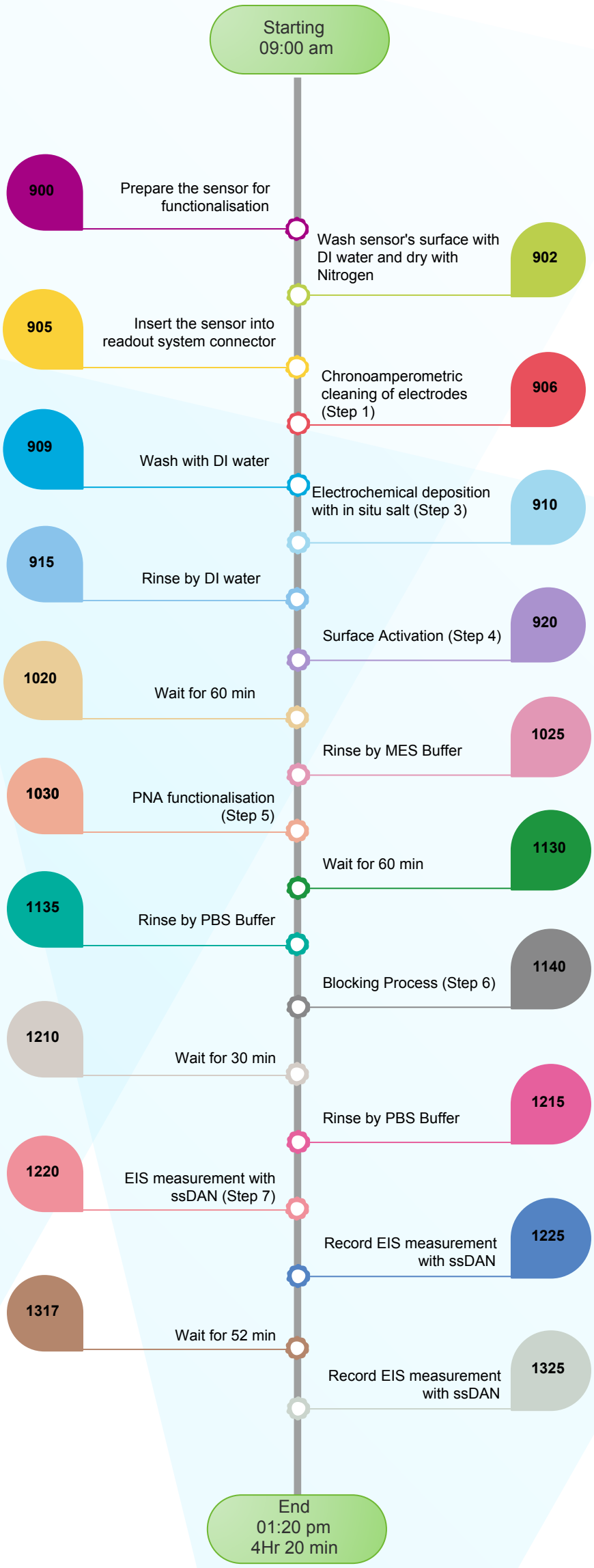
Final volumes:

- 20 µM = 2.985 mL. (49 Tests)
- 10 µM = 2 mL. (33 Tests)
- 5 µM = 1 mL. (16).
- 2.5 µM = 2 mL. (33 Tests).

Mol to gram Converter					
Enter Data				Result	
Chemical Compound	Molar mass (g/mol)	Solvent in kg (V)	Molarity (M)	g	mg
Na_2CO_3	105.9888	0.1	2.07	21.9396816	21939.6816
4-aminobenzoic acid	137.14	0.02	0.002	0.0054856	5.4856
Sodium Nitrite	68.9953	0.02	0.002	0.00275981	2.759812
EDC	191.7	0.1	0.25	4.7925	4792.5
NHS	115.09	0.1	0.05	0.57545	575.45
MES	195.2	0.1	0.1	1.952	1952
NaOH	39.997	0.1	10	39.997	39997
Potassium hexacyanoferrate (III) - K3	329.24	0.1	0.001	0.032924	32.924
Potassium hexacyanoferrate (II) trihydrate - K4	422.39	0.1	0.001	0.042239	42.239
KCL	74.5513	0.1	0.001	0.00745513	7.45513

Appendix 4: SPCE Functionalisation process timeline

SPCE Functionalisation and EIS Measurement Process Timer



Overlapping Functionalisation for multi sensors

	900	915	930	945	1000	1015	1030	1045	1100	1115	1130	1145	1200	1215	1230	1245	1300	1315	1330	1345	1400	1415	1430	1445	1500	1515	1530	1545	1600	1615	1630	1645	1700	1715	1730	1745	1800	1815	1830	1845	1900	1915
Sensor_01	Cleaning		Activation				PNA Immobolisation				Blocking		EIS																													
Sensor_02							Cleaning		Activation				PNA Immobolisation				Blocking		EIS																							
Sensor_03													Cleaning		Activation				PNA Immobolisation				Blocking		EIS																
Sensor_04																			Cleaning		Activation				PNA Immobolisation				Blocking		EIS										
Sensor_05																									Cleaning				Activation						PNA Immobolisation				Blocking		EIS	

Appendix 5: SPCE Functionalisation process Flowchart

SPCE Functionalisation and EIS measurement process Flowchart

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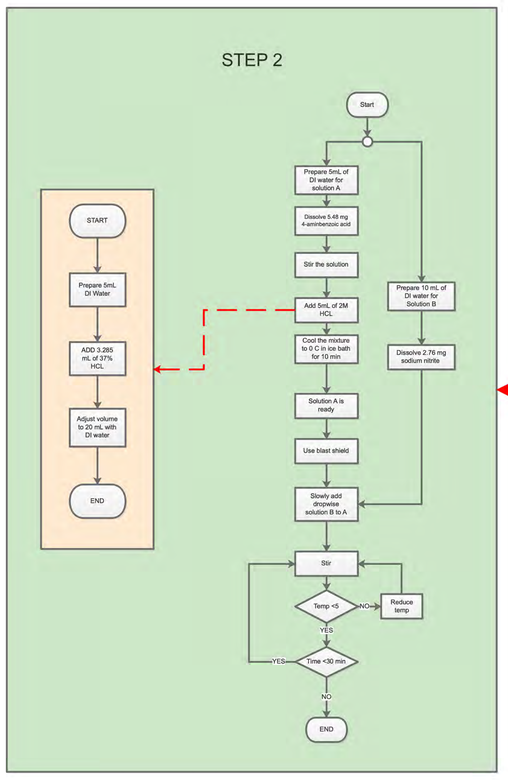
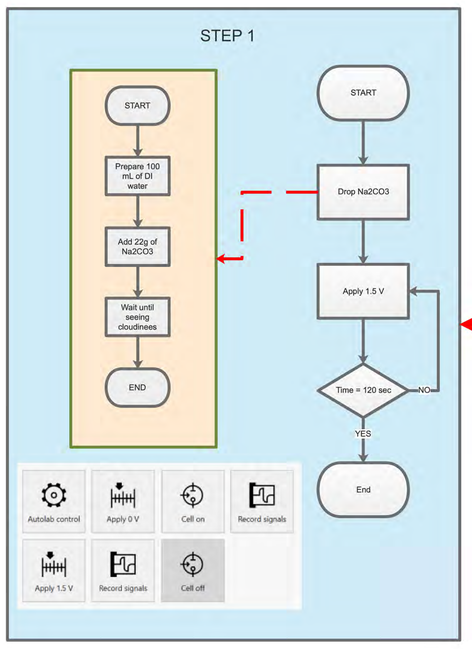
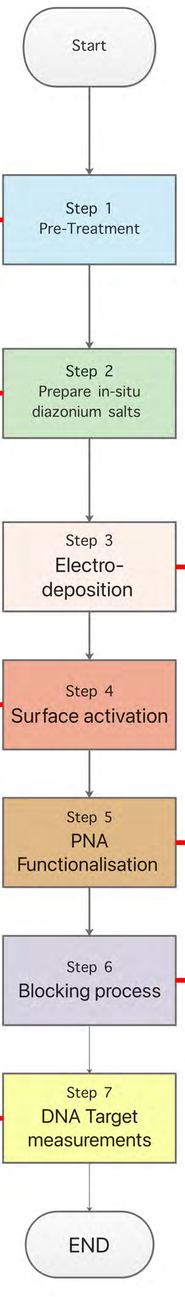
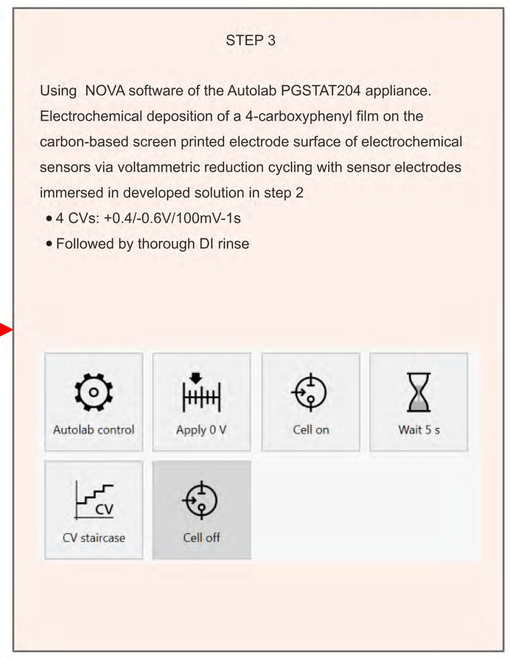
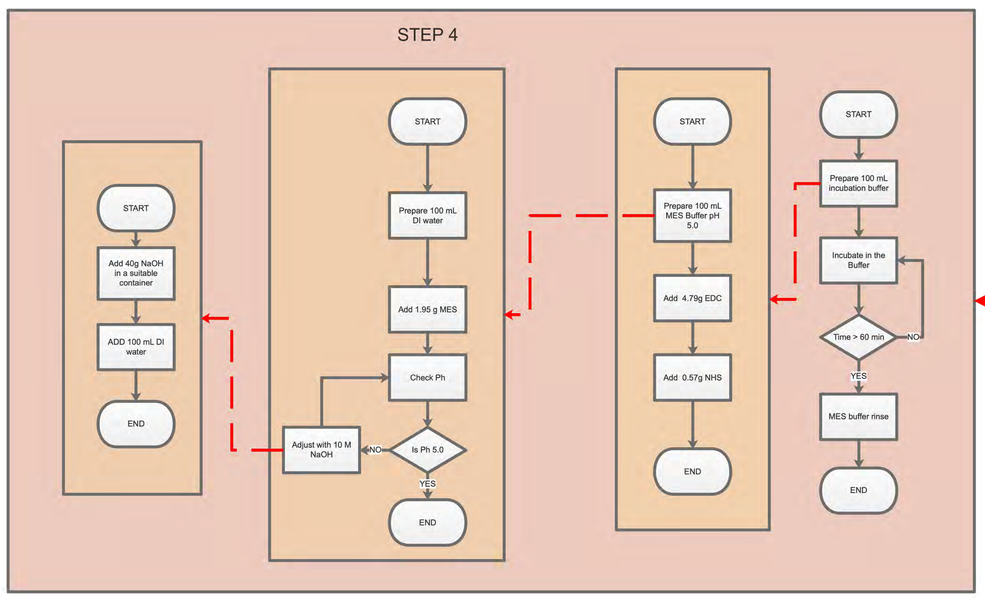
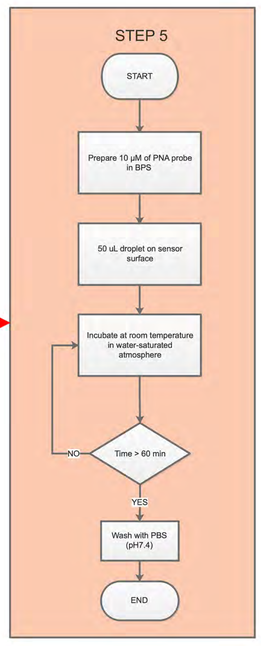


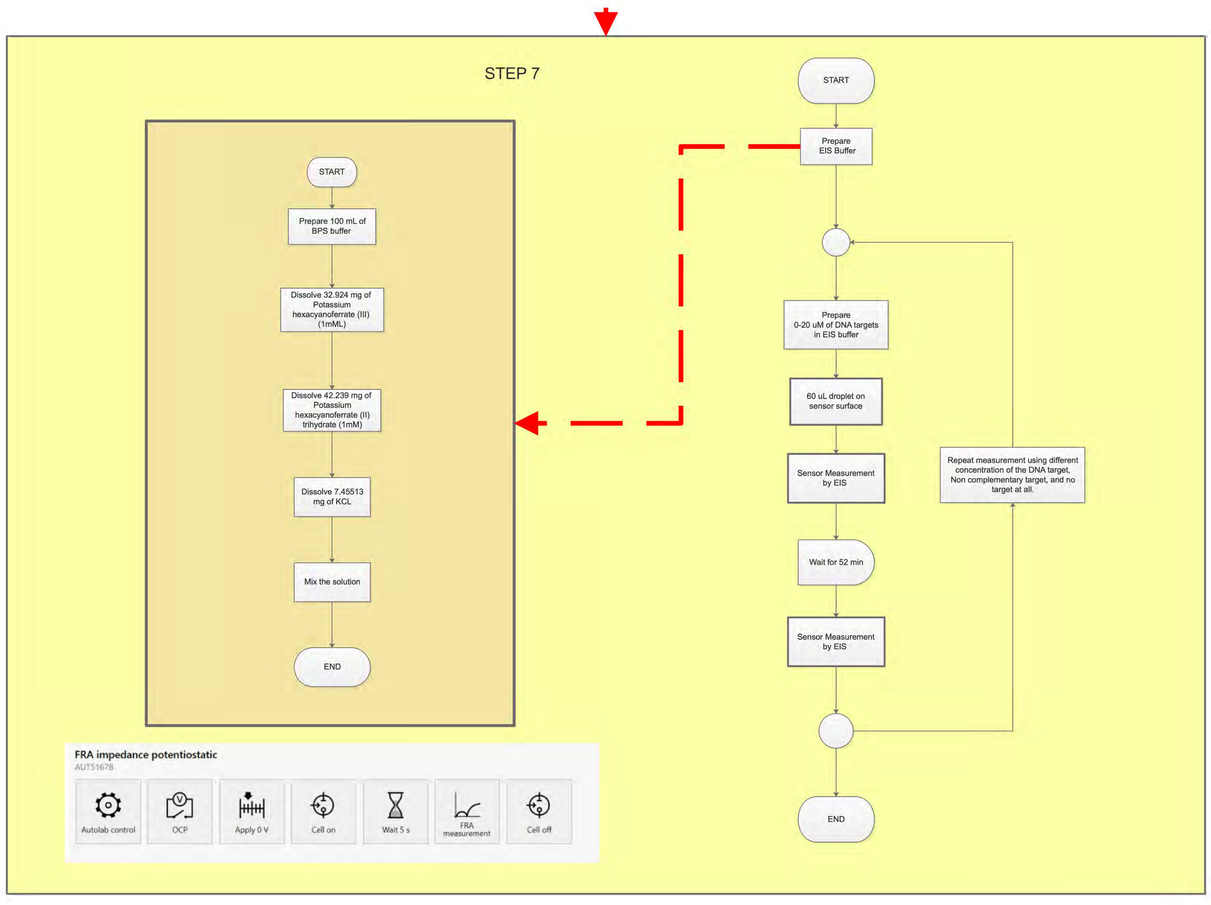


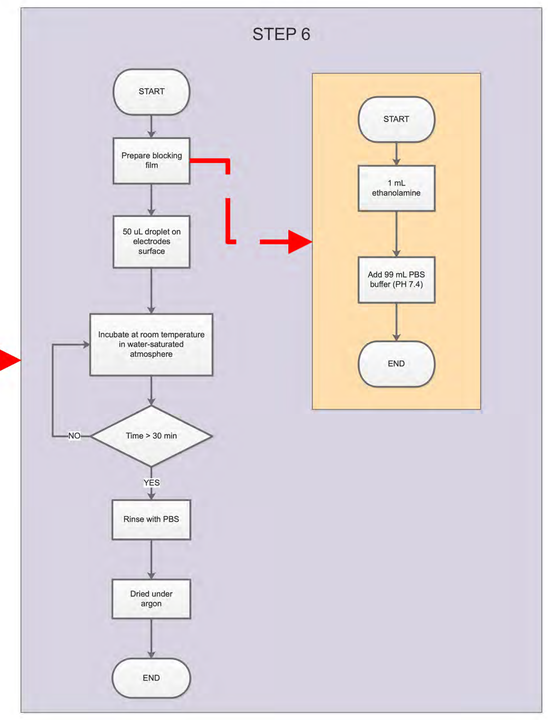












Mol to gram Converter					
Enter Data				Result	
Chemical Compound	Molar mass (g/mol)	Solvent in kg (V)	Molarity (M)	g	mg
Na_2CO_3	105.9888	0.1	2.07	21.9396816	21939.6816
4-aminobenzoic acid	137.14	0.02	0.002	0.0054856	5.4856
Sodium Nitrite	68.9953	0.02	0.002	0.00275981	2.759812
EDC	191.7	0.1	0.25	4.7925	4792.5
NHS	115.09	0.1	0.05	0.57545	575.45
MES	195.2	0.1	0.1	1.952	1952
NaOH	39.997	0.1	10	39.997	39997
Potassium hexacyanoferrate (III) - K3	329.24	0.1	0.001	0.032924	32.924
Potassium hexacyanoferrate (II) trihydrate - K4	422.39	0.1	0.001	0.042239	42.239
KCL	74.5513	0.1	0.001	0.00745513	7.45513

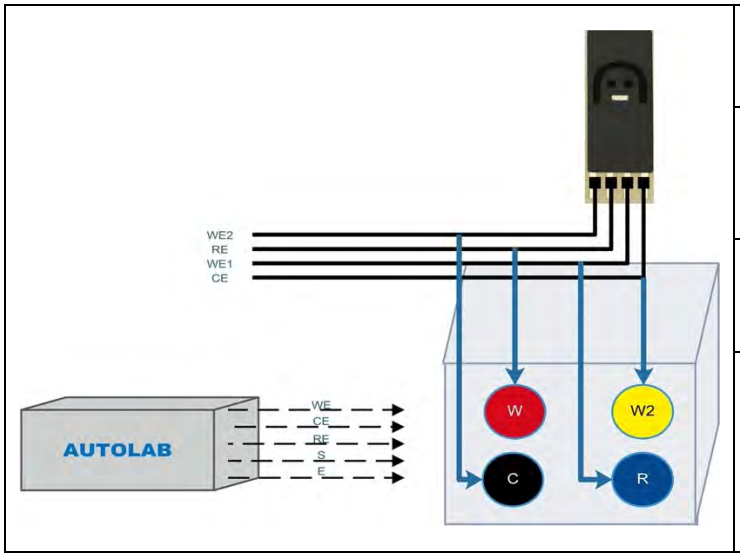
Appendix 6: Autolab – Connector, wiring arrangement

The Autolab **DRP-BIDSC** connector has four terminals, two for working electrodes, one for the counter electrode, and one for the reference electrode, as shown in **Figure Ap2.**

W	W2
C	R

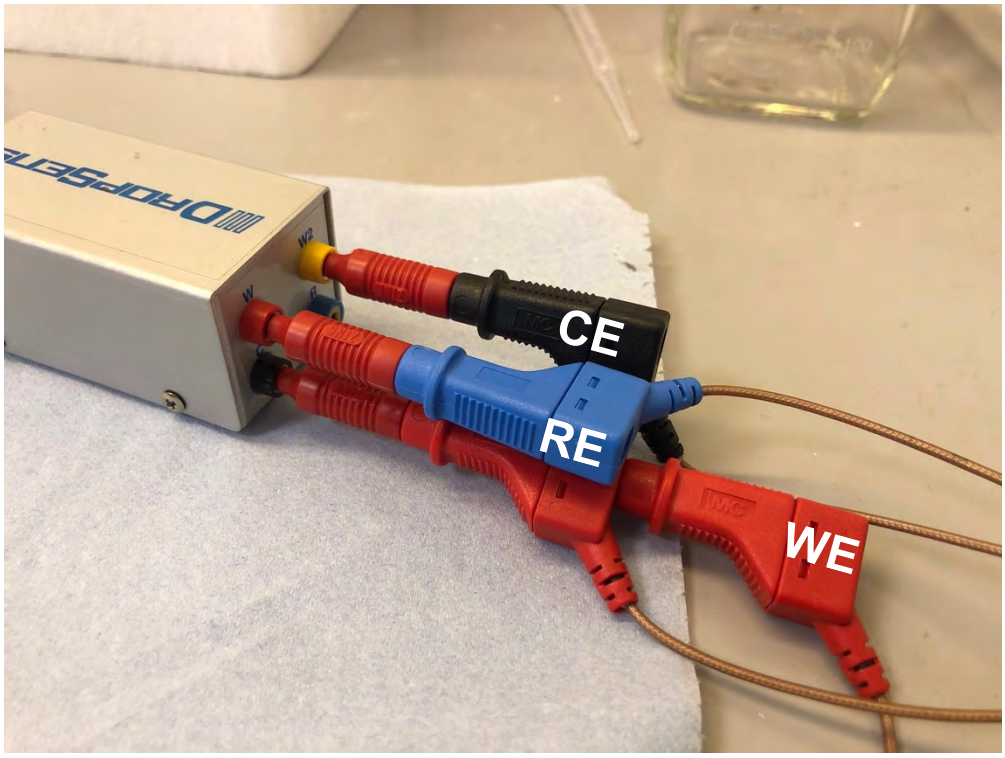
Figure AP 2 *DRP-BIDSC connector*

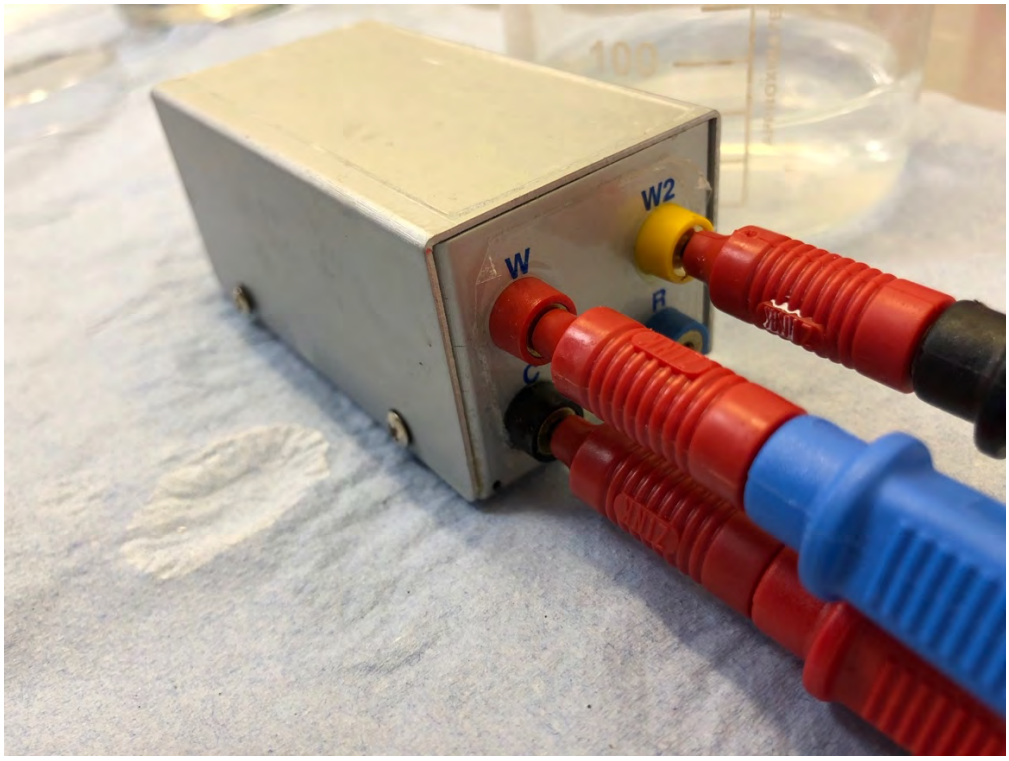
It is not necessarily that the sensor has the same electrodes order like the connector. For example, HW SPCE electrodes' order, left to right, is WE2, RE, WE1, and CE. Therefore, the user shall swap Autolab's wire to meet the sensor's terminals as shown in **Figure AP 2** and pictures below.



Autolab Wire label	→	Connector label
WE	→	C / R
RE	→	W
CE	→	W2

Figure AP 3 *HW SPCE to DRP-BIDSC connector wire connecting management*


CE
RE
WE


W2
W

Appendix 7: Material inventory

A. DNA and PNA

	Item	Location	Notes
1	OXA-1 ssDAN complementary target.	JN1.33 – Freezer 1	Appendix 2
2	ssDAN non-complementary target.		
3	OXA-1 PNA.		
4	Sul1, Int1, DNA and plasmid		

B. Chemical Compounds

	Item	Molar mass (g/mol)	CAS	Location	Notes
1	Sodium Carbonate	105.9888	497-19-8	JN1.33 – Fume cupboard cabinet	
2	Sodium Nitrite	68.9953	7632-00-0		
3	NHS	115.09	6066-82-6		
4	MES	195.2	4432-31-9		
5	Sodium Hydroxide	39.997	1310-73-2		
6	Potassium hexacyanoferrate (III)-K3-	329.24	13746-66-2		
7	Potassium hexacyanoferrate (II) trihydrate -K4-	422.39	14459-95-1		
8	KCL	74.5513	7447-40-7		
9	Hydrochloric acid	36.46	7647-01-0		
10	Dulbecco's Phosphate Buffered Saline (PBS)	NA	NA		
11	4-aminobenzoic acid	137.14	150-13-0	JN1.33 – Freezer 2	
12	EDC	191.7	25952-53-8	JN1.33 – Freezer 1	
13	**C2141021D4**: Silver Topcoat Paste:BG04	NA	NA	EM 2.23	
14	**D2070423P5**: GREY DIELECTRIC INK:BG04	NA	NA		
15	**C2030519P4**: CARBON SENSOR PASTE:BG04	NA	NA		
16	**ZT639** screenwash	NA	NA		
17	Ethylene glycol diacetate	NA	NA		

C: Sensors

	Sensor	Location	Notes
1	HW SPCEs Sensor	JN1.33 – Fume cupboard cabinet	
2	Commercial Sensor		

D. Instruments and equipment

	Item	Location	Notes
1	HW SPCEs Sensor	JN1.33 – Fume cupboard cabinet	
2	Commercial Sensor (DRP-C110)		
3	Autolab PGSTAT12	JN1.33	
4	Autolab dummy load	JN1.33- table Next to fume cupboard	
5	DRP-BIDSC connector	JN1.33 - Fume cupboard cabinet	Appendix 6
6	Epilog Mini 18, 40W CO_2 laser	EM 2.23	
7	Dek Horizon 03i Screenprinter		
8	Hot plate		
9	Vacuum		
10	Keko ILS-4 Isostatic Press		
11	Furnace	EM G.17	
12	DISCO DAD-640 dicing saw (glass blade)	DB G.52	

Appendix 8: SPCE Functionalisation and EIS Measurement Process Checklists

Date: / /

Chemical Compounds checklist	
Na_2CO_3	
4-aminobenzoic acid	
Sodium Nitrite	
EDC	
NHS	
MES	
NaOH	
Potassium hexacyanoferrate (III)-K3	
Potassium hexacyanoferrate (II) trihydrate- K4	
KCL	
BPS	

Chemical Solutions checklist						
Na_2CO_3	In-Situ salt	Activation buffer	MES buffer	PBS buffer	Blocking buffer	EIS buffer

Processes checklist							
Sensor - 01	Clean with Na_2CO_3	Electro deposition	surface activation	PNA functionalisation	Blocking	EIS Measurement at min 0	EIS Measurement at min 52
Sensor-2							
Sensor-3							
Sensor-4							
Sensor-5							

Appendix 9: Change in Charge Transfer Resistance vs Target concentration and incubation time

Code	Identifier	Date	Experiment Number	Target Type	Target Concentration	Incubation time	R_{CT} (MΩ)
1	150721_EX01_CT_20_30	15/07/2021	EX01	CT	20	30	4.45E+04
2	150721_EX01_CT_20_52	15/07/2021	EX01	CT	20	52	1.44E+05
3	160721_EX01_CT_20_0	16/07/2021	EX01	CT	20	0	6.68E+04
4	160721_EX01_CT_20_30	16/07/2021	EX01	CT	20	30	3.95E+05
5	160721_EX01_CT_20_52	16/07/2021	EX01	CT	20	52	1.66E+06
6	160721_EX02_NCT_20_0	16/07/2021	EX02	NCT	20	0	1.25E+04
7	160721_EX02_NCT_20_30	16/07/2021	EX02	NCT	20	30	1.18E+04
8	160721_EX02_NCT_20_52	16/07/2021	EX02	NCT	20	52	1.08E+04
9	190721_EX03_CT_0.25_0	19/07/2021	EX03	CT	0.25	0	7.63E+04
10	190721_EX03_CT_0.25_52	19/07/2021	EX03	CT	0.25	52	7.80E+04
11	190721_EX04_CT_0.25__0	19/07/2021	EX04	CT	0.25	0	6.66E+03
12	190721_EX04_CT_0.25_52	19/07/2021	EX04	CT	0.25	52	8.94E+03
13	200721_EX01_CT_20_0	20/07/2021	EX01	CT	20	0	1.12E+04
14	200721_EX01_CT_20_52	20/07/2021	EX01	CT	20	52	6.13E+05
15	200721_EX01_NCT_20_0	20/07/2021	EX01	NCT	20	0	2.97E+04
16	200721_EX01_NCT_20_52	20/07/2021	EX01	NCT	20	52	3.69E+04
17	200721_EX02_CT_20_0	20/07/2021	EX02	CT	20	0	6.97E+03
18	200721_EX02_CT_20_52	20/07/2021	EX02	CT	20	52	1.46E+06
19	200721_EX02_NCT_20_0	20/07/2021	EX02	NCT	20	0	1.90E+04
20	200721_EX02_NCT_20_52	20/07/2021	EX02	NCT	20	52	7.84E+04
21	210721_EX01_CT_0.25_0	21/07/2021	EX01	CT	0.25	0	1.27E+04

22	210721_EX01_CT_0.25_52	21/07/2021	EX01	CT	0.25	52	1.34E+04
23	210721_EX01_CT_2.50_0	21/07/2021	EX01	CT	2.50	0	8.86E+03
24	210721_EX01_CT_2.50_62	21/07/2021	EX01	CT	2.50	62	4.38E+05
25	210721_EX02_CT_0.50_20	21/07/2021	EX02	CT	0.50	20	2.37E+04
26	210721_EX02_CT_0.50_52	21/07/2021	EX02	CT	0.50	52	3.26E+05
27	210721_EX02_CT_2.50_0	21/07/2021	EX02	CT	2.50	0	1.91E+04
28	210721_EX02_CT_2.50_52	21/07/2021	EX02	CT	2.50	52	6.05E+05
29	210721_EX03_CT_0.50_0	21/07/2021	EX03	CT	0.5	0	1.05E+04
30	210721_EX03_CT_0.50_52	21/07/2021	EX03	CT	0.5	52	2.51E+05
31	220721_EX01_CT_0.5_0	22/07/2021	EX01	CT	0.5	0	4.79E+03
32	220721_EX01_CT_0.5_52	22/07/2021	EX01	CT	0.5	52	4.46E+05
33	220721_EX02_CT_0.5_0	22/07/2021	EX02	CT	0.5	0	7.81E+03
34	220721_EX02_CT_0.5_52	22/07/2021	EX02	CT	0.5	52	3.08E+05
35	220721_EX05_CT_0_0	22/07/2021	EX05	CT	0	0	1.47E+04
36	220721_EX05_CT_0_60	22/07/2021	EX05	CT	0	60	9.88E+04
37	220721_EX06_CT_0_0	22/07/2021	EX06	CT	0	0	3.50E+04
38	220721_EX06_CT_0_70	22/07/2021	EX06	CT	0	70	6.58E+03
39	260721_EX03_CT_1_0	26/07/2021	EX03	CT	1	0	3.00E+04
40	260721_EX03_CT_1_60	26/07/2021	EX03	CT	1	60	3.99E+05
41	270721_EX01_CT_1_0	27/07/2021	EX01	CT	1	0	2.43E+04
42	270721_EX01_CT_1_52	27/07/2021	EX01	CT	1	52	1.79E+05
43	270721_EX02_CT_1_0	27/07/2021	EX02	CT	1	0	2.57E+04
44	270721_EX02_CT_1_52	27/07/2021	EX02	CT	1	52	8.63E+04
45	270721_EX05_CT_0.5_0	27/07/2021	EX05	CT	0.5	0	8.65E+03
46	270721_EX05_CT_0.5_52	27/07/2021	EX05	CT	0.5	52	4.07E+05
47	270721_EX06_CT_0.5_0	27/07/2021	EX06	CT	0.5	0	1.44E+04
48	270721_EX06_CT_0.5_52	27/07/2021	EX06	CT	0.5	52	3.41E+05

49	280721_EX03_CT_0.5_0	28/07/2021	EX03	CT	0.5	0	5.96E+03
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51	280721_EX04_CT_0.5_0	28/07/2021	EX04	CT	0.5	0	5.96E+03
52	280721_EX04_CT_0.5_52	28/07/2021	EX04	CT	0.5	52	7.37E+03
53	290721_EX01_CT_0.6_0	29/07/2021	EX01	CT	0.6	0	16182
54	290721_EX01_CT_0.6_52	29/07/2021	EX01	CT	0.6	52	96976
55	290721_EX04_CT_0.5_0	29/07/2021	EX04	CT	0.5	0	9303
56	290721_EX04_CT_0.5_52	29/07/2021	EX04	CT	0.5	52	66477