



# Electrochemical Biosensors for Rapid Diagnostics to fight Antimicrobial Resistance

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University of Edinburgh  
25<sup>th</sup> January 2017

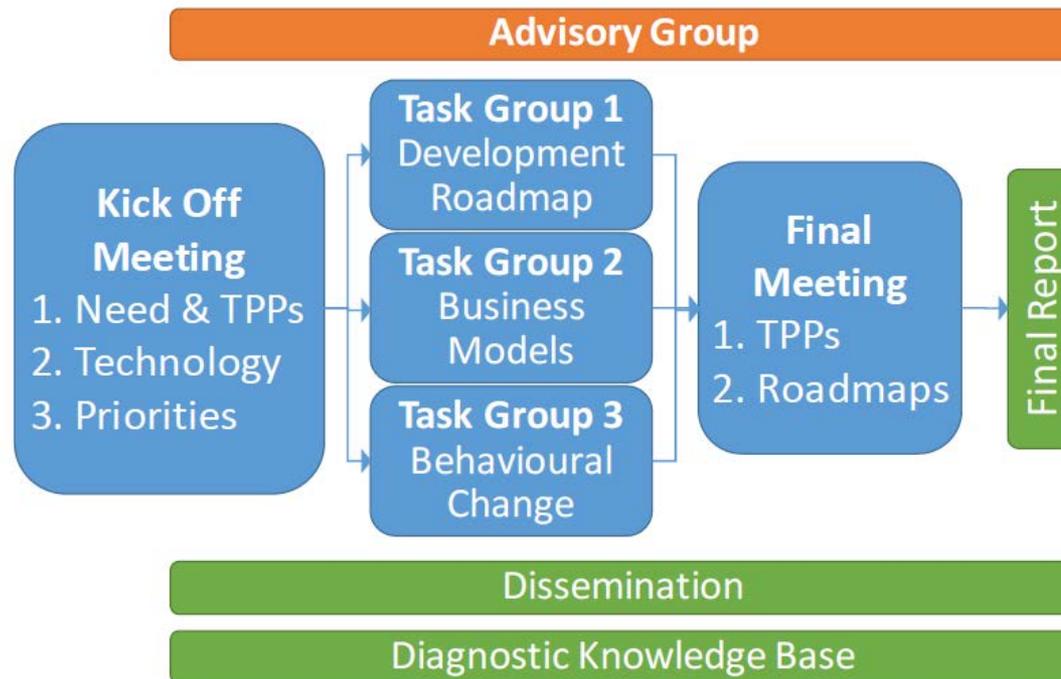
NIHR Oxford DEC/Leeds/KTN  
Antimicrobial Resistance Workshop Programme  
25 January 2017, St Hugh's College, Oxford



# Joint Programming Initiative on AMR Working Group on Rapid Diagnostics AMR-RDT



- Coordinator Till Bachmann, University of Edinburgh
- 50 partners globally
- Identify barriers for development and implementation of rapid diagnostic tests to tackle AMR





# AMR Diagnostics Prizes to Win

- *EU Reducing the Mis-use of Antibiotics Prize*
  - **€1 million**
  - *Avoid antibiotics for viral upper respiratory tract infections*
  - <http://ec.europa.eu/research/horizonprize/index.cfm>
- **UK Longitude Prize (Discovery Award)**
  - **£8 million** (£20,000 each)
  - Point of Care Test
  - Any type of bacterial infection
  - <http://www.longitudeprize.org/>
- **US**
  - **\$20 million**
  - Rapid, Point-of-Care Diagnostic Tests
  - Identify highly resistant bacterial infections
  - <http://www.cccinnovationcenter.com/challenges/antimicrobial-resistance-diagnostic-challenge/>





**challenge**  
advancing the next generation of AMR Dx

**bilateral**  
student teams

**international**  
expert judge panel

2017 UK-India competition

2018 global competition



CONNECT TO INSPIRE FOR IMPACT



**AMR DxC Winter School 2017 UK**  
*20 – 24 February 2017, Edinburgh*

**AMR DxC Winter School 2017 INDIA**  
*3 – 7 April 2017, Bangalore*

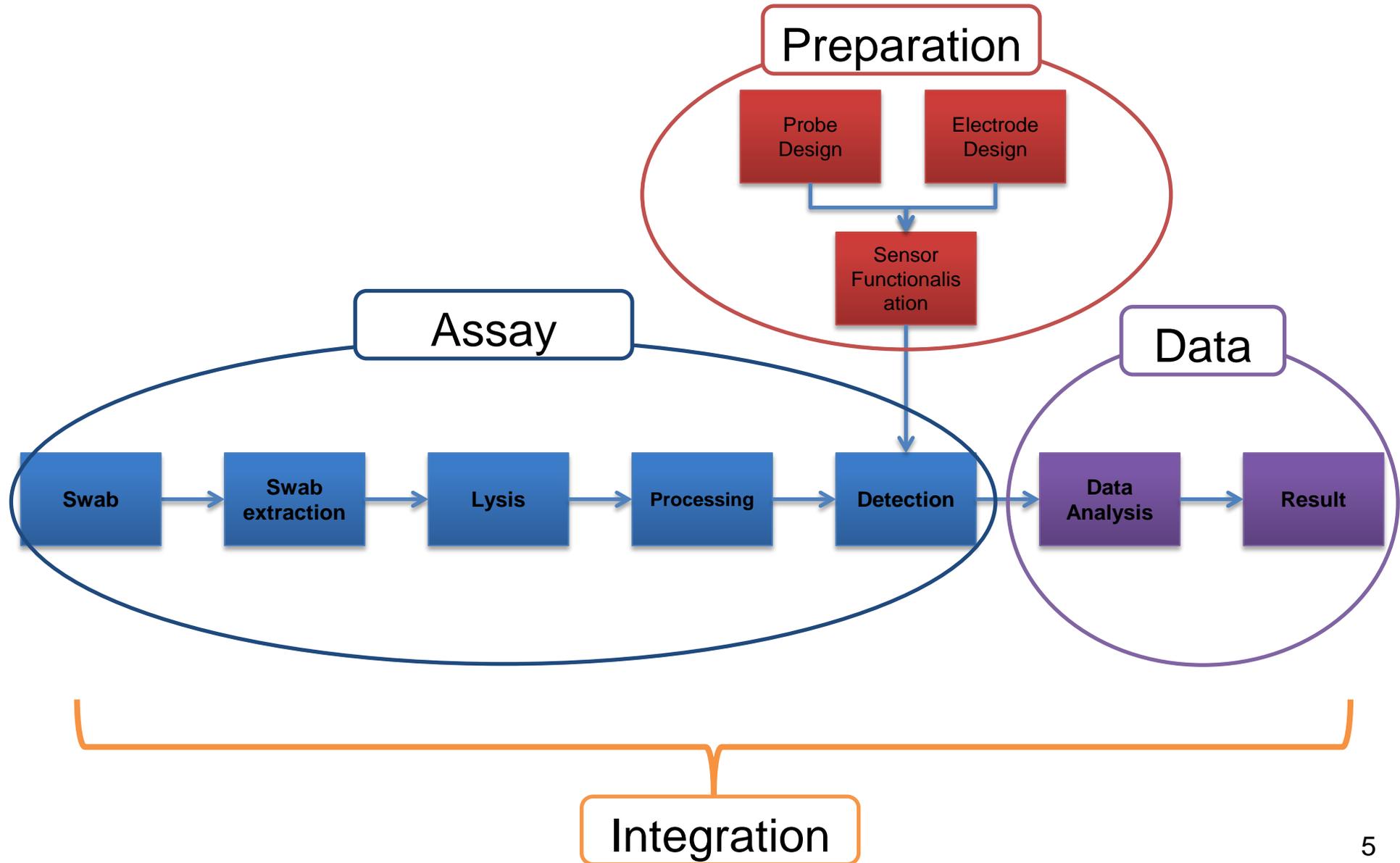
<http://www.ed.ac.uk/pathway-medicine/antimicrobial-resistance/amr-dxc>



@AMR\_DxC



# Building a POCT Assay & Cartridge



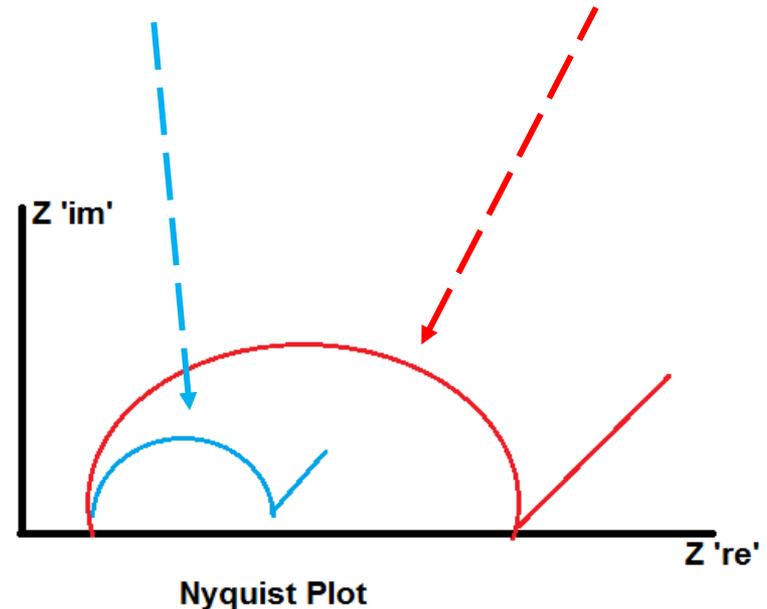
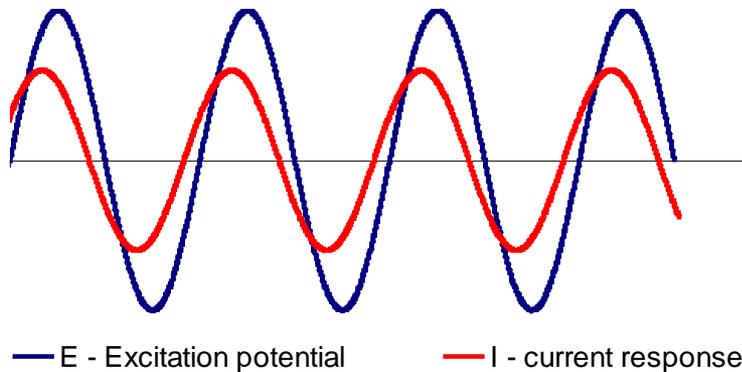
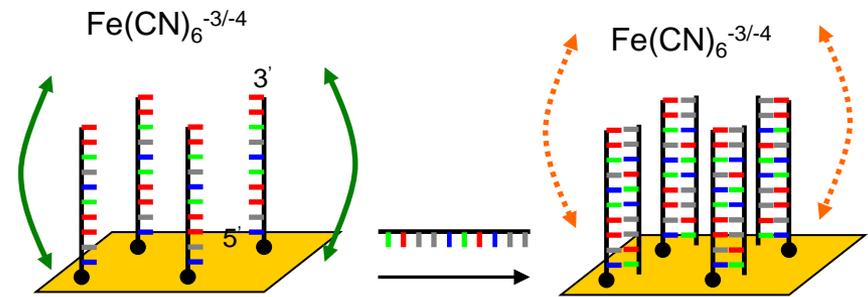
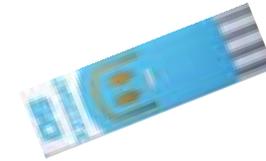
# Chronic Wound Care Programme

- Development of a an easy-to-use, portable medical device that can be readily applied to diagnose chronic wounds in a clinical environment and in the community.
- University of Edinburgh, NHS Lothian, Zisys Ltd. research provider
- Aim: **Molecular MRSA detection from clinical specimen without PCR**

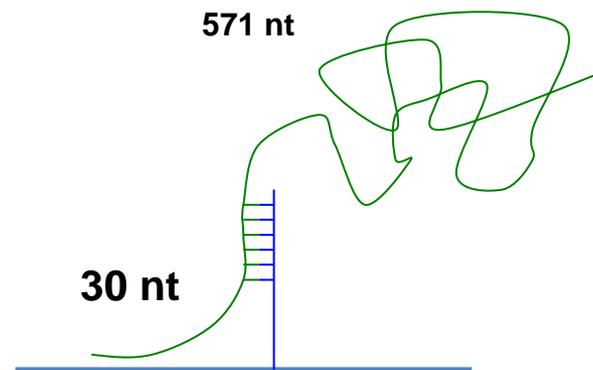
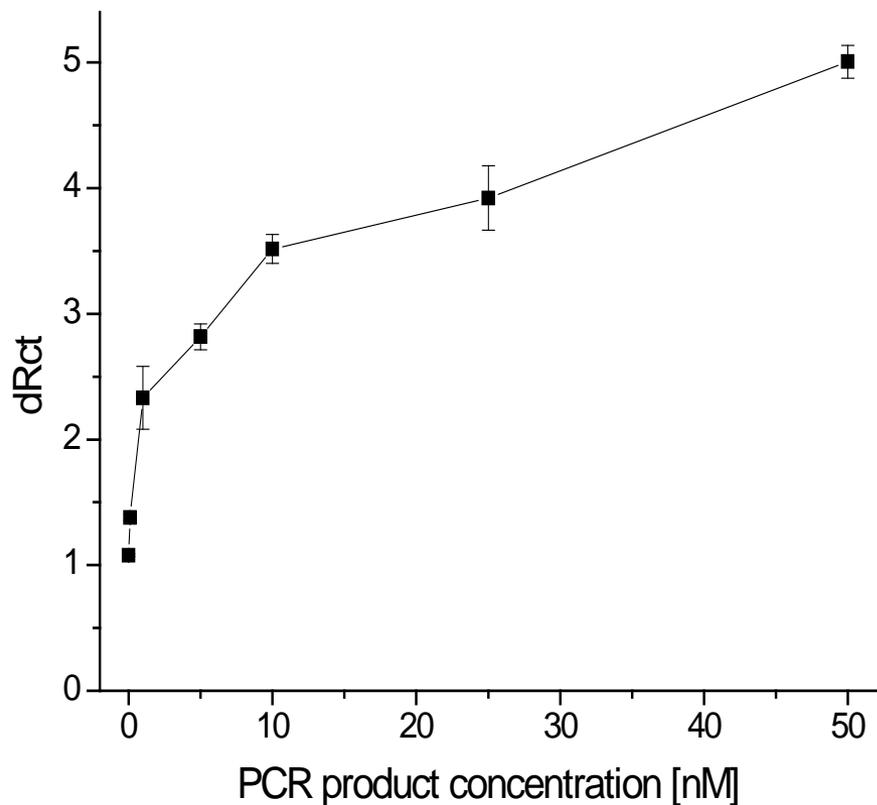


# Electrochemical Impedance Spectroscopy for Point of Care Testing

- EIS
- Label free detection
- Small AC potential is applied to an electrochemical cell and the current response measured
- Interrogate different frequencies ranging from 0.1 -100,000 Hz
- Platform technology



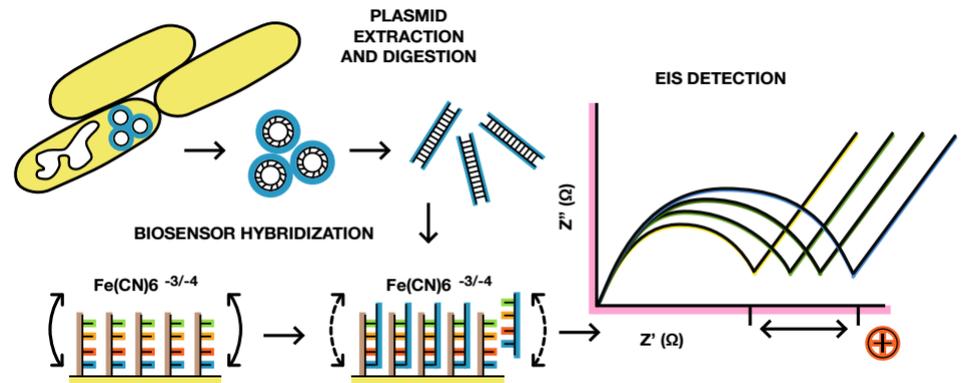
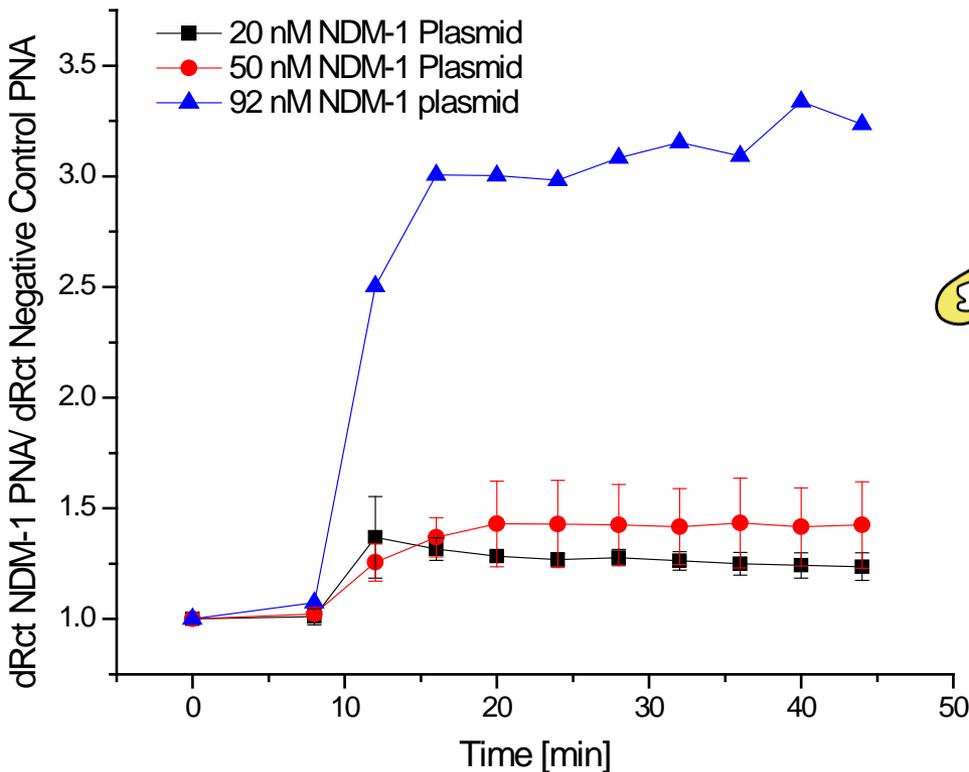
# EIS Assay Performance – NDM-1 PCR Products



Molarity (mol/L)		mol	N/ml
milli	1.00E-03	6.00E+23	600,000,000,000,000,000
micro	1.00E-06	6.00E+23	600,000,000,000,000,000
nano	1.00E-09	6.00E+23	600,000,000,000,000,000
<b>pico</b>	1.00E-12	6.00E+23	600,000,000,000,000,000
femto	1.00E-15	6.00E+23	600,000,000,000,000,000
atto	1.00E-18	6.00E+23	600,000,000,000,000,000
zepto	1.00E-21	6.00E+23	600,000,000,000,000,000
yocto	1.00E-24	6.00E+23	600,000,000,000,000,000

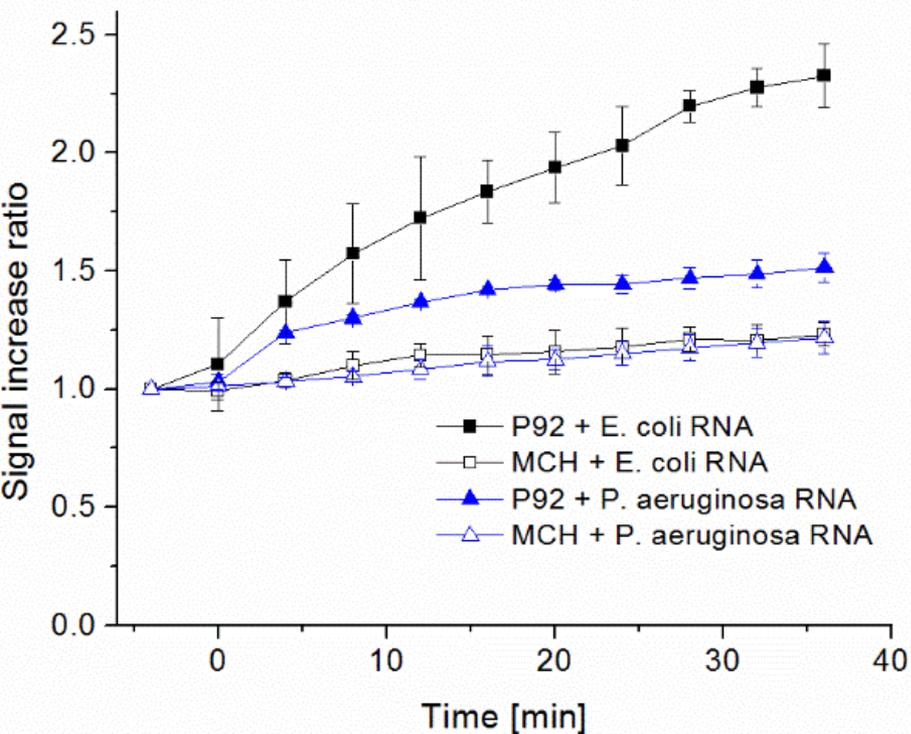
- NDM-1 PCR product generated from *Citrobacter freundii* clinical isolate (Edinburgh Royal infirmary, NHS Lothian)
- Limit of Detection = 100 pM

# Amplification-free Detection of NDM-1 Plasmids

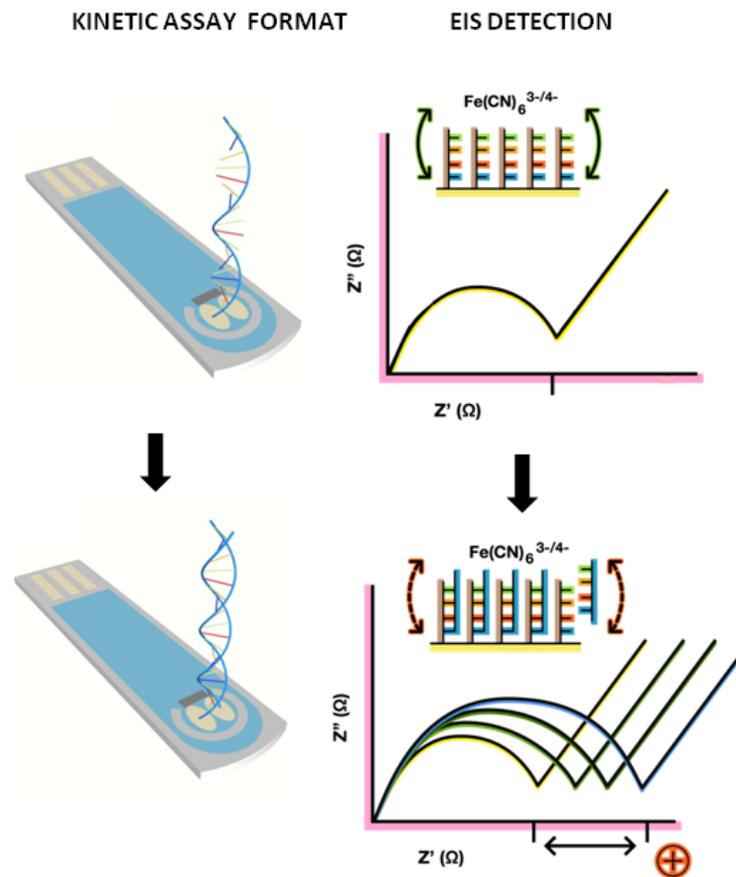


- Direct amplification-free detection of NDM-1 plasmid DNA extracted from *Citrobacter freundii* clinical isolate
- DNase used to generate fragments for better hybridization efficiency
- First example of direct EIS-based plasmid detection

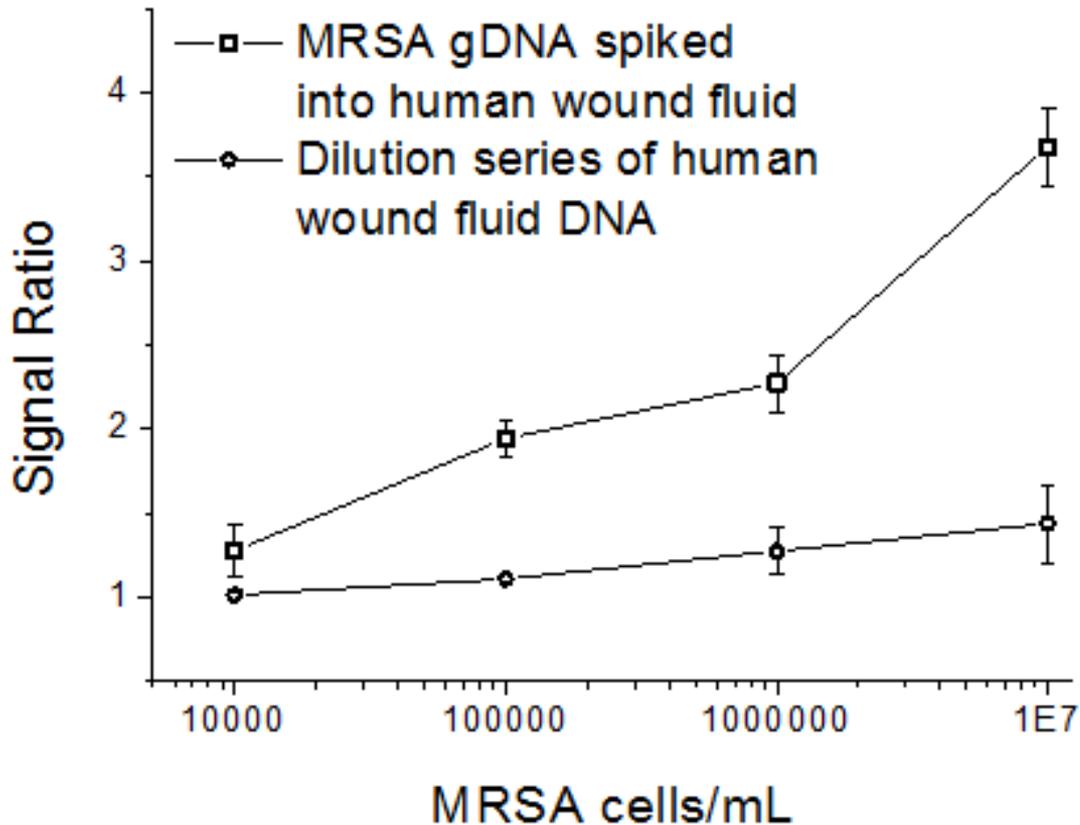
# Amplification-free Detection of Ribosomal RNA



Signal increase ratios obtained at screen-printed electrodes functionalised with E. coli specific PNA probe P92 and MCH only (negative control), respectively, following exposure to 750 pM E. coli and P. aeruginosa rRNA in EIS measurement buffer, respectively. EIS measurements were continuously repeated in the presence of the target solution without any washing steps.



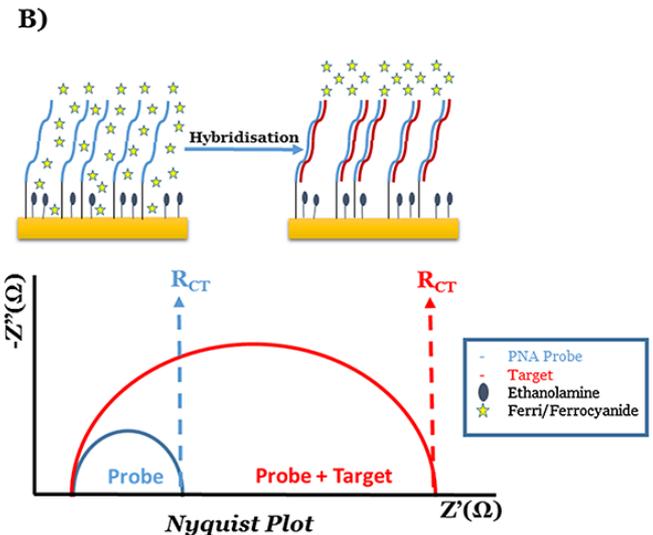
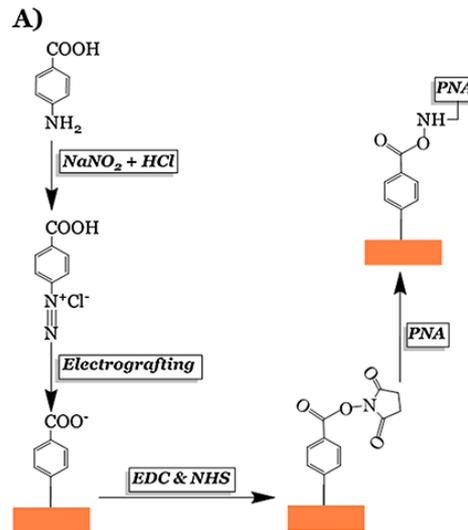
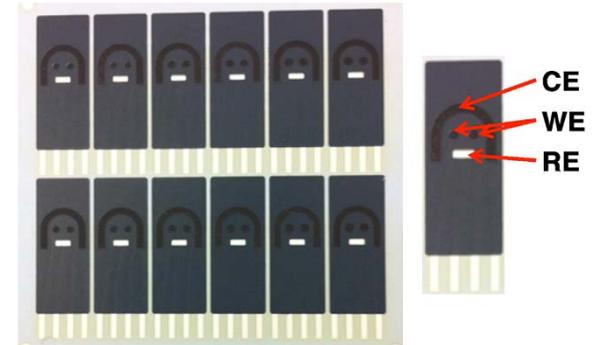
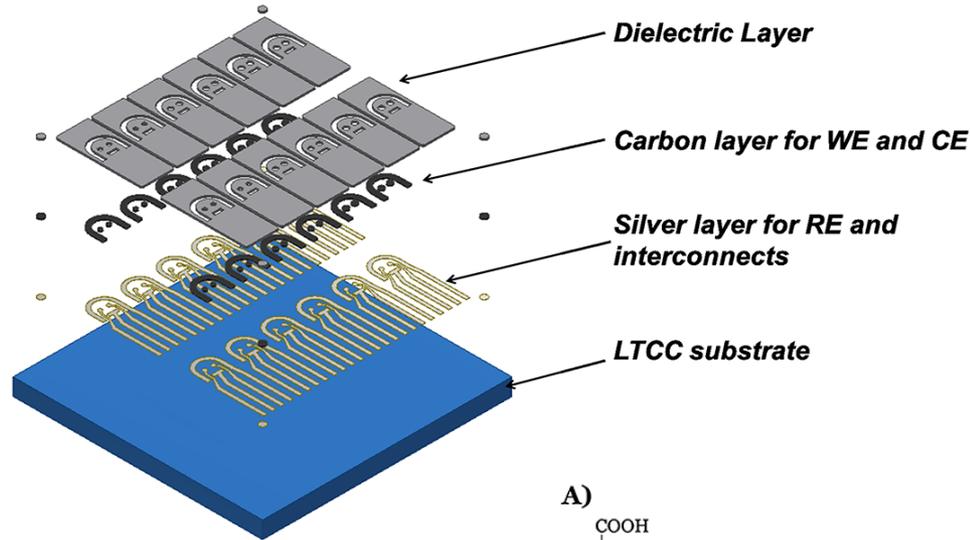
# Amplification-free Detection of **Genomic DNA**



Signal Ratios caused by incubation with gDNA extracted from MRSA cells spiked into human wound fluid and uninoculated human wound fluid. Signal Ratio measured 10 min after sample addition  $N = 3$  and error bars = S.D.

Molarity (mol/L)		mol	N/ml
milli	1.00E-03	6.00E+23	600,000,000,000,000,000
micro	1.00E-06	6.00E+23	600,000,000,000,000,000
nano	1.00E-09	6.00E+23	600,000,000,000,000,000
pico	1.00E-12	6.00E+23	600,000,000,000,000,000
femto	1.00E-15	6.00E+23	600,000,000,000,000,000
atto	1.00E-18	6.00E+23	600,000,000,000,000,000
zepto	1.00E-21	6.00E+23	0.6
yocto	1.00E-24	6.00E+23	0.0006

# Streamlining Sensor Manufacturing for POCT



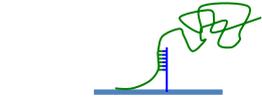
# One Detection Technology – Many Targets



[<http://www.bio.nite.go.jp/dogan/>]

## MRSA gDNA

Corrigan, D. K.; Schulze, H.; Henihan, G.; Hardie, A.; Ciani, I.; Giraud, G.; Terry, J. G.; Walton, A. J.; Pethig, R.; Ghazal, P.; Crain, J.; Campbell, C. J.; Templeton, K. E.; Mount, A. R.; Bachmann, T. T. Development of a PCR-free electrochemical point of care test for clinical detection of methicillin resistant *Staphylococcus aureus* (MRSA). *Analyst* 2013, 138 (22), 6997-7005.



*mecA*, NDM-1, PCR products, *mcr-1*

Corrigan, D. K.; \*, Schulze, H.; \*, Henihan, G.; Ciani, I.; Giraud, G.; Terry, J. G.; Walton, A. J.; Pethig, R.; Ghazal, P.; Crain, J.; Campbell, C. J.; Mount, A. R.; Bachmann, T. T. Impedimetric detection of single-stranded PCR products derived from methicillin resistant *Staphylococcus aureus* (MRSA) isolates. *Biosens. Bioelectron.* 2012, 34 (1), 178-184.

Huang JM, Henihan G, Macdonald D, Michalowski A, Templeton K, Gibb AP, Schulze H, Bachmann TT. Rapid Electrochemical Detection of New Delhi Metallo-beta-lactamase Genes To Enable Point-of-Care Testing of Carbapenem-Resistant Enterobacteriaceae. *Anal. Chem.* 2015, 87 (15), 7738-7745.



[Behrens S. et al. (2003) *Appl. Environ. Microbiol.* 69, 1748-58]

## Pathogen rRNA

Grace Henihan\*, Holger Schulze\*, Ilenia Ciani, Damion Corrigan, Gerard Giraud, Jonathan G. Terry, Colin J. Campbell, Anthony J. Walton, Jason Crain, Ronald Pethig, Peter Ghazal, Andrew R. Mount and Till T. Bachmann. Label- and amplification-free electrochemical detection of bacterial ribosomal RNA. *Biosens. Bioelectron.* 2016 DOI: <http://dx.doi.org/10.1016/j.bios.2016.03.037>.



## microRNA

Ongoing, In collaboration with Dr. James Dear, University of Edinburgh

# EIS platform



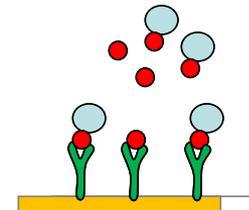
## TREM-1, MMP9 protein infection biomarker

Ciani, I.; Schulze, H.; Corrigan, D. K.; Henihan, G.; Giraud, G.; Terry, J. G.; Walton, A. J.; Pethig, R.; Ghazal, P.; Crain, J.; Campbell, C. J.; Bachmann, T. T.; Mount, A. R. Development of immunosensors for direct detection of three wound infection biomarkers at point of care using electrochemical impedance spectroscopy. *Biosens. Bioelectron.* 2012, 31 (1), 413-418.



## VEGF Aptamer

Ongoing, In collaboration with Prof. Kazunori Ikebukuro, Tokyo University of Agriculture and Technology



## Homo Serine Lactone (HSL) quorum sensing biomarker

Ciani, I.; Schulze, H.; Corrigan, D. K.; Henihan, G.; Giraud, G.; Terry, J. G.; Walton, A. J.; Pethig, R.; Ghazal, P.; Crain, J.; Campbell, C. J.; Bachmann, T. T.; Mount, A. R. Development of immunosensors for direct detection of three wound infection biomarkers at point of care using electrochemical impedance spectroscopy. *Biosens. Bioelectron.* 2012, 31 (1), 413-418.



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