

Biosensing and Bioanalysis Group

UAB
Universitat Autònoma de Barcelona

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SUSNANO SPRING SCHOOL Electrochemical Nanobiosensors for Environmental Diagnostics 15-16/04/2024



ExoSenS PoC



ExoSenS



ECHILIBRIST



Funded by
the European Union

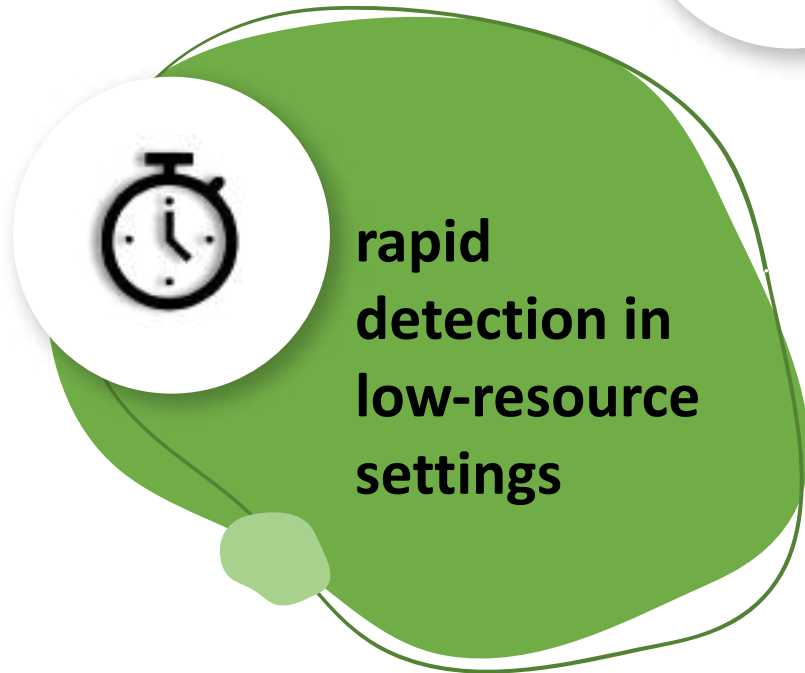


The aim of our research is to contribute to the development of the next generation of **Rapid Diagnostic Tests (RDTs)** for low-resource settings in order to address societal challenges, such as global health issues, by improving the **analytical performance** of RDTs, including sensitivity, specificity, and analytical simplification.

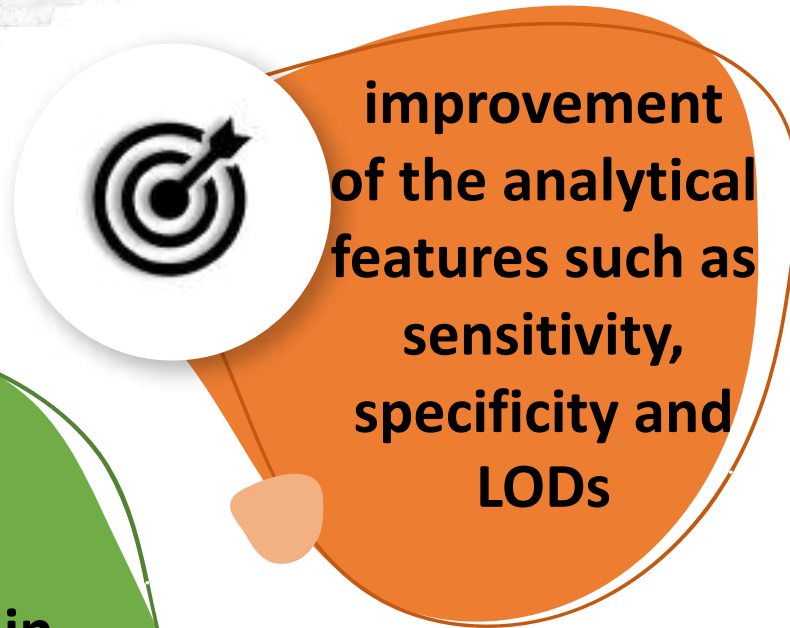
UAB Universitat Autònoma de Barcelona



Goals



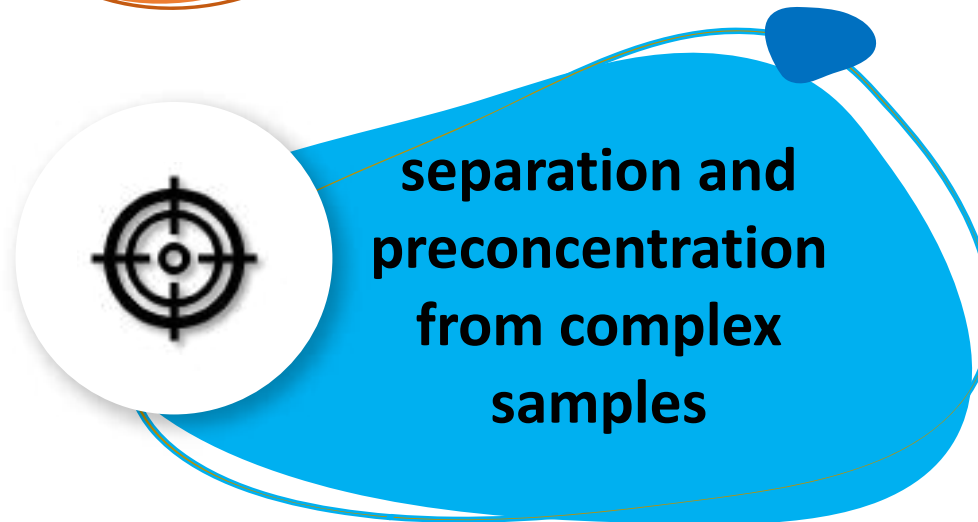
**rapid
detection in
low-resource
settings**



**improvement
of the analytical
features such as
sensitivity,
specificity and
LODs**



**analytical
simplification**



**separation and
preconcentration
from complex
samples**

Features

A

AFFORDABLE

S

SENSITIVE

S

SPECIFIC

U

**USER
FRIENDLY**

R

**RAPID &
ROBUST**

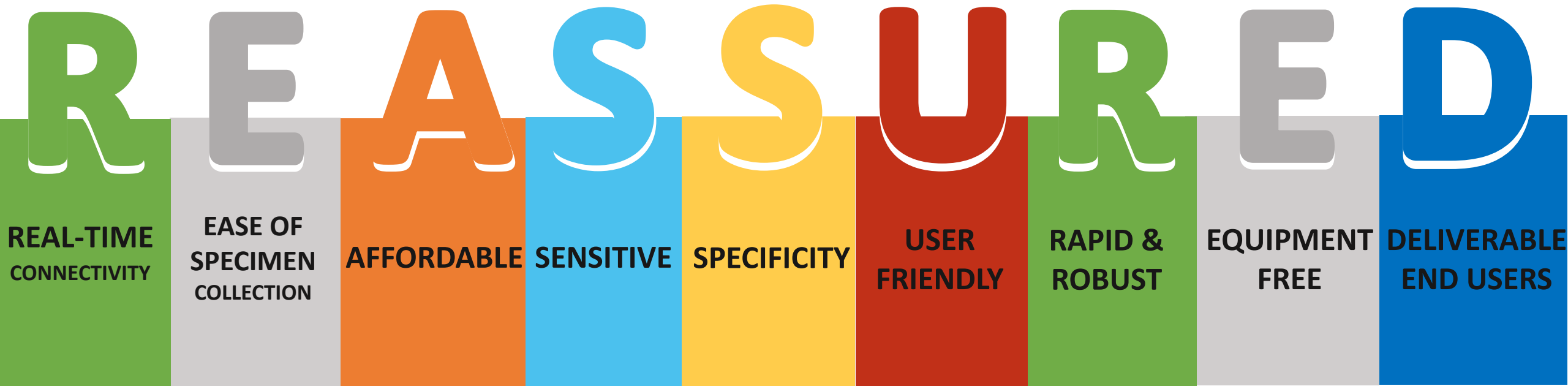
E

**EQUIPMENT
FREE**

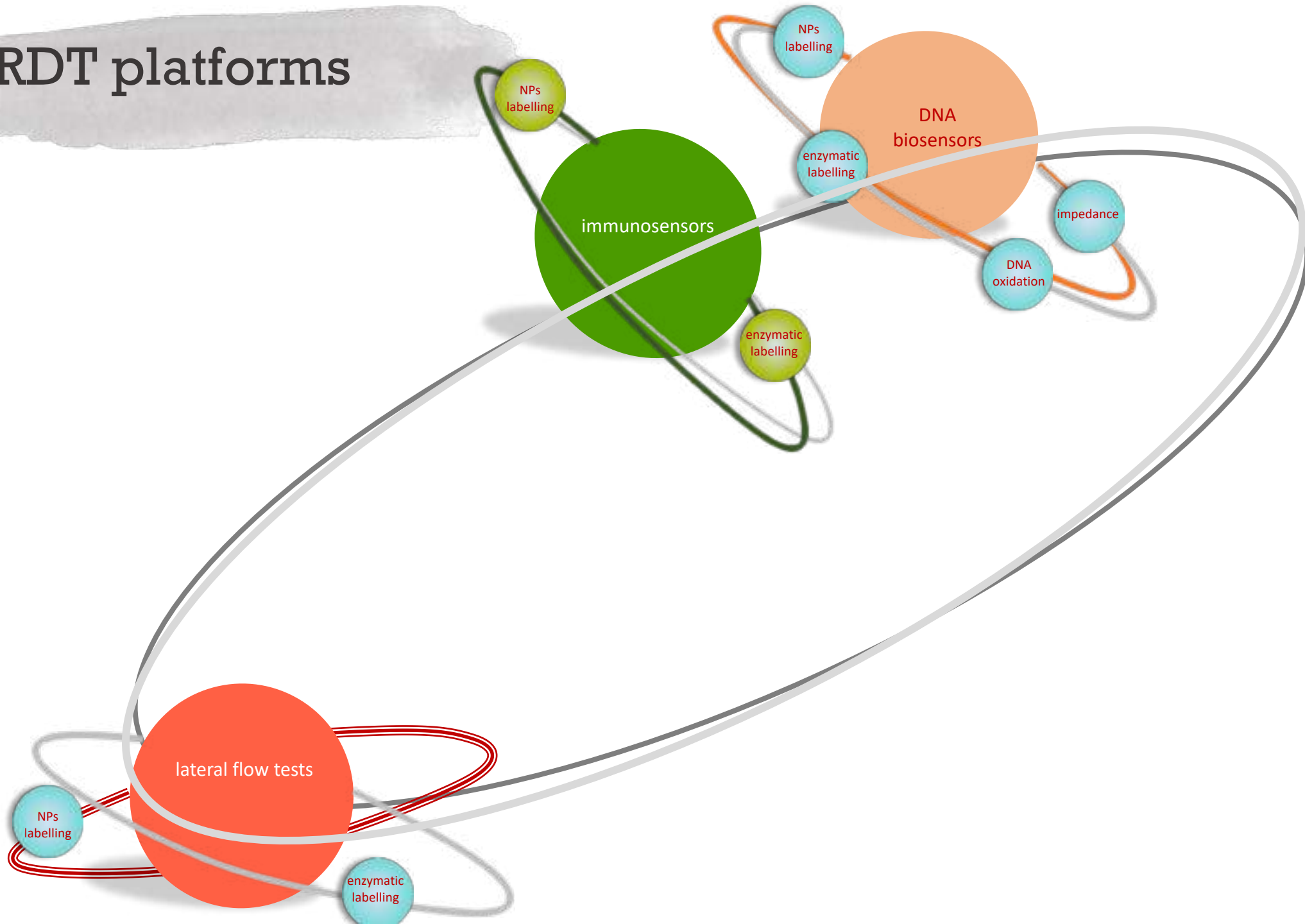
D

**DELIVERABLE
END USERS**

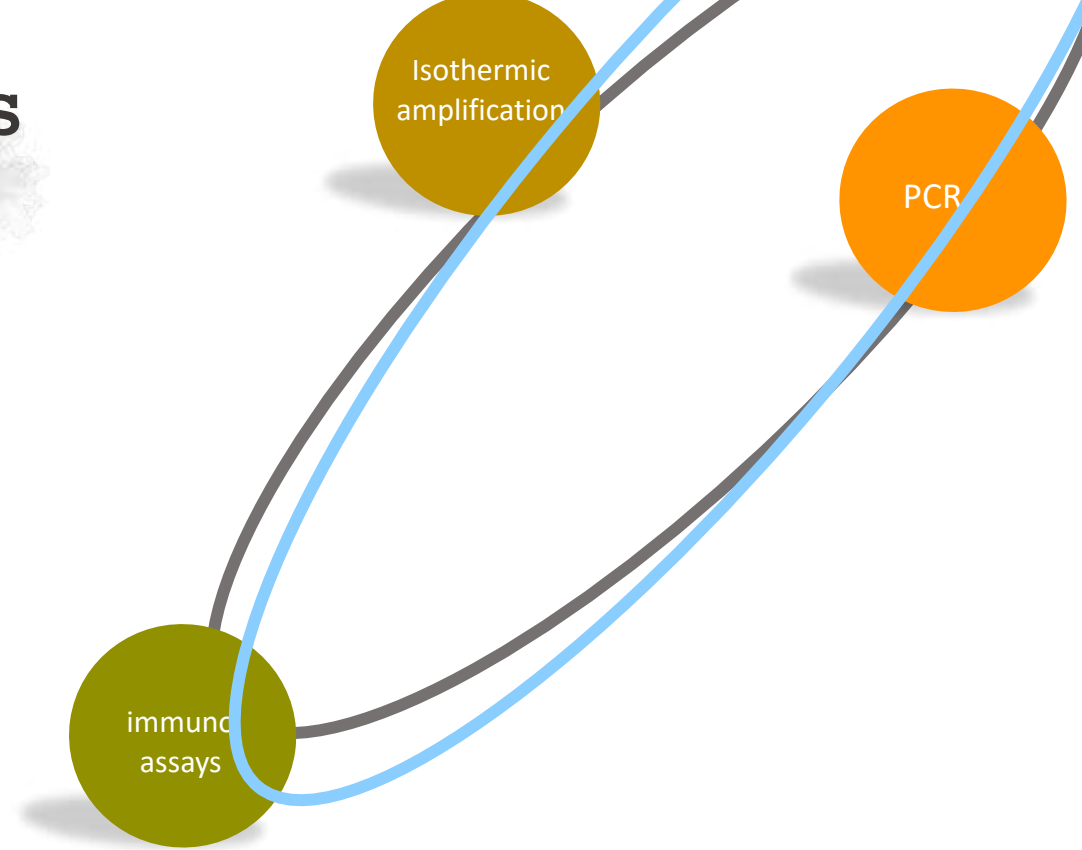
Features



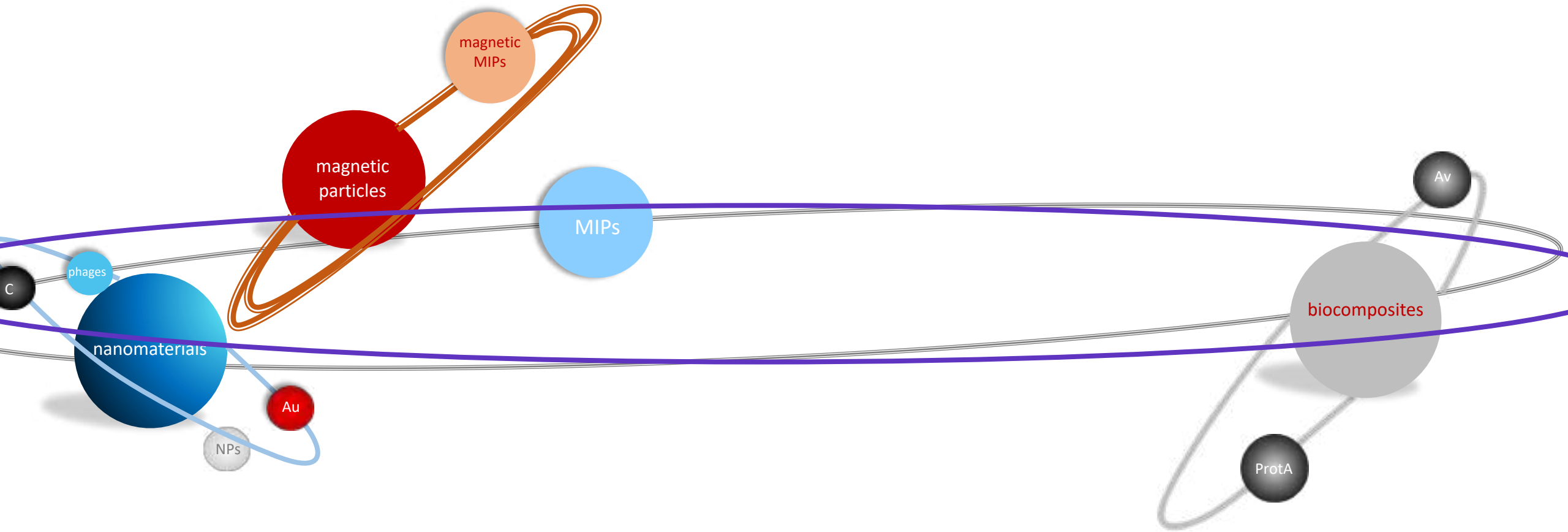
RDT platforms



Improved bioanalytical techniques



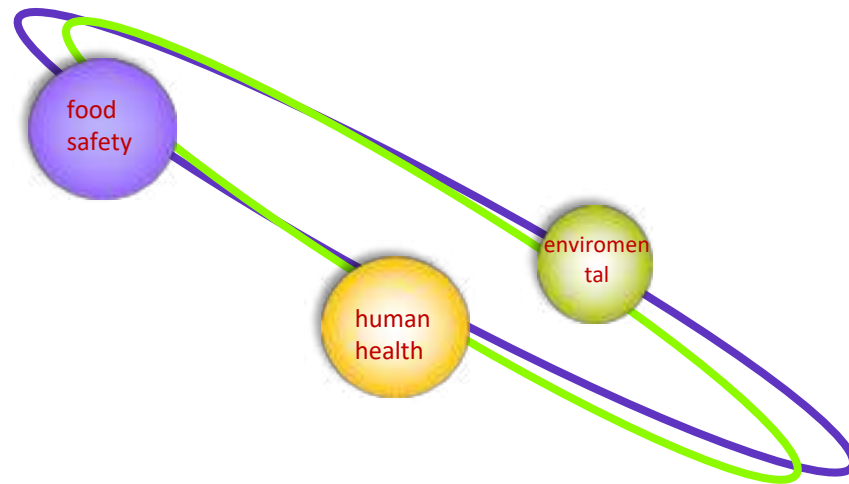
Materials

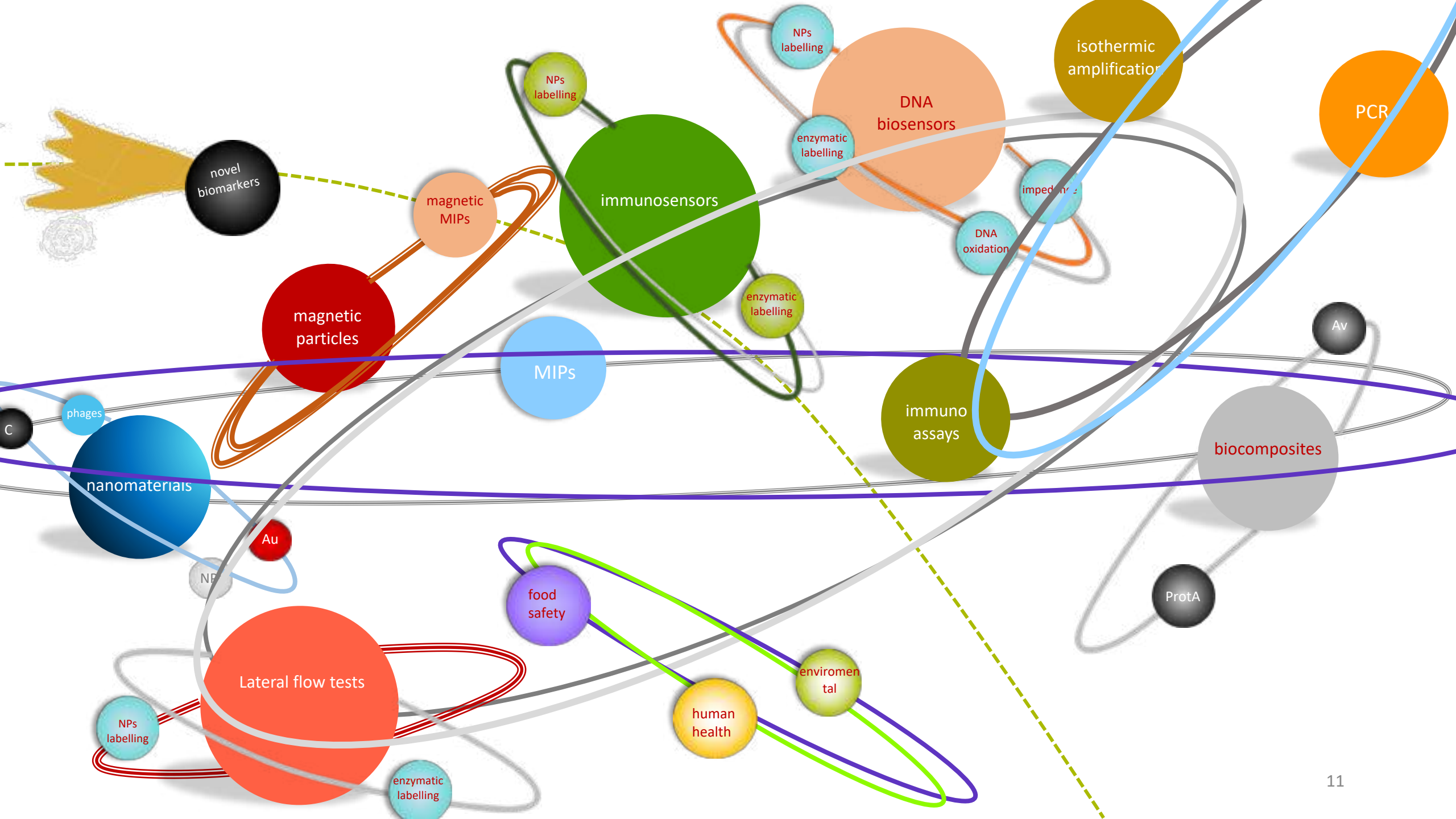


Biomarkers

novel
biomarkers

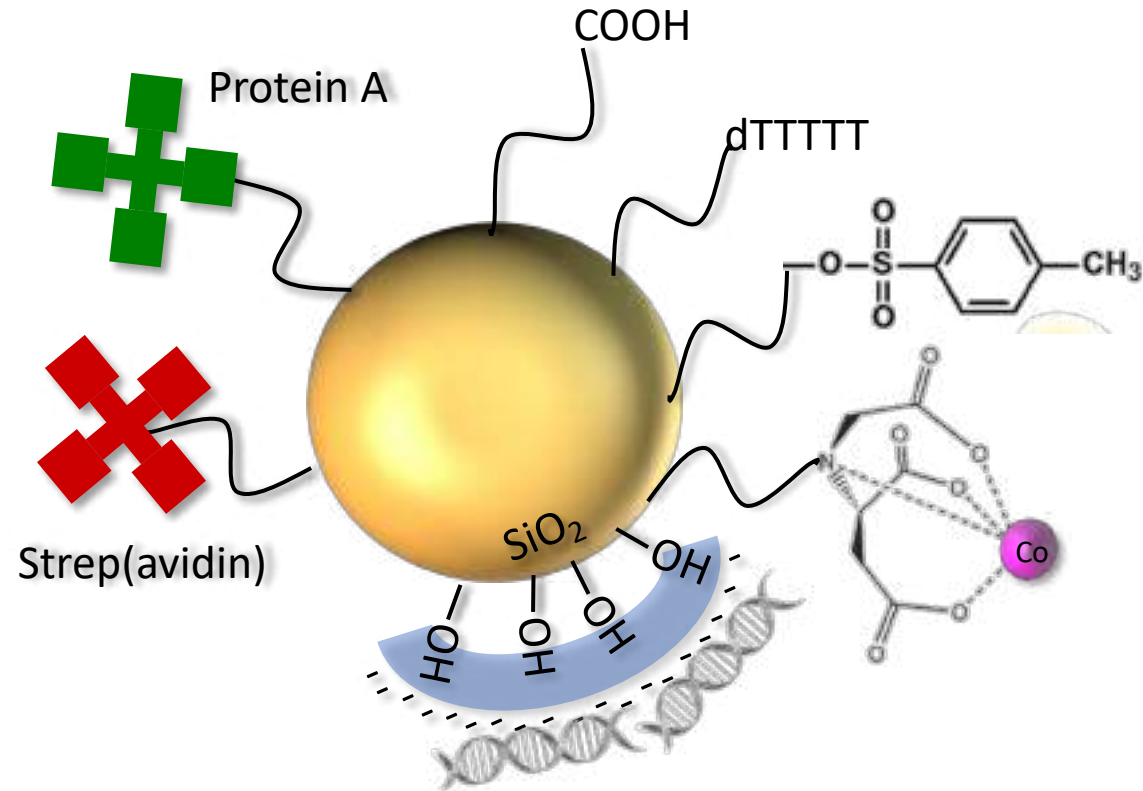
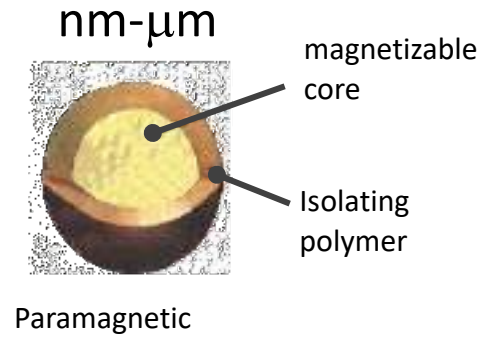
Applications





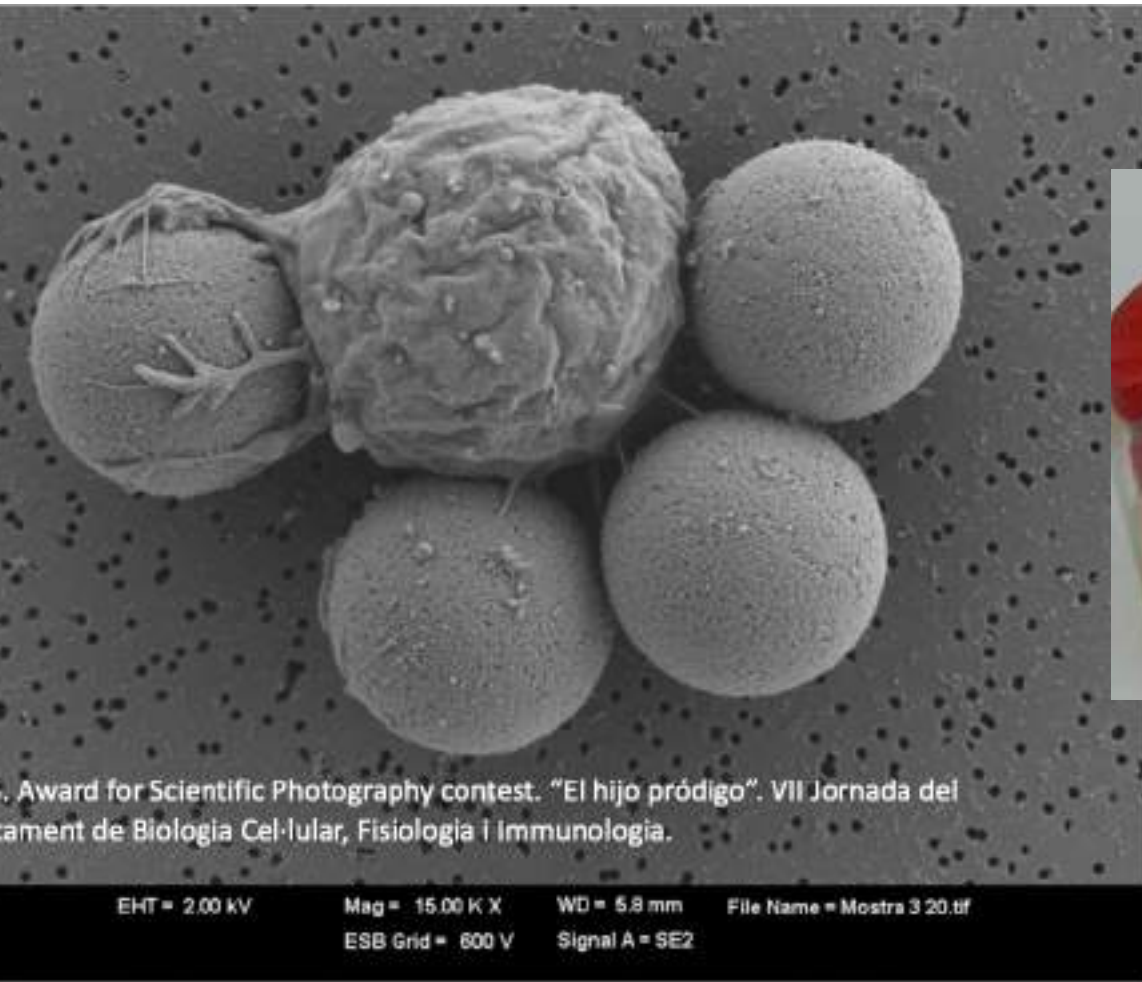
Materials

m-GEC: magneto-actuated transducer with the integration of MPs





Materials

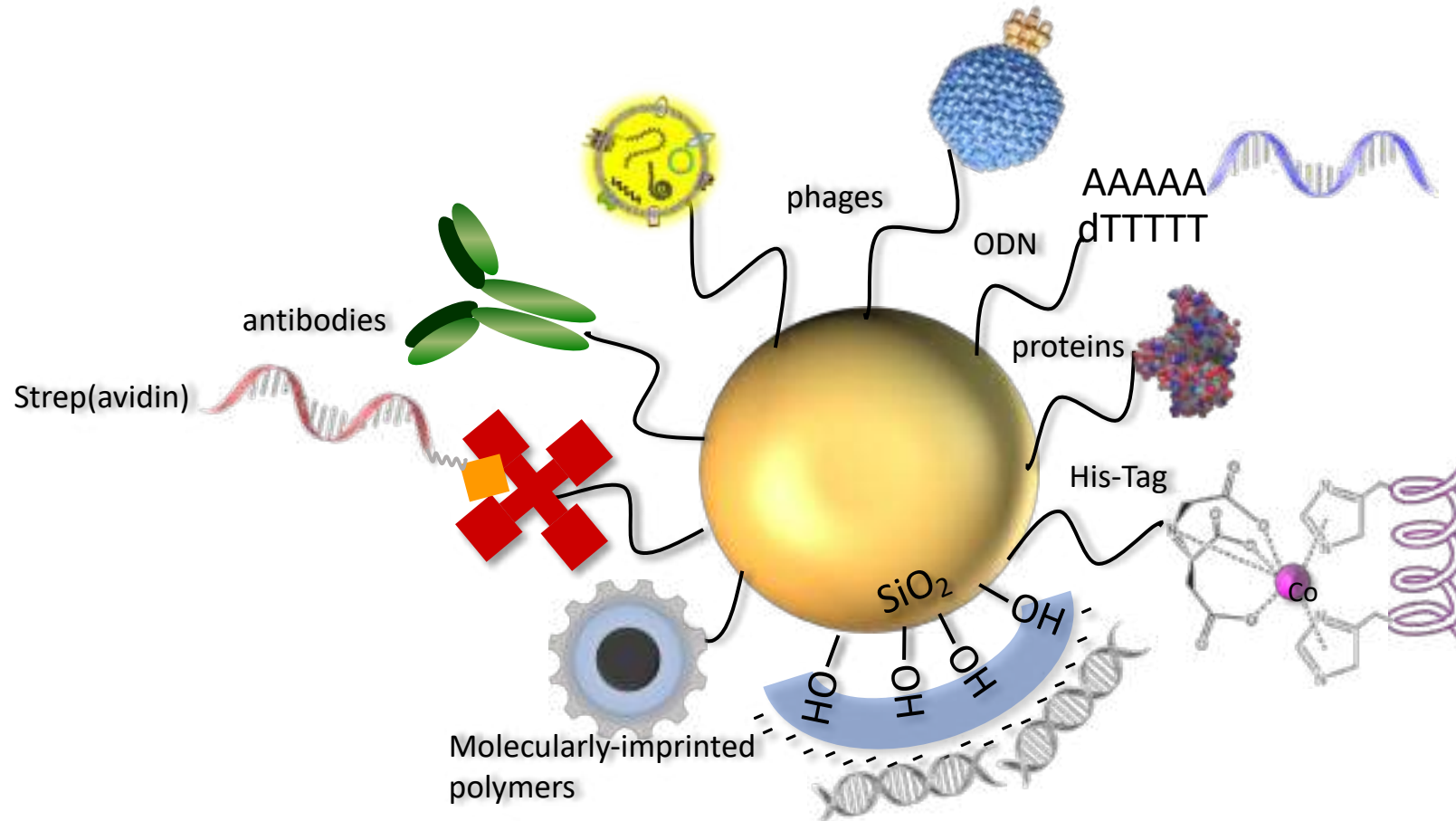


30

- 2015. S Carinelli, C Xufré Ballesteros, M Martí, S Alegret, MI Pividori. «Electrochemical magneto-actuated biosensor for CD4 count in AIDS diagnosis and monitoring». Biosensors and Bioelectronics 74, 974-980.

Materials

m-GEC: magneto-actuated transducer with the integration of MPs



Materials

m-GEC: magneto-actuated transducer with the integration of MPs

- 2006. Anal Chem 78:1789-98
- 2007. Biosens Bioelectron 22: 2184-91
- 2009. Biosens Bioelectron 24:2057-63
- 2009. Biosens Bioelectron 25:510-3
- 2011. Anal Chem 83:5570-7
- 2009. Anal Chem 81, 5812-20
- 2015. Talanta 143:198-204
- 2015. Biosens Bioelectron 74, 974-980.
- 2016. Talanta 153:38-44
- 2016. Talanta 160, 36-45.
- 2018. Journal of Extracellular Vesicles 7, 240
- 2022 Biosens Bioelectron 198, 113826

- 2018. Journal of Extracellular Vesicles 7, 240
- 2020. Biosensors & Bioelectronics 150, 111882
- 2020. Materials Science and Engineering C, 110931
- 2020 Talanta 211, 120657
- 2020. Sensors 20, 965. Feature article.
- 2022 Biosens Bioelectron 198, 113826

exosomes



phages



ODN

- 2013. Anal Chem 85:3079-86

- 2014. Appl Microbiol Biotechnol 98:1795-1805

AAAAA
dTTTTT



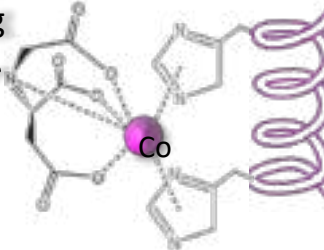
- 2018. Biosens Bioelectron 117, 183-90.

proteins



- 2011. Biosens Bioelectron 27:46-52
- 2013. Biosens Bioelectron 48:203-9
- 2018. Environmental Pollution 242, 863-871.

His-Tag



US15/118,516



CA 2939476

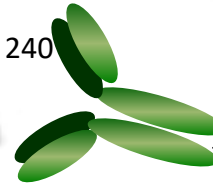


EP15749357.8

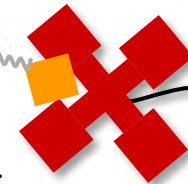
Strep(avidin)



- 2007. Biosens Bioelectron 22:2010-17
- 2008. Biosens Bioelectron 23,1805-11
- 2015. Biosens Bioelectron 74:652-9
- 2017. Biosensors & Bioelectronics 93, 65-71.
- 2017. Biosensors & Bioelectronics 88, 265-272

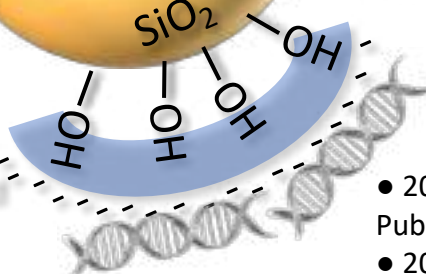


antibodies



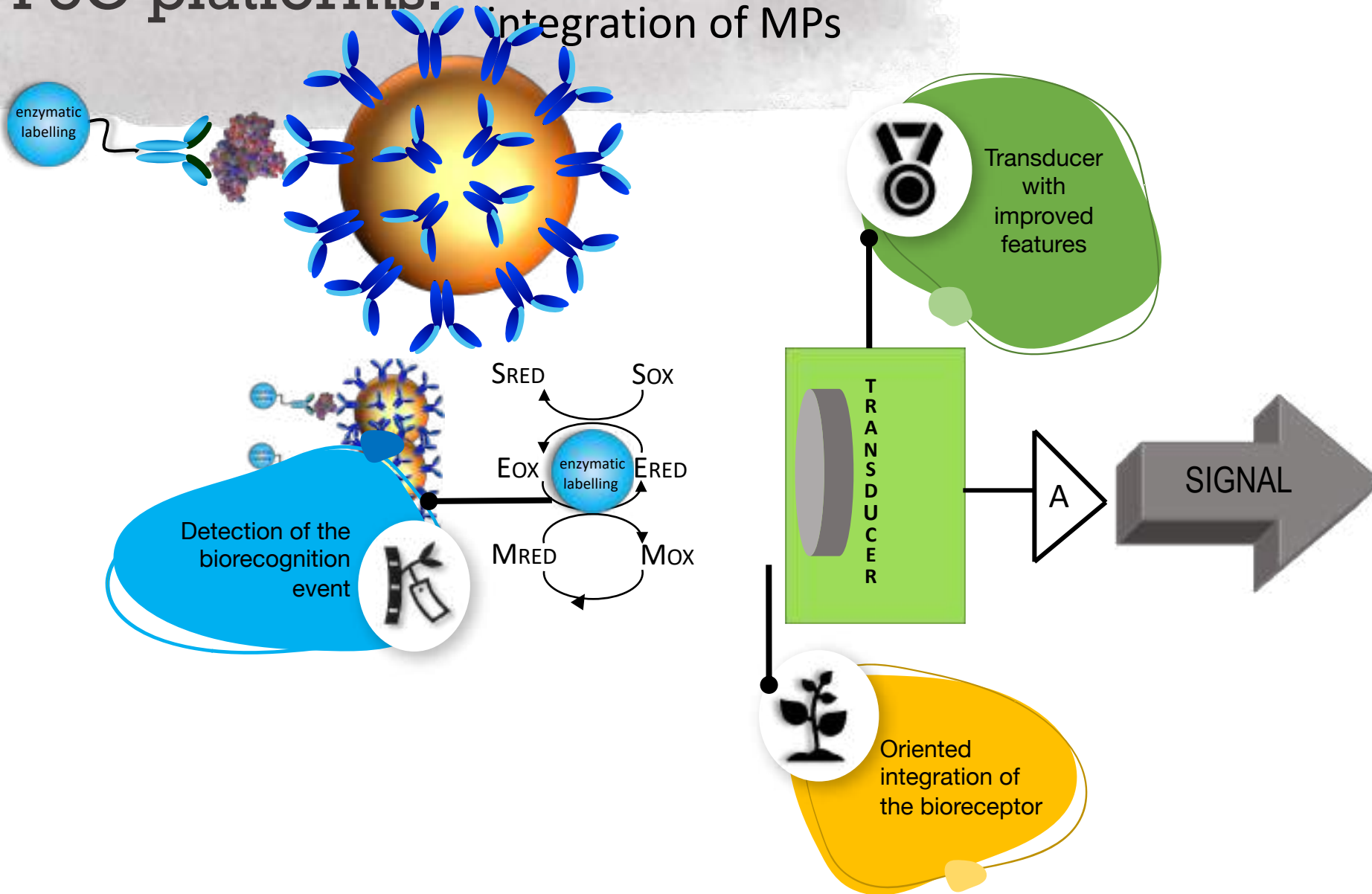
- 2018. Biosensors & Bioelectronics 107, 203-10
- 2018. Biosensors & Bioelectronics 118, 181-7
- 2018. Talanta 181, 19-23
- 2019. Talanta 194, 997-1004.

Molecularly-imprinted
polymers



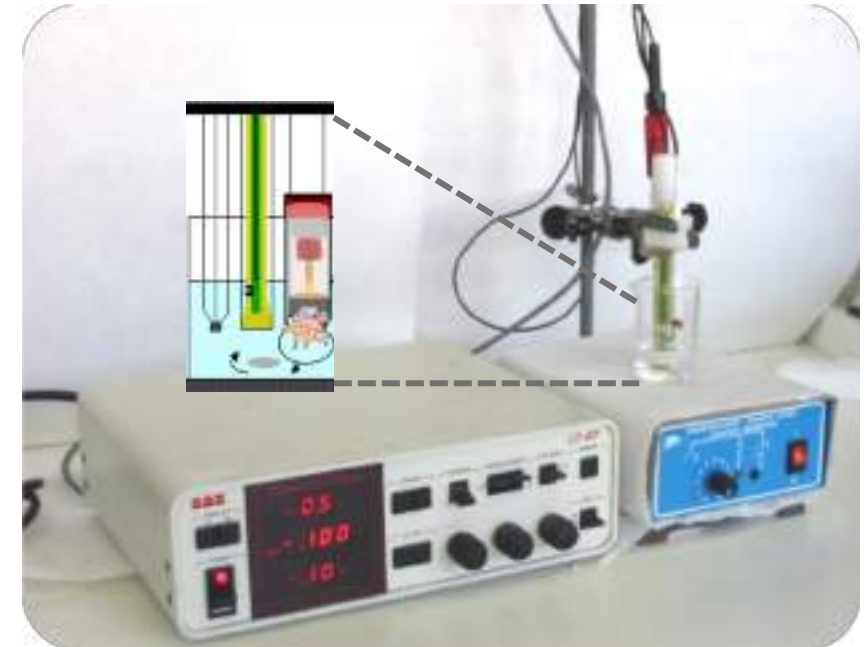
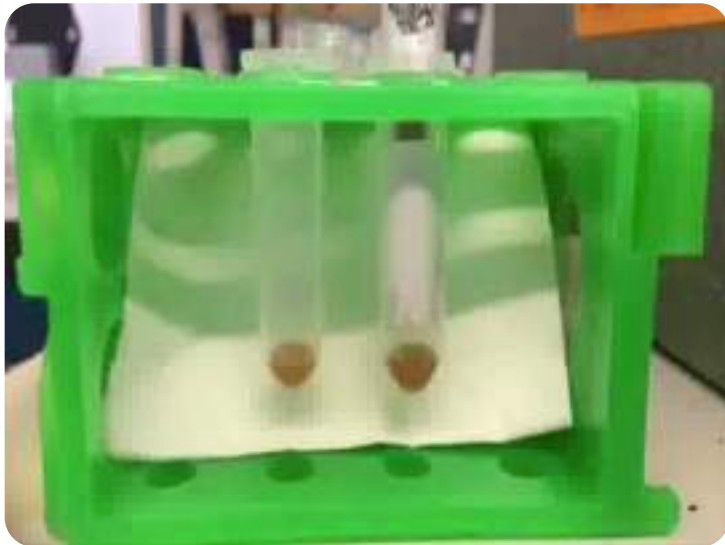
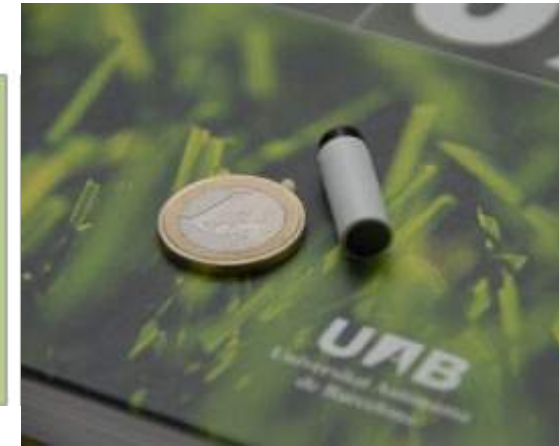
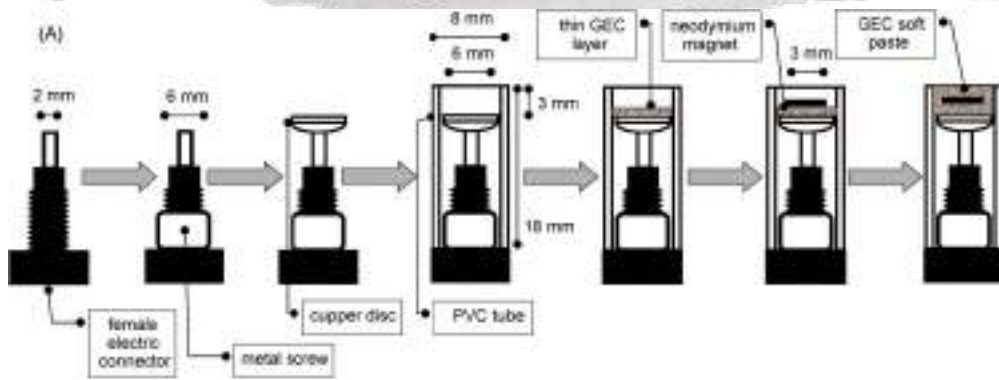
- 2016. Anal Chim Acta 904:1-9. Selected as a "featured article". Published on the front hard cover of the issue.
- 2015. Biosensors and Bioelectronics 74, 652-659.

PoC platforms. m-GEC: magneto-actuated transducer with the integration of MPs



Lab prototype 1

m-GEC: magneto-actuated transducer with the integration of MPs. 2005.

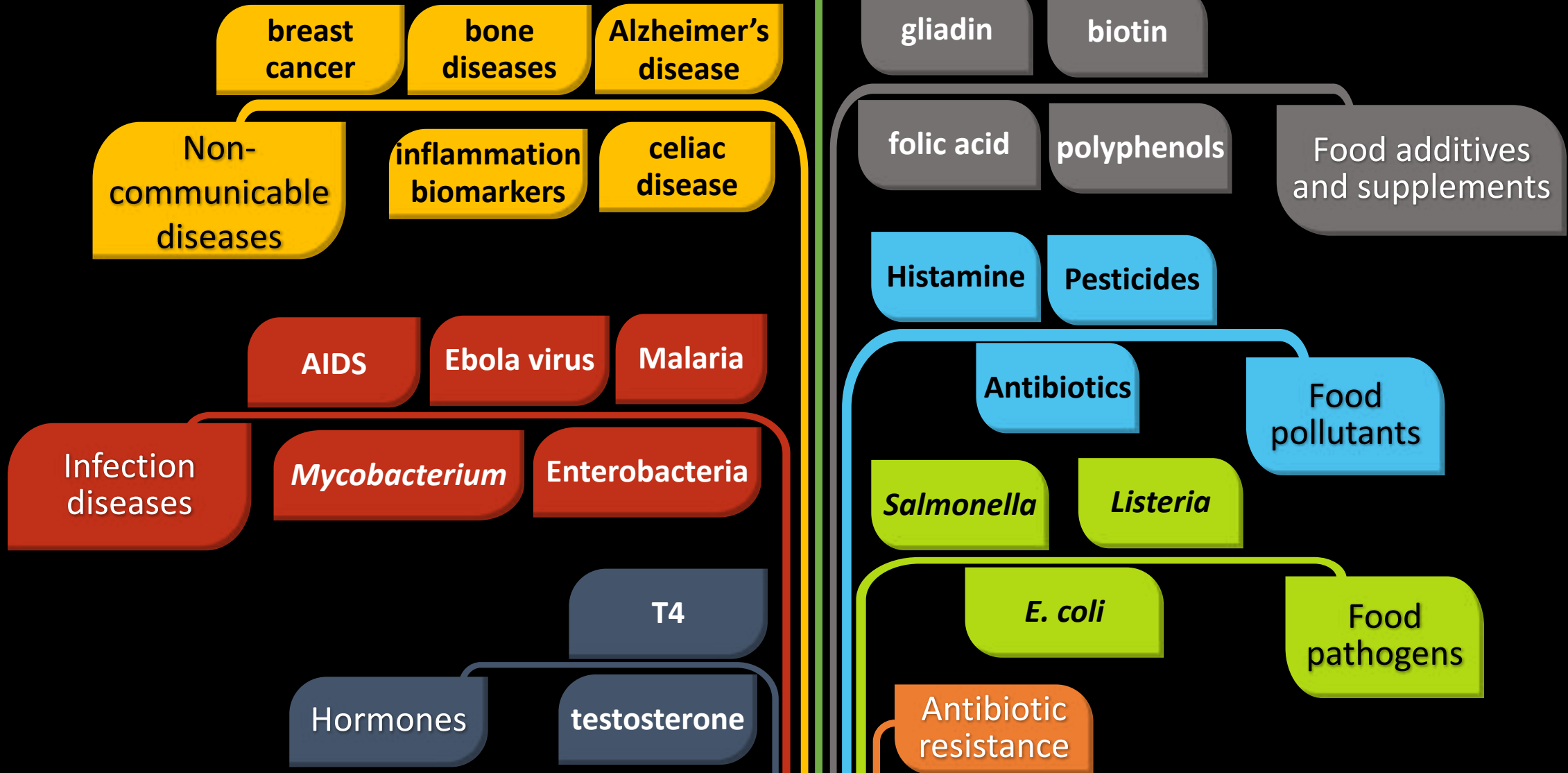


Lab prototype 2

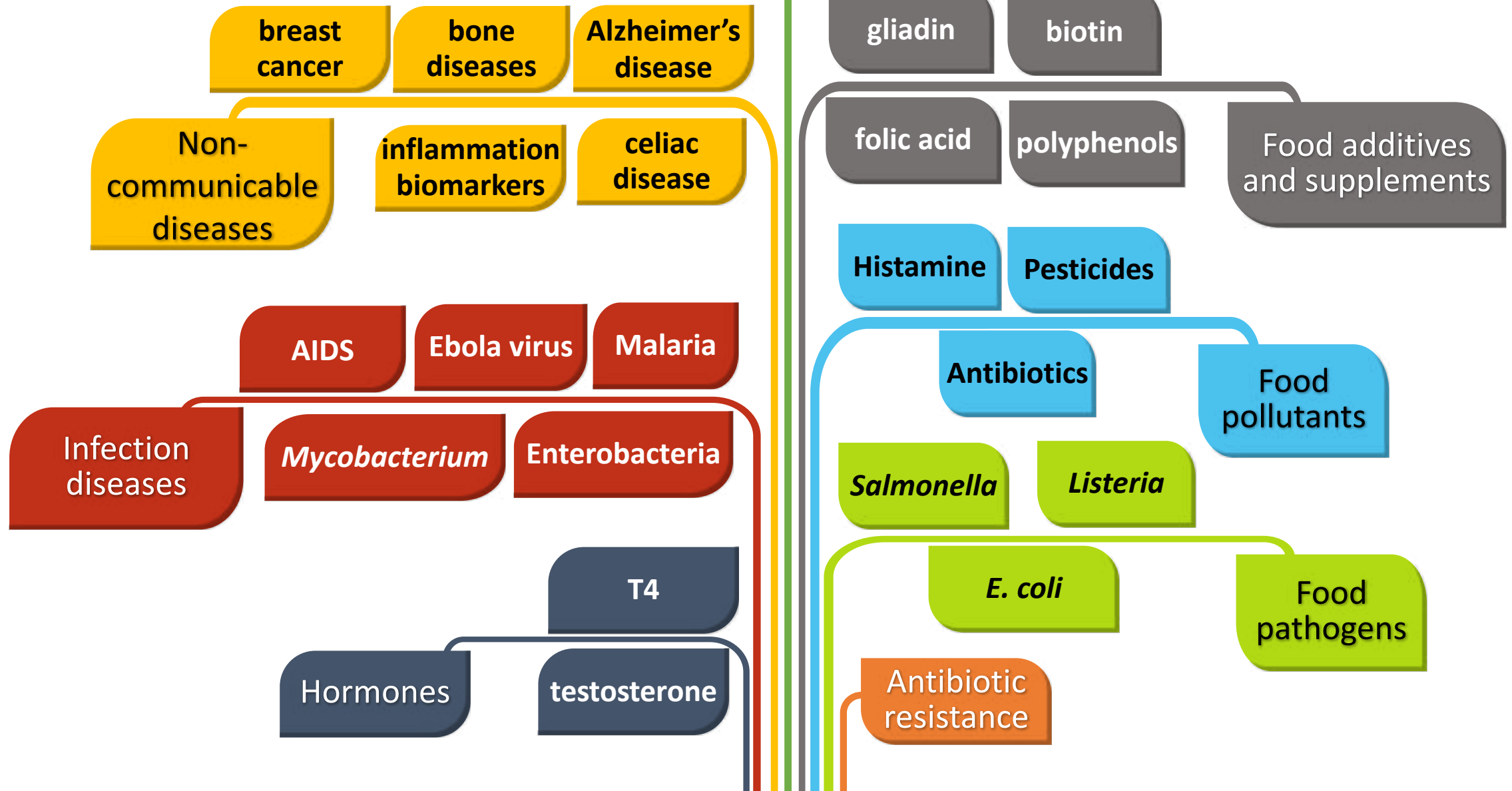
m-GEC: screen-printed, disposable, portable, magneto-actuated transducer with the integration of MPs.



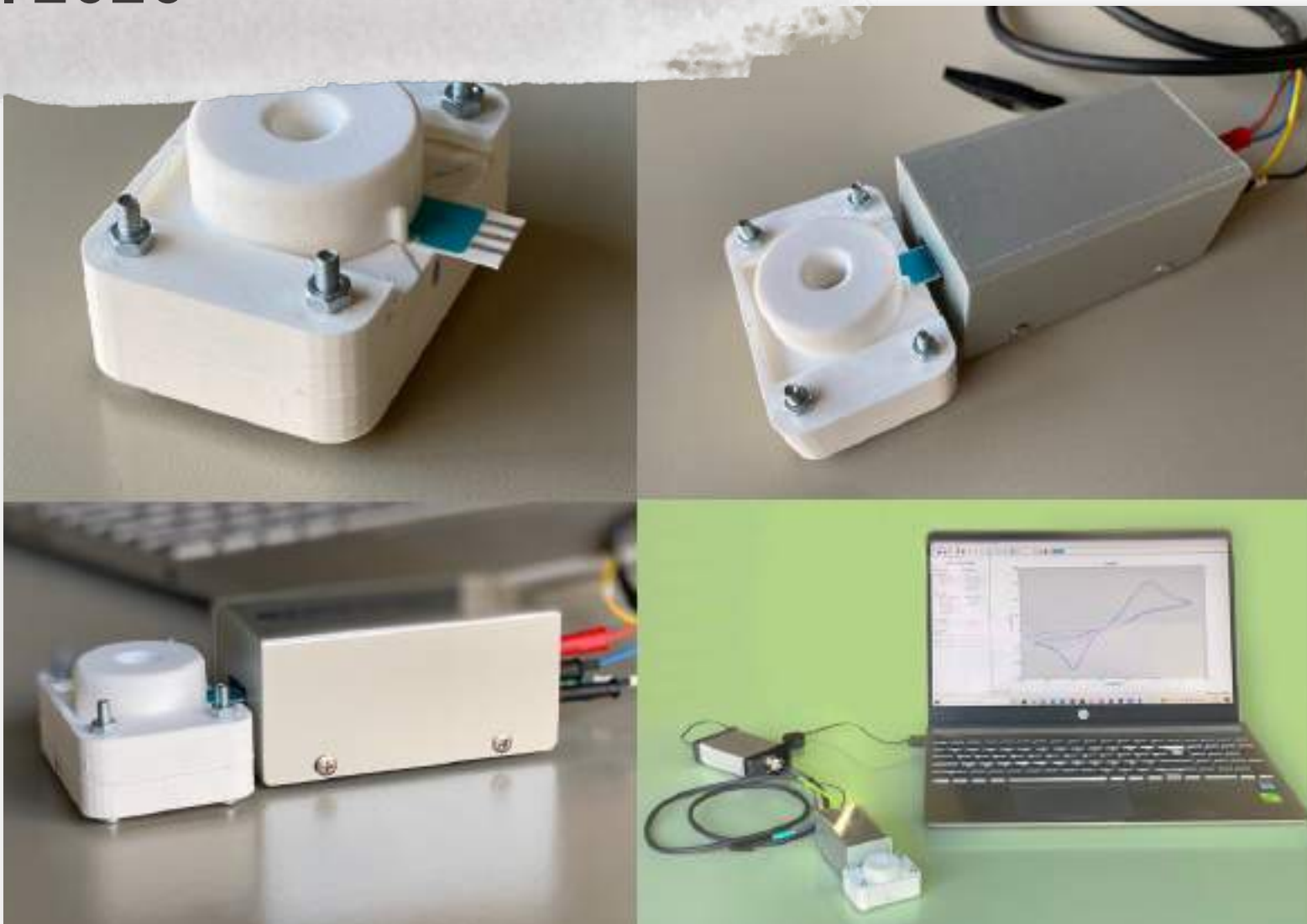
Aplicaciones

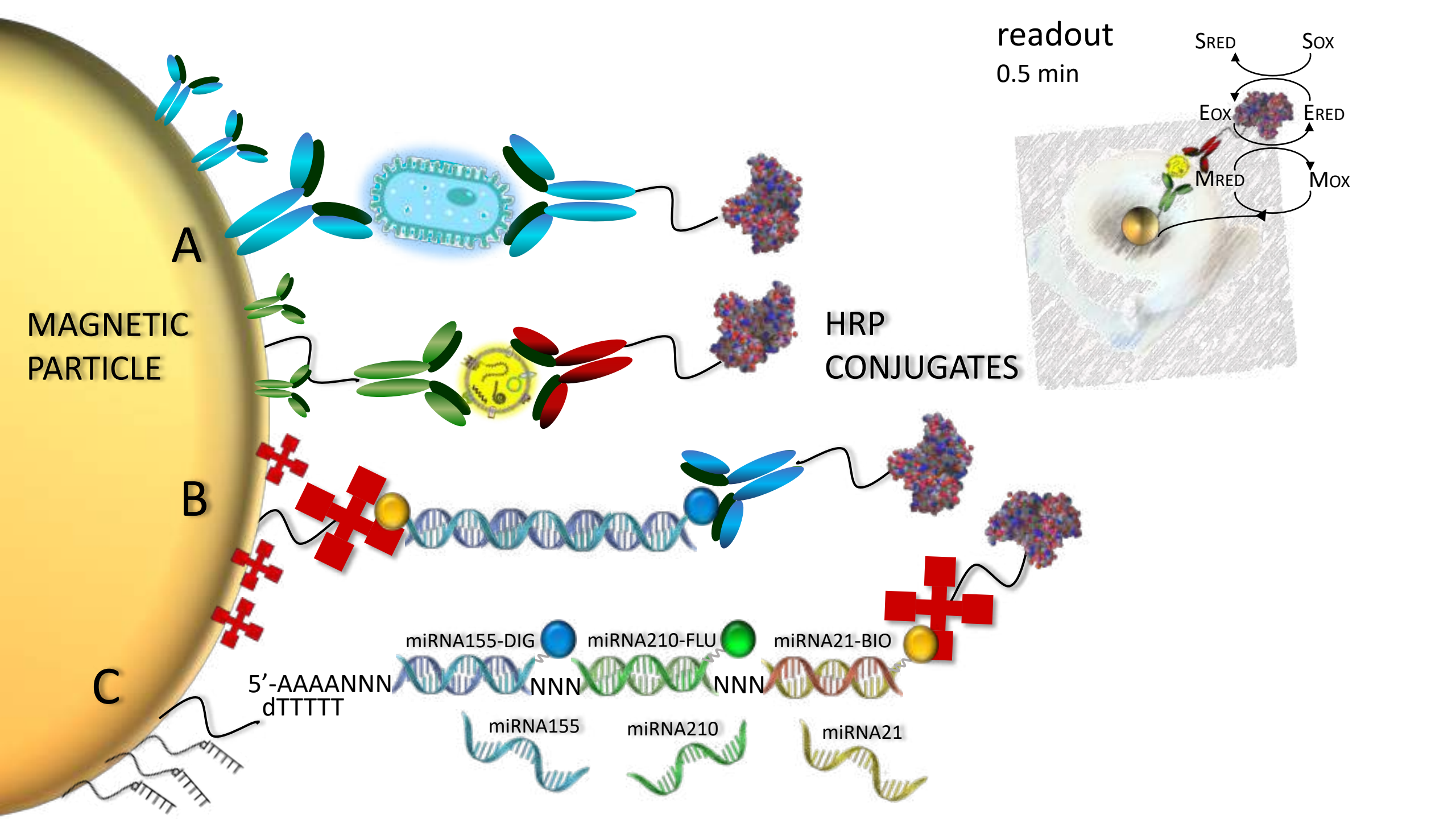


Aplicaciones



MVP. 2020





MAGNETIC PARTICLE

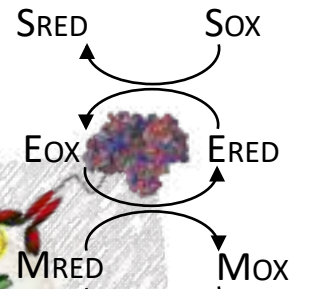
A

B

C

HRP CONJUGATES

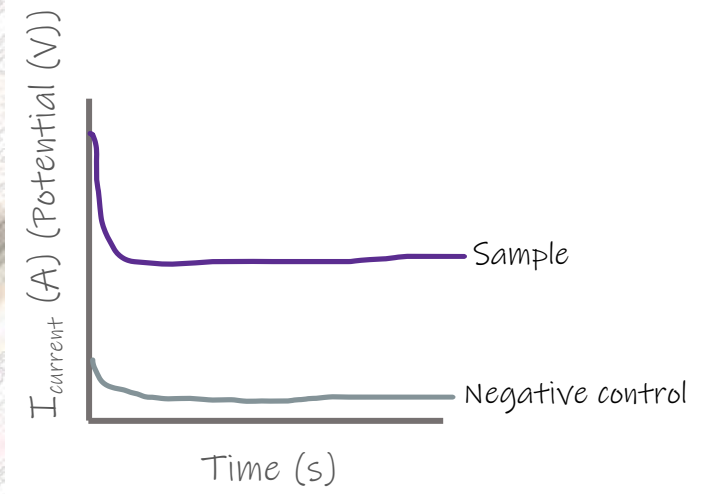
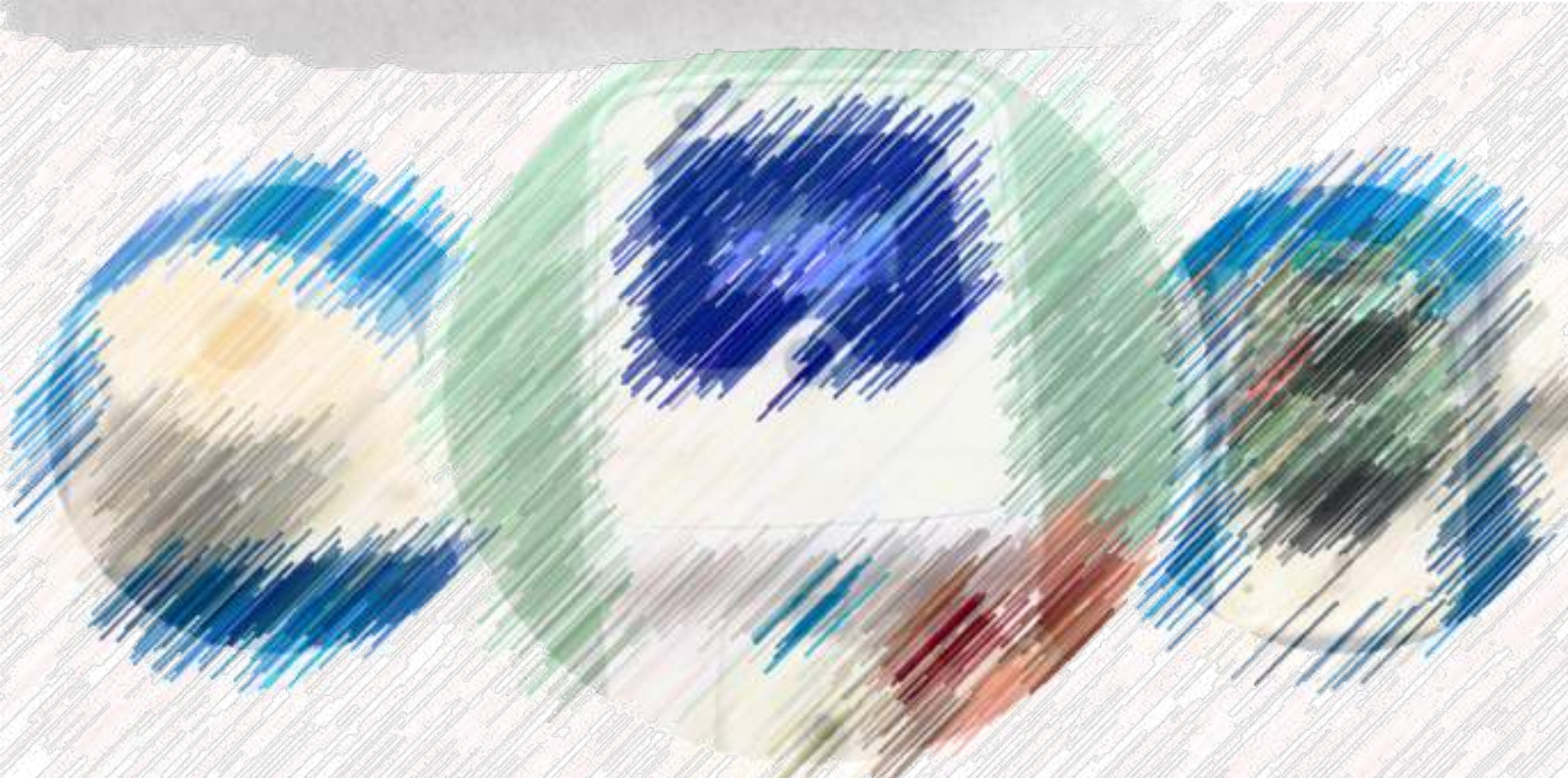
readout
0.5 min



5'-AAAANNN
dTTTTT

miRNA155-DIG miRNA210-FLU miRNA21-BIO
NNN NNN NNN
miRNA155 miRNA210 miRNA21

MVP. 2021



Legionella

in tap water samples



Legionella pneumophila

Major responsible of infections in humans
Legionnaire's disease and Pontiac fever



Inhalation of contaminated aerosols
Waterborne pathogen



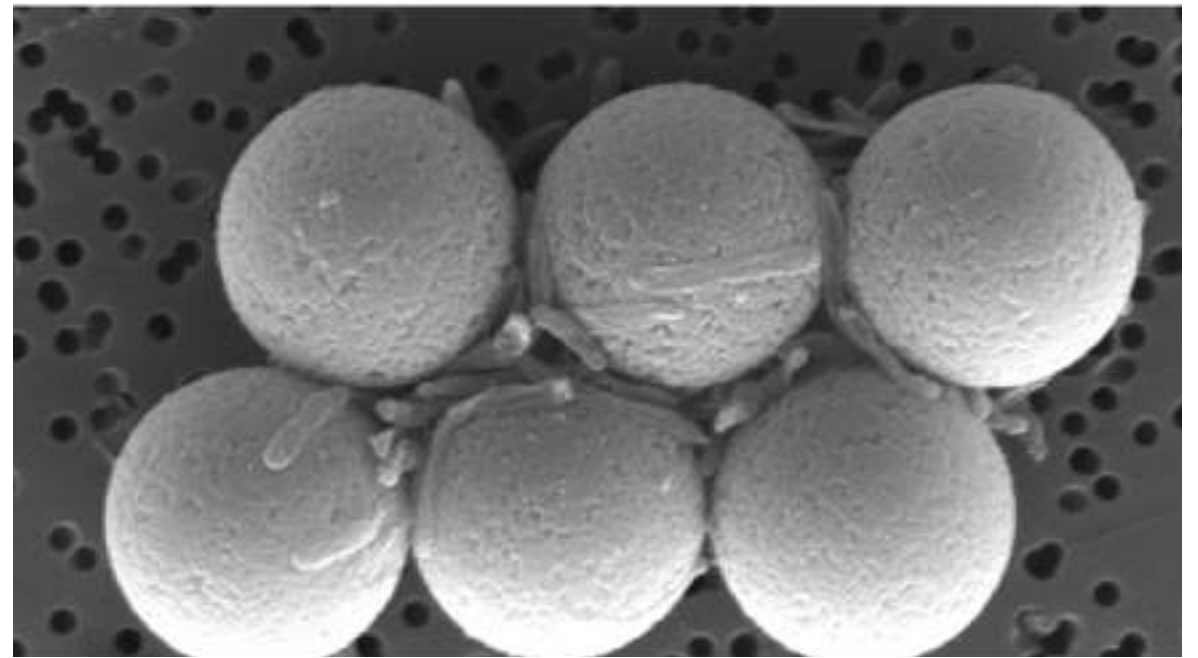
Cooling towers, industrial cooling,
evaporative condensers, hospitals



Non-harmonization of detection
protocols and thresholds (LOD)



- Collection of high-volumes of samples
- Preenrichment steps/culturing procedures
- Time-consuming procedures



Legionella

in tap water samples



Legionella pneumophila

Major responsible of infections in humans
Legionnaire's disease and Pontiac fever



Inhalation of contaminated aerosols
Waterborne pathogen



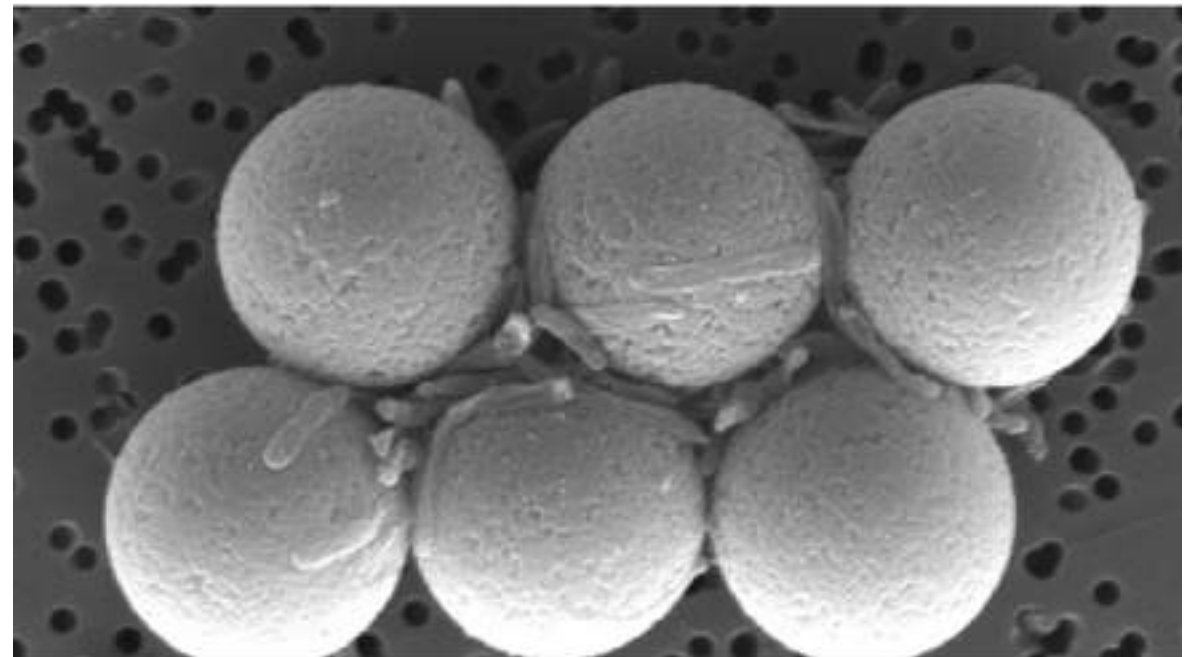
Cooling towers, industrial cooling,
evaporative condensers, hospitals



Non-harmonization of detection
protocols and thresholds (LOD)



- Collection of high-volumes of samples
- Preenrichment steps/culturing procedures
- Time-consuming procedures



European Technical Guidelines for the
Prevention, Control, and Investigation of
Infections caused by *Legionella*

100 CFU L⁻¹

General agreement: corrective actions

1 x 10⁴ CFU L⁻¹

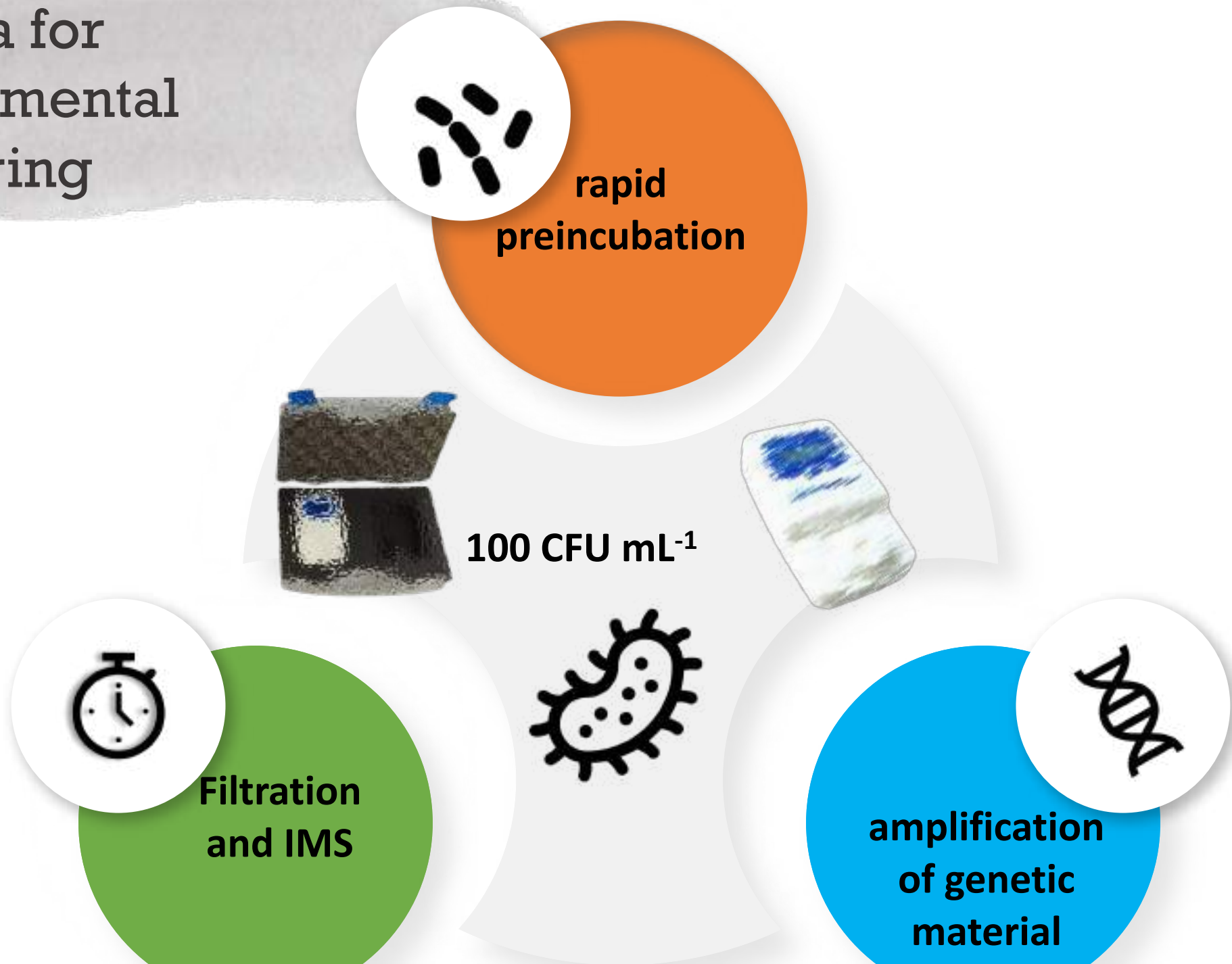
Minor interventions
(spa pools and cooling towers)

> 100 CFU L⁻¹

Corrective actions (cooling towers)

> 10⁵-10⁶ CFU L⁻¹

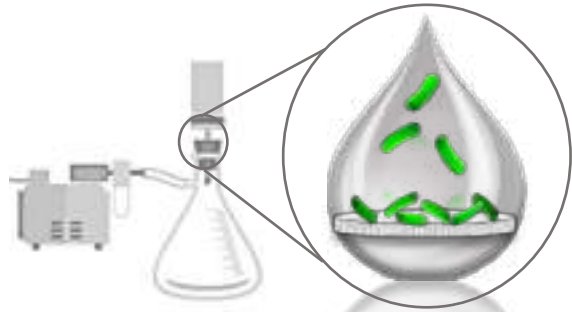
Bacteria for environmental monitoring



Legionella

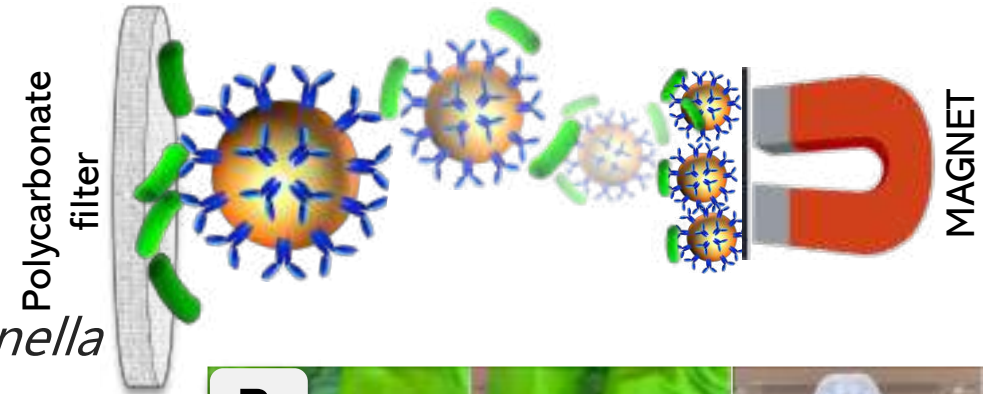
A

Filtration of high volumes at low concentration samples to retain the bacteria on the filters (1-10 min)



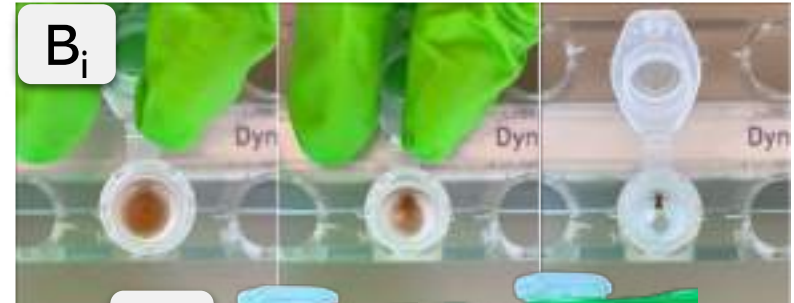
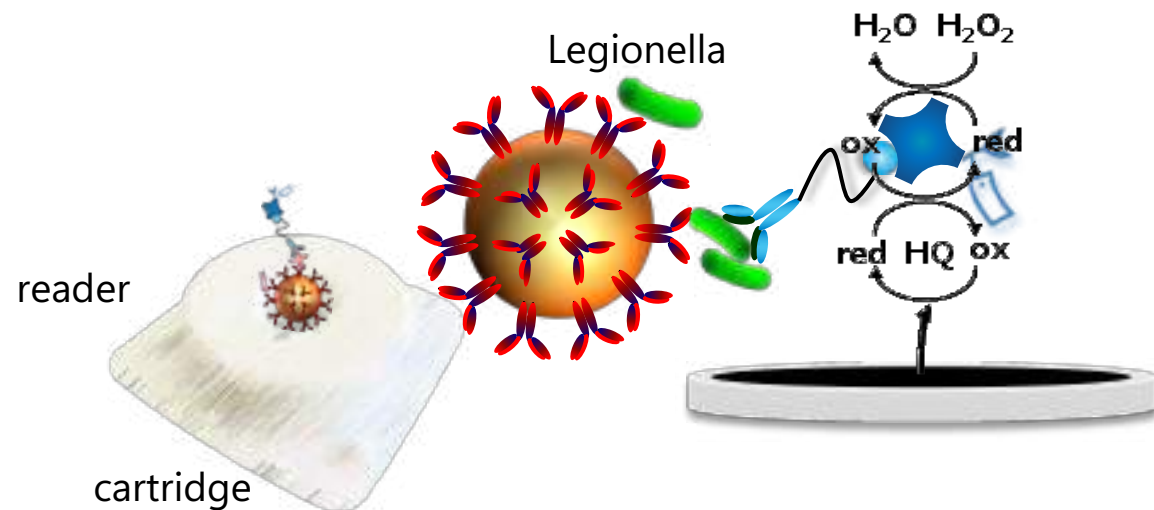
B

Direct immunomagnetic separation of the bacteria retained in the filter and magnetic actuation



C

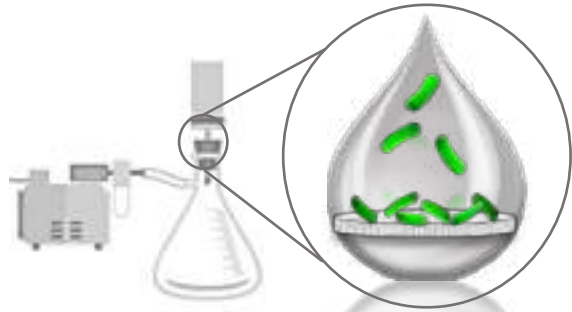
Incubation with HRP-antibody against *Legionella* and electrochemical in a handheld reader operated by batteries



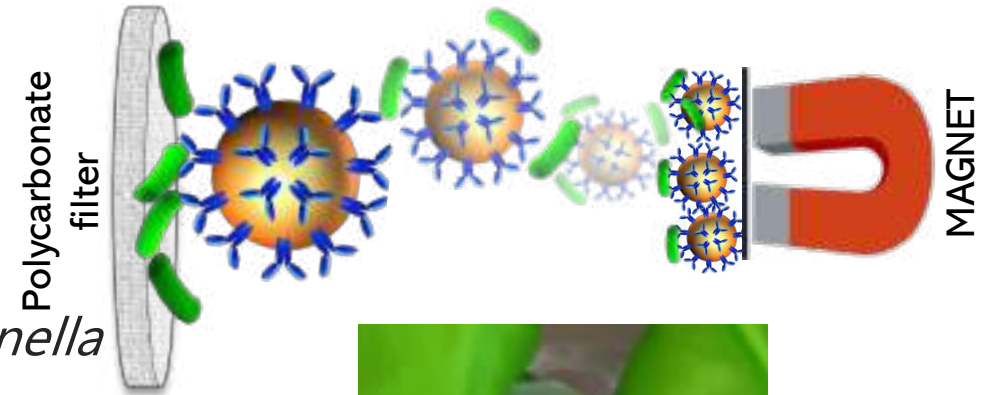
Legionella

in tap water samples

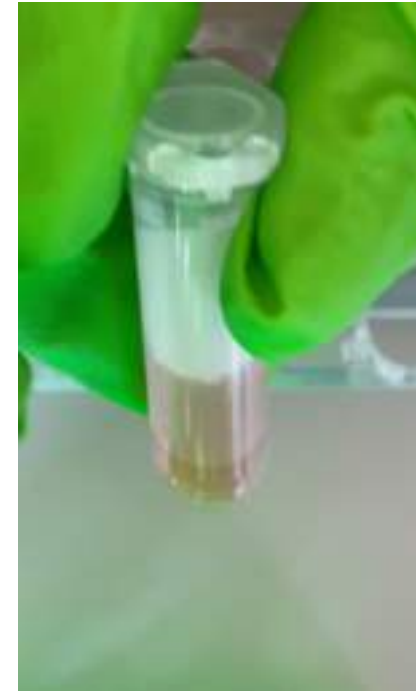
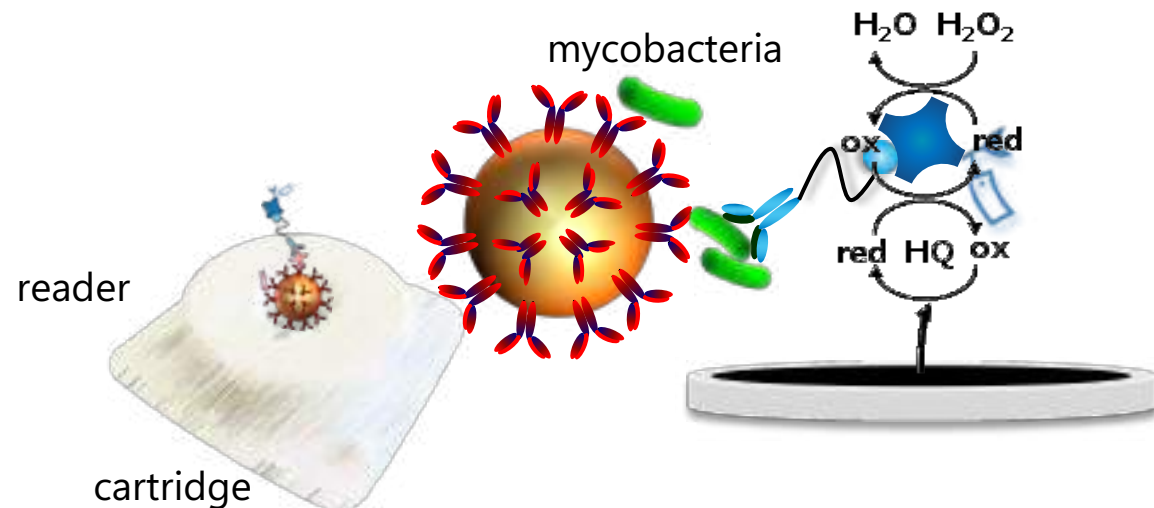
A Filtration of high volumes at low concentration samples to retain the bacteria on the filters (1-10 min)



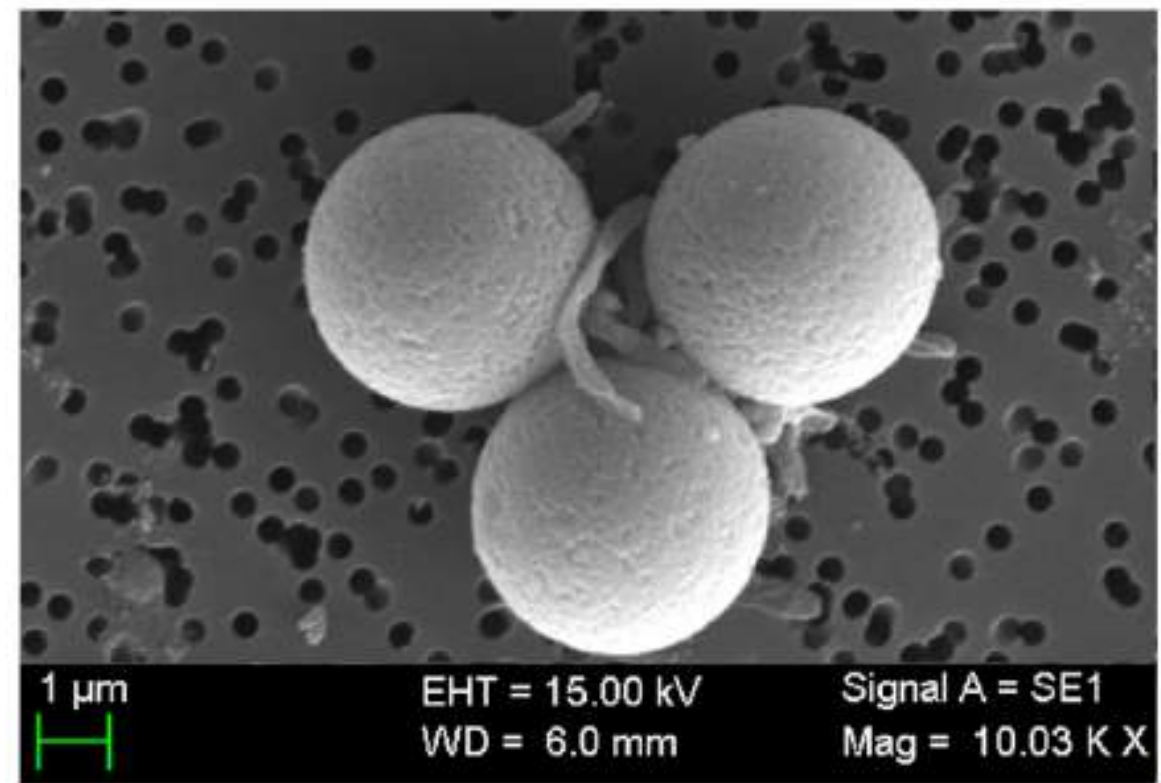
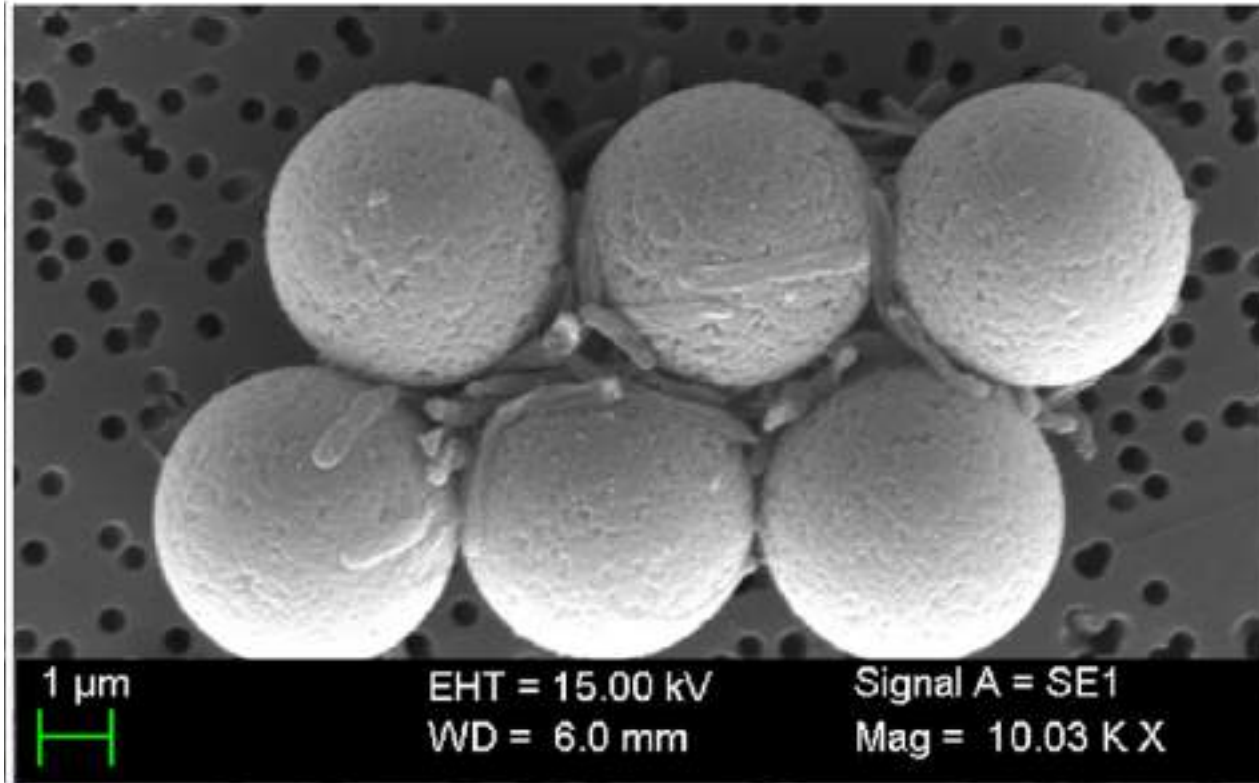
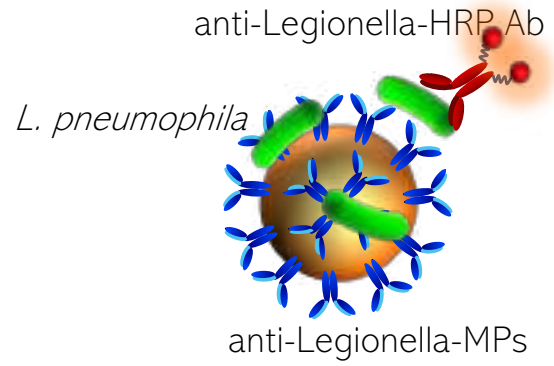
B Direct immunomagnetic separation of the bacteria retained in the filter and magnetic actuation



C Incubation with HRP-antibody against *Legionella* and electrochemical in a handheld reader operated by batteries

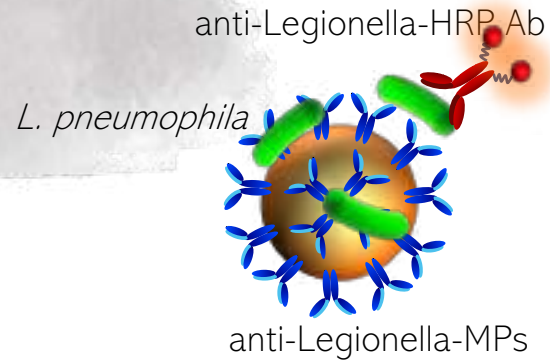


Legionella in tap water samples



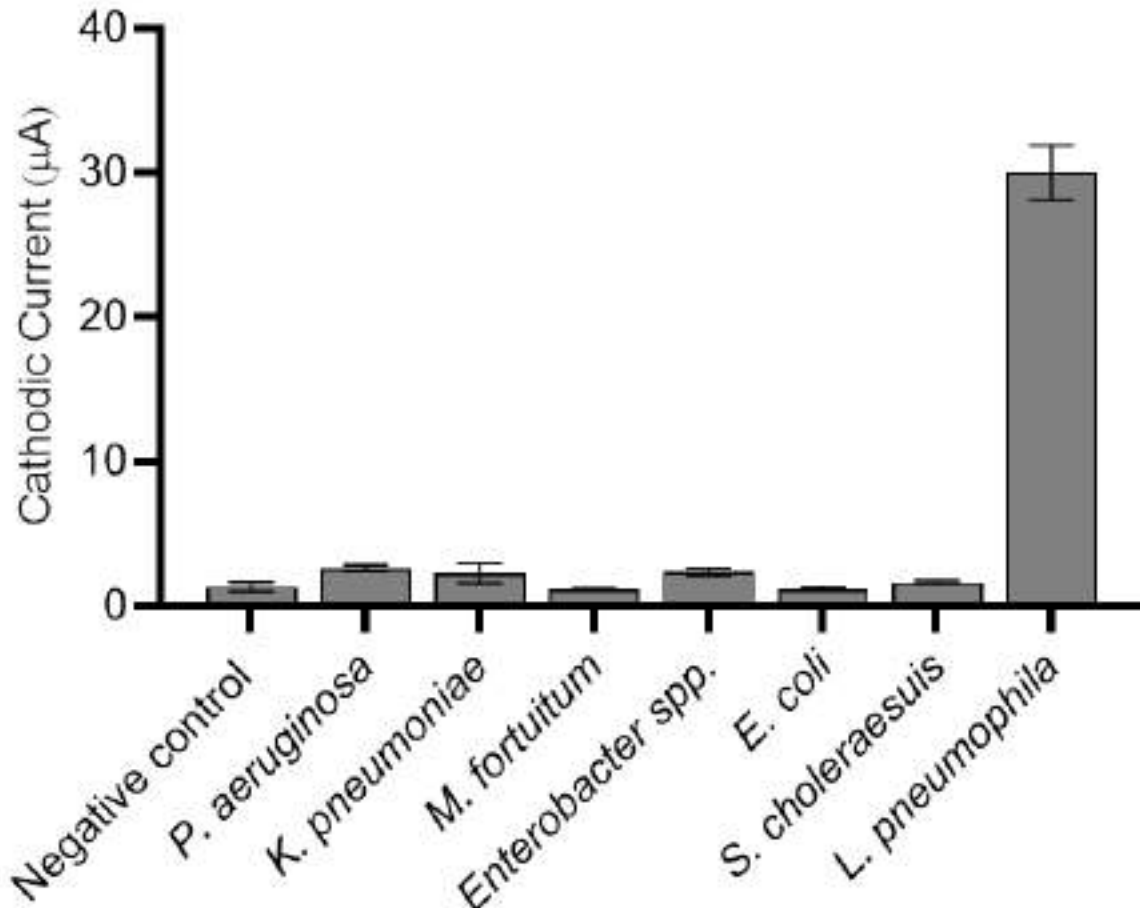
Legionella

in tap water samples



Specificity study

Immunomagnetic separation of different bacterial strains by the modified anti-Legionella-MPs and detection with the anti-legionella-HRP Ab.



The error bars show the standard deviation for n=3.

- Negative control, 0 CFU mL⁻¹
- Pseudomonas aeruginosa*, 2 x 10⁸ CFU mL⁻¹
- Klebsiella pneumoniae*, 2 x 10⁸ CFU mL⁻¹
- Mycobacterium fortuitum*, 2 x 10⁵ CFU mL⁻¹
- Enterobacter spp.*, 2 x 10⁸ CFU mL⁻¹
- Escherichia coli*, 2 x 10⁸ CFU mL⁻¹
- Salmonella choleraesuis*, 3 x 10⁸ CFU mL⁻¹
- L. pneumophila* positive control, 8 x 10⁴ CFU mL⁻¹

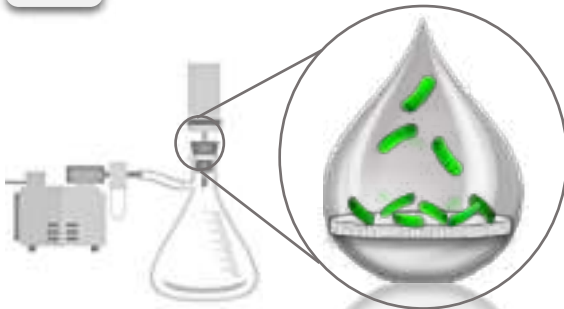
Legionella in tap water samples

Study of the filtering material

Optimization of the novel combined
preconcentration strategy

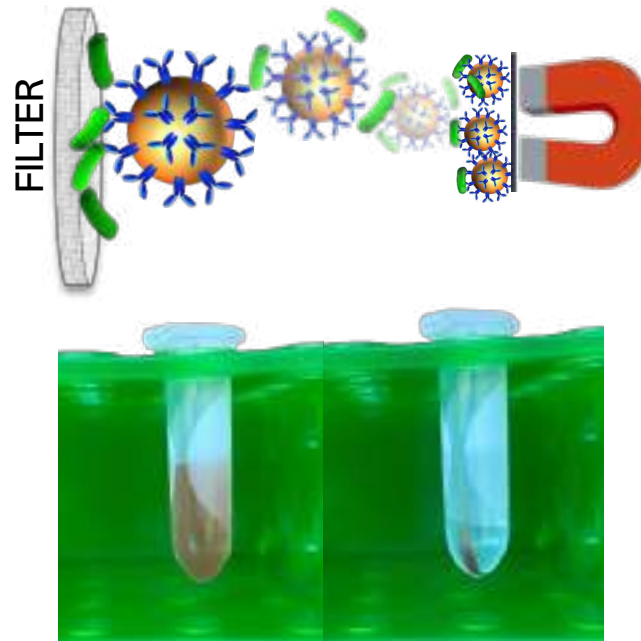
Preconcentration strategy

A Filtration



- Polycarbonate
- Nylon
- Cellulose acetate
- Cellulose nitrate
- Mixed cellulose ester

B Direct IMS of the bacteria retained in the filter and magnetic actuation

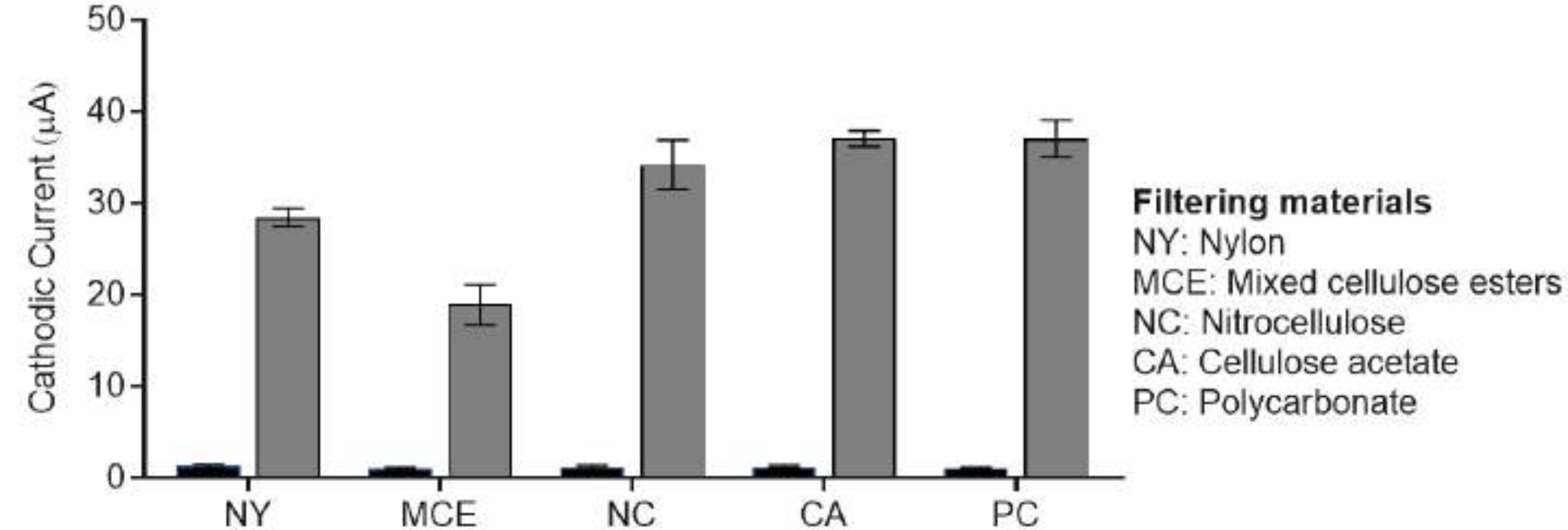


Pore size 0.45 μm
Diameter 25 mm


Legionella in tap water samples

Study of the filtering material

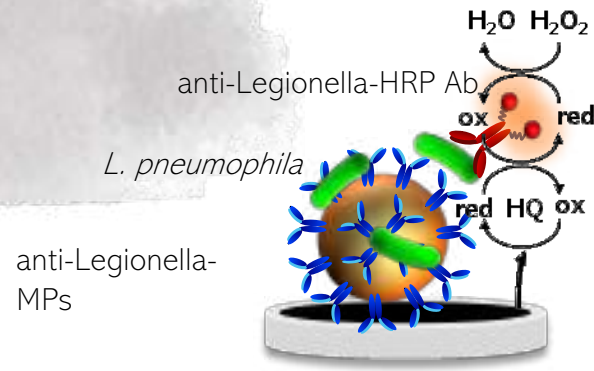
Optimization of the novel combined
preconcentration strategy



The error bars show the standard deviation for n=3.

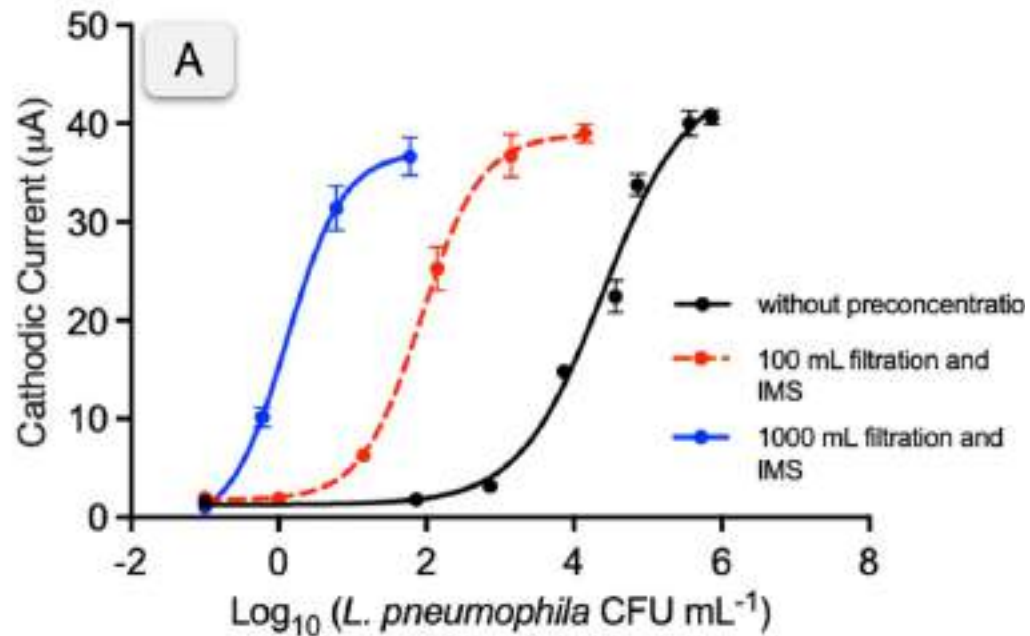
- 
- Recommended material
 - Low protein binding capacity
 - Workflow time

Legionella in tap water samples



Electrochemical magneto immunosensing

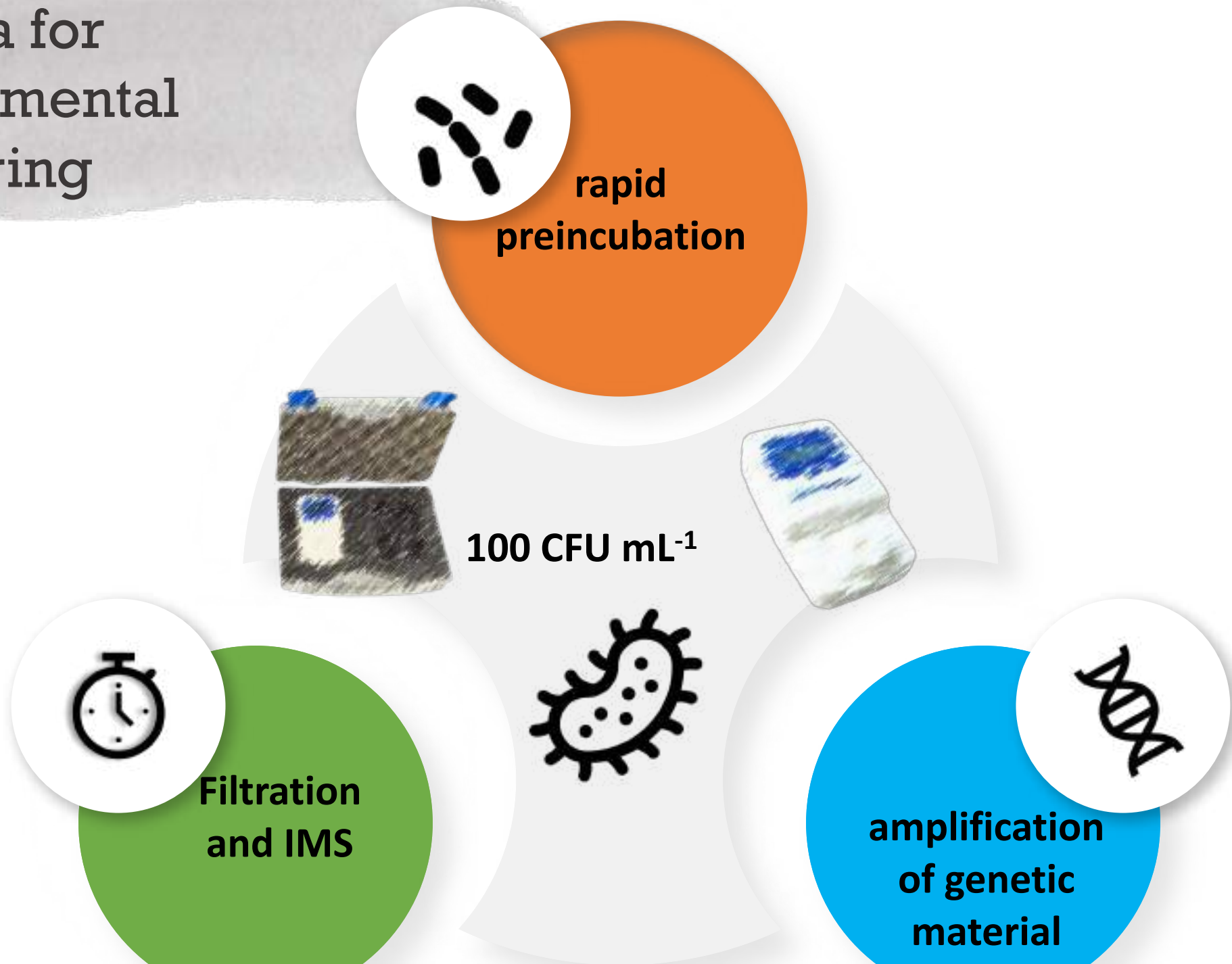
Calibration plots for the detection of *L. pneumophila* with/without the preconcentration strategy



The data was fitted with a nonlinear regression (Sigmoidal 4PL, GraphPad Prism Software v 10.0.1; Black: $R^2= 0.9886$, red: $R^2= 0.9950$, blue: $R^2= 0.9923$). The error bars show the standard deviation for $n=3$.

Sample volume	1000 mL	100 mL	0.1 mL
Preconcentration method	✓	✓	✗
IMS	✓	✓	✓
Filtration	✓	✓	✗
LOD (CFU mL ⁻¹)	0.1	2	100

Bacteria for environmental monitoring



portable thermocycler
operated by batteries



AmpliFAST
Rapid Test
for communicable diseases



Based on double-tagging
end-point PCR

RDT platform



UAB eurecat

ISGlobal

BioEcllosion
ENHANCING RAPID TESTS FOR WIDESPREAD DIAGNOSTICS

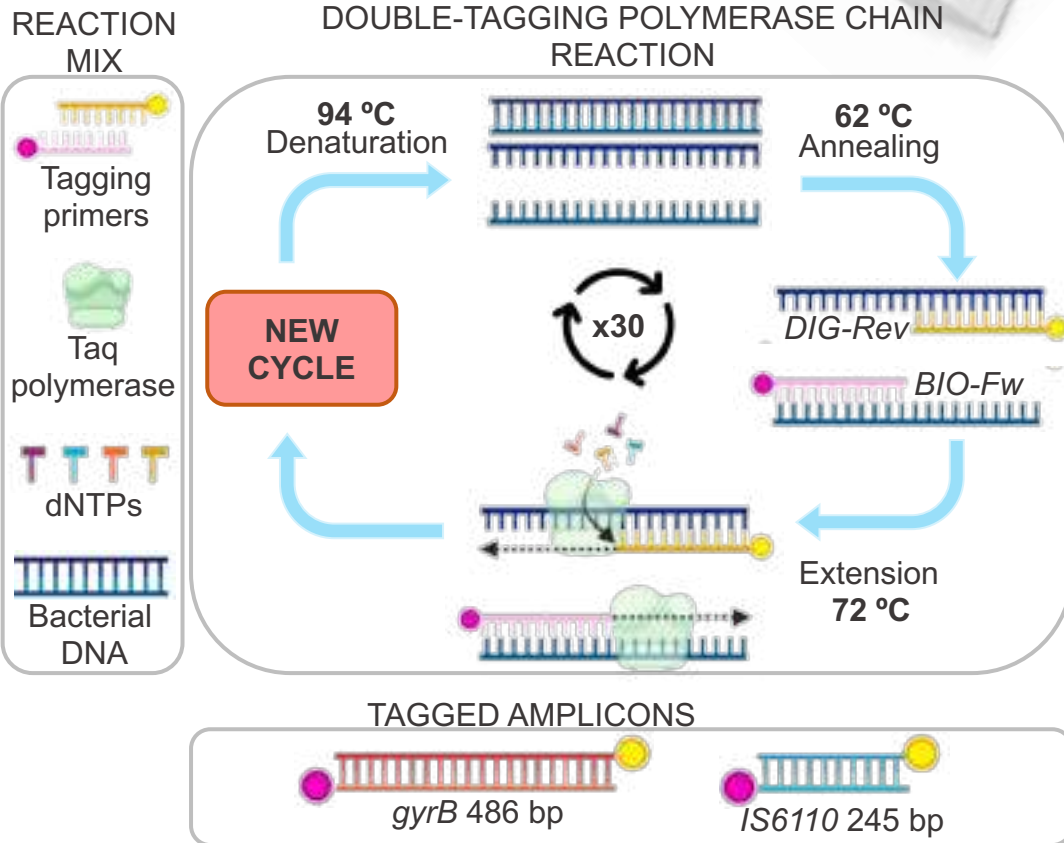
AmpliSens



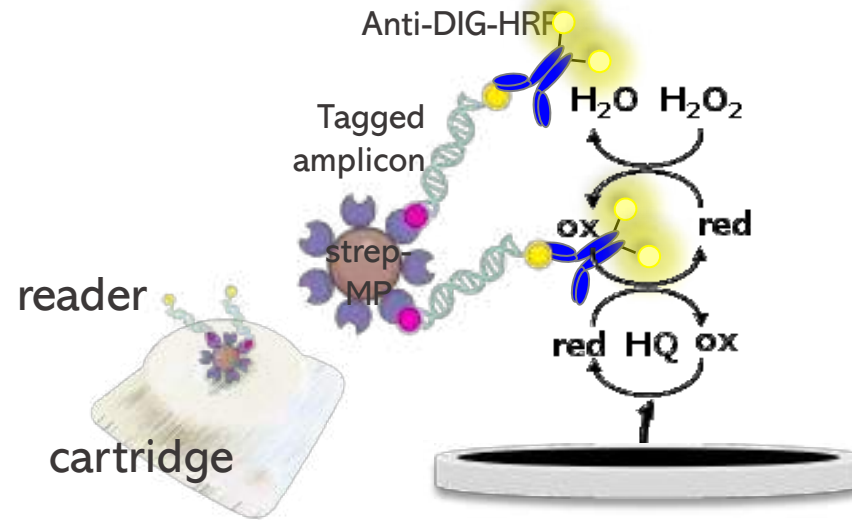
TBC



A Thermal heat and Double-tagging PCR

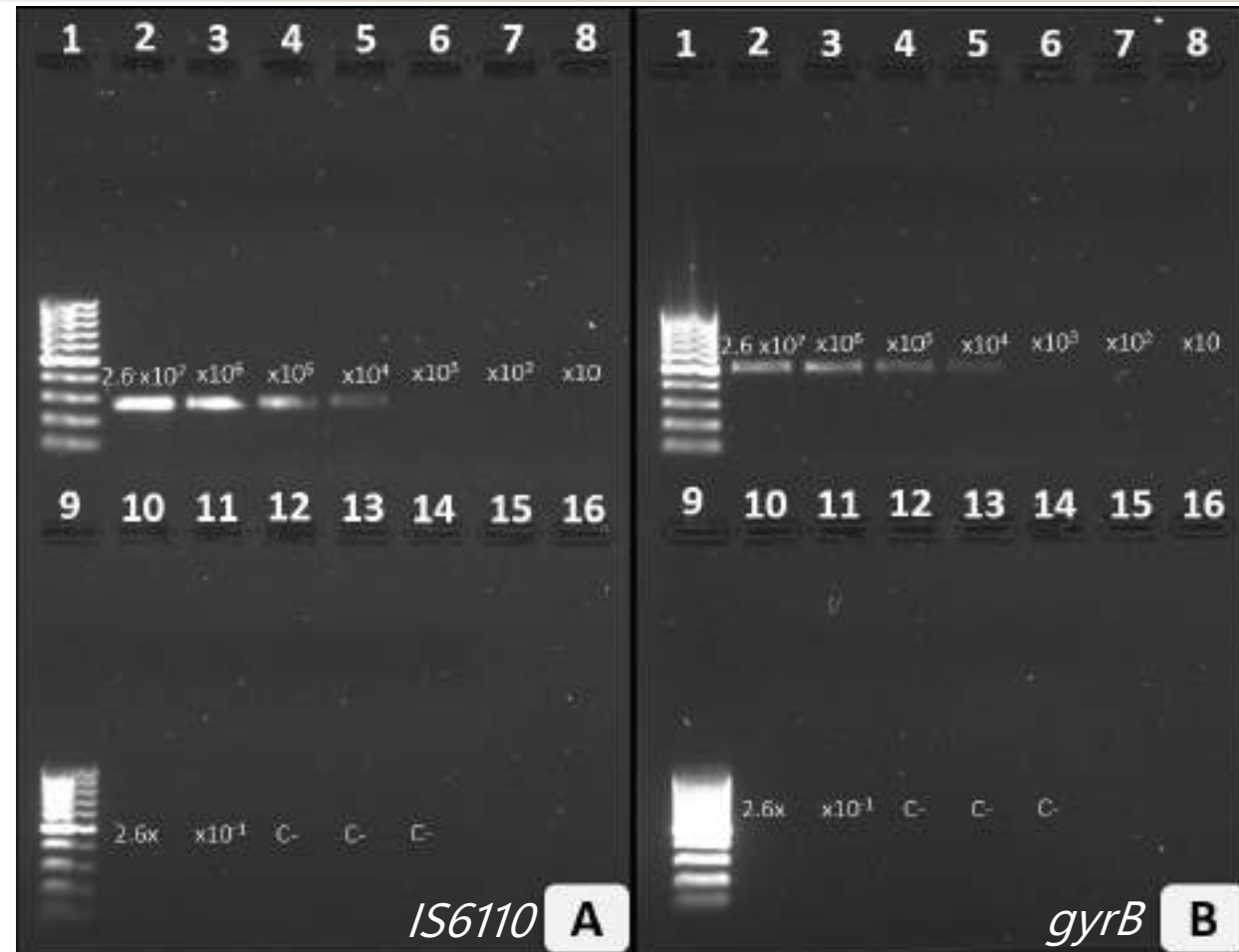
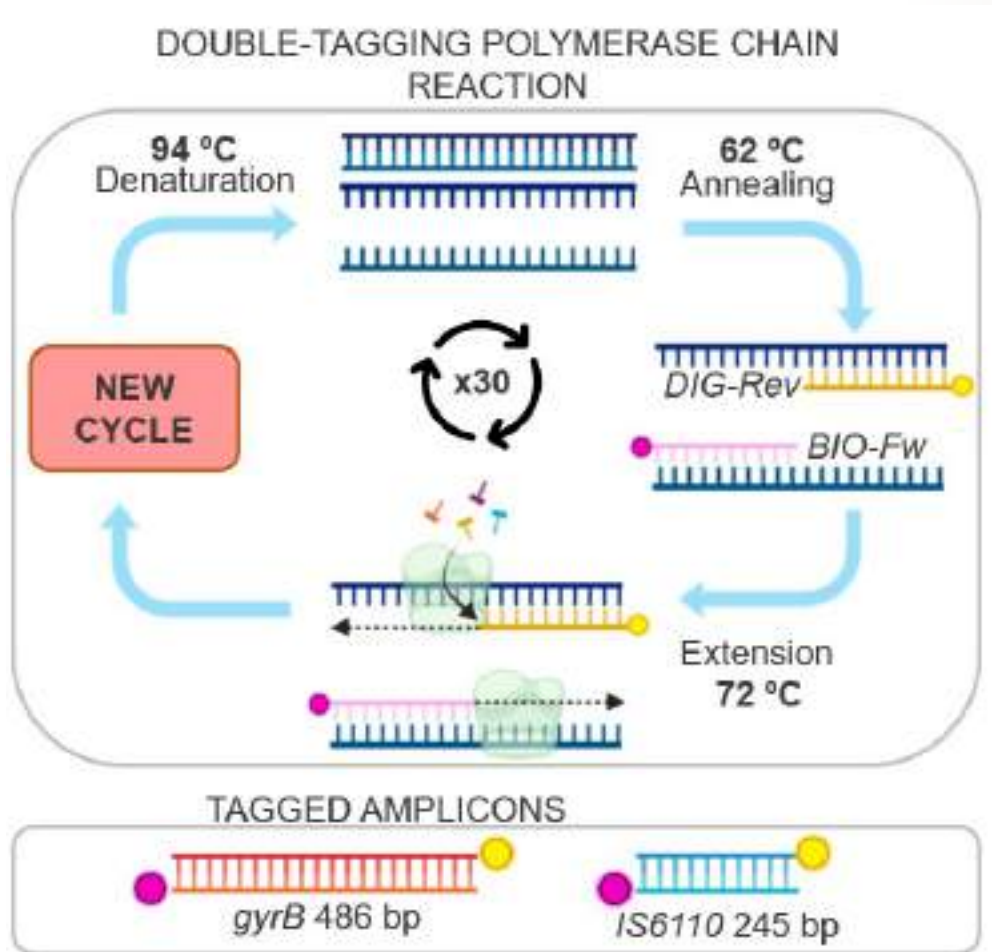


B Electrochemical magneto-genosensing of tagged amplicons

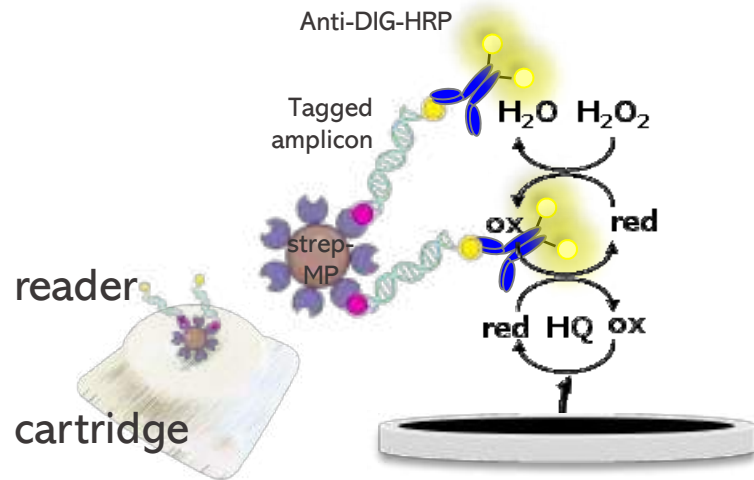


Double-tagging PCR

Primer selection and electrophoretic gels

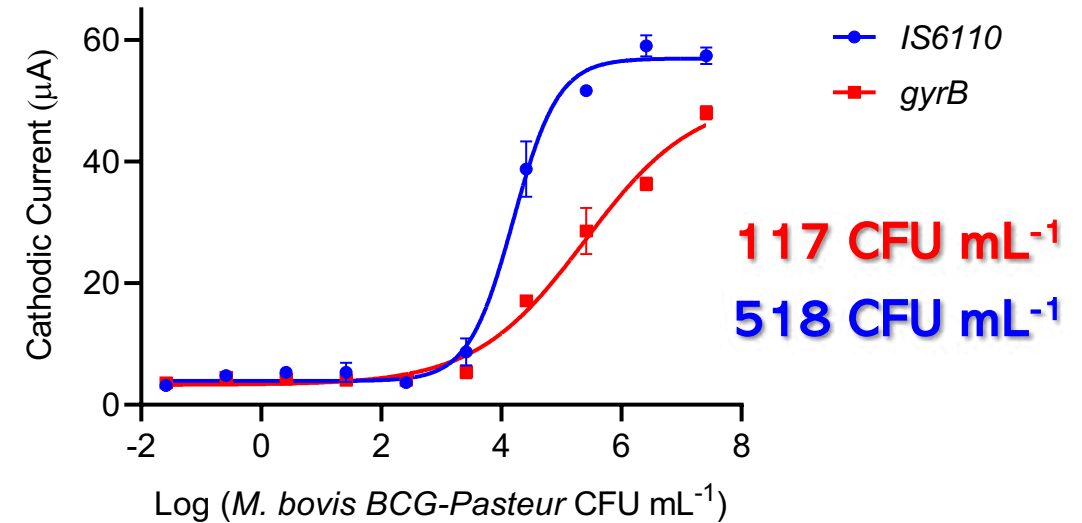


TBC

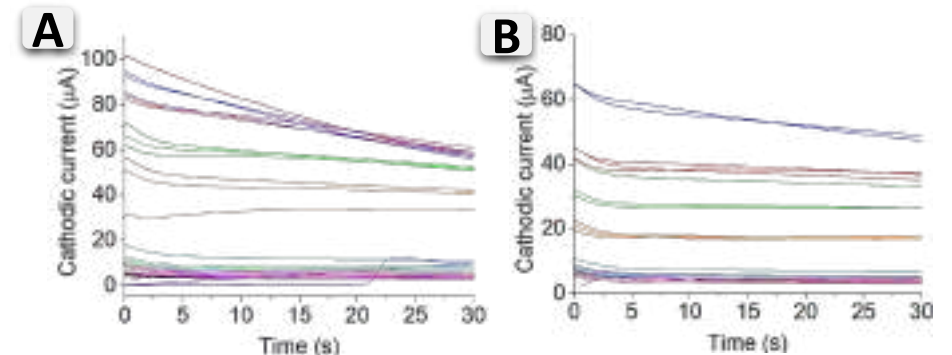


15 min one-step incubation
30 s electrochemical readout

Electrochemical magneto-genosensing

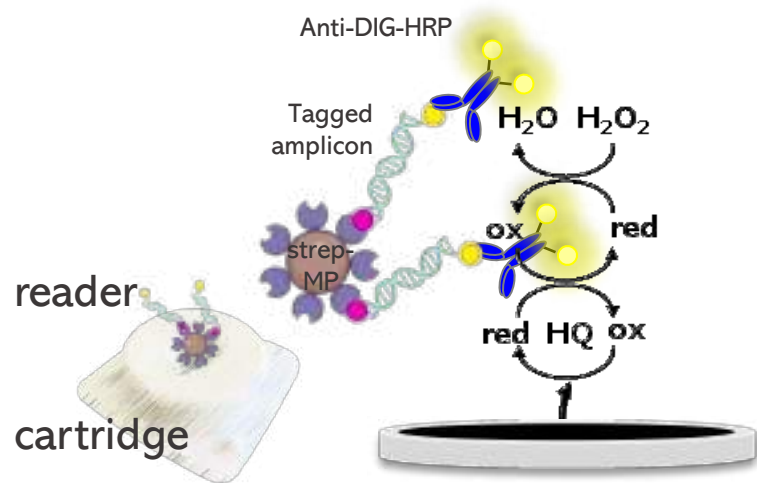


The data were analyzed using a nonlinear regression, Sigmoidal 4-PL, GraphPad Prism Software, v.8.0; Blue: $R^2 = 0.9909$ and red: $R^2 = 0.9815$. The error bars show the standard deviation for $n=3$.



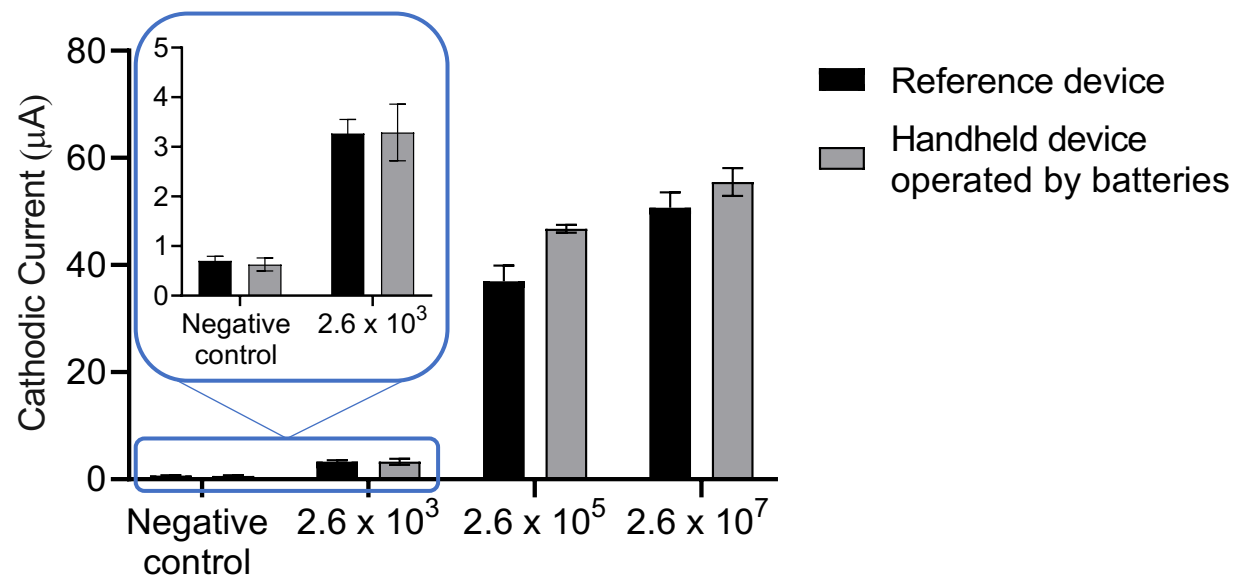
TBC

Electrochemical magnetosensing

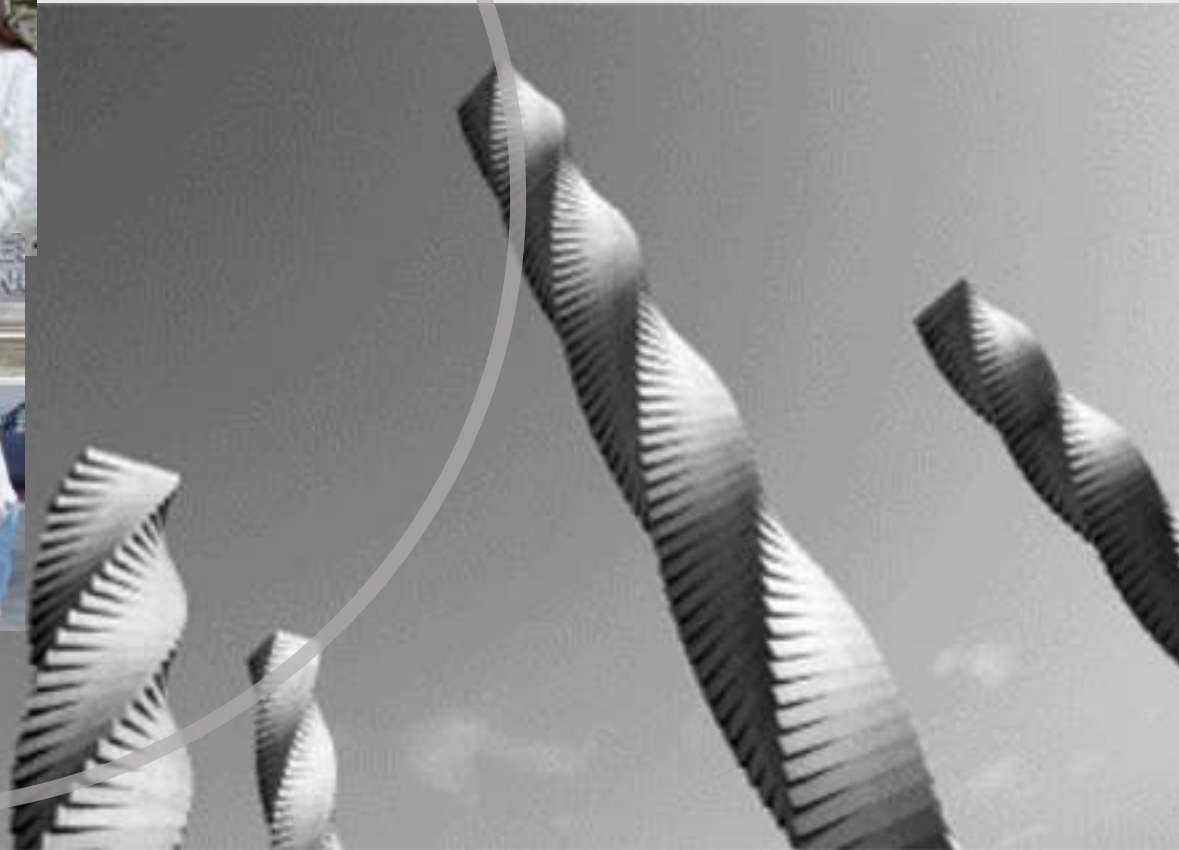
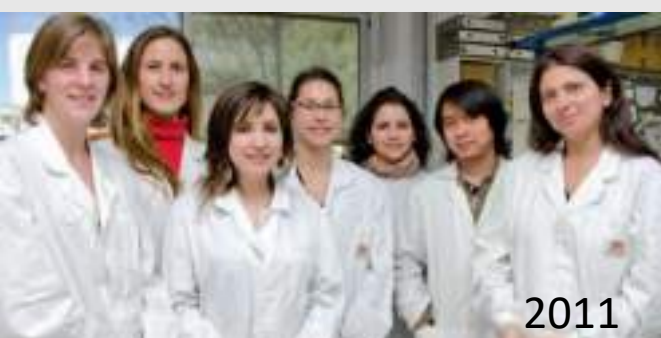


15 min one-step incubation
30 s electrochemical readout

Comparison between devices



The error bars show the standard deviation for n=3.



Biosensing and Bioanalysis Group



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<https://isabelpividori.net/>



SUSNANO SPRING SCHOOL

Electrochemical Nanobiosensors for Environmental Diagnostics

15-16/04/2024



ExoSenS PoC



ExoSenS



ECHILIBRIST



Funded by the European Union