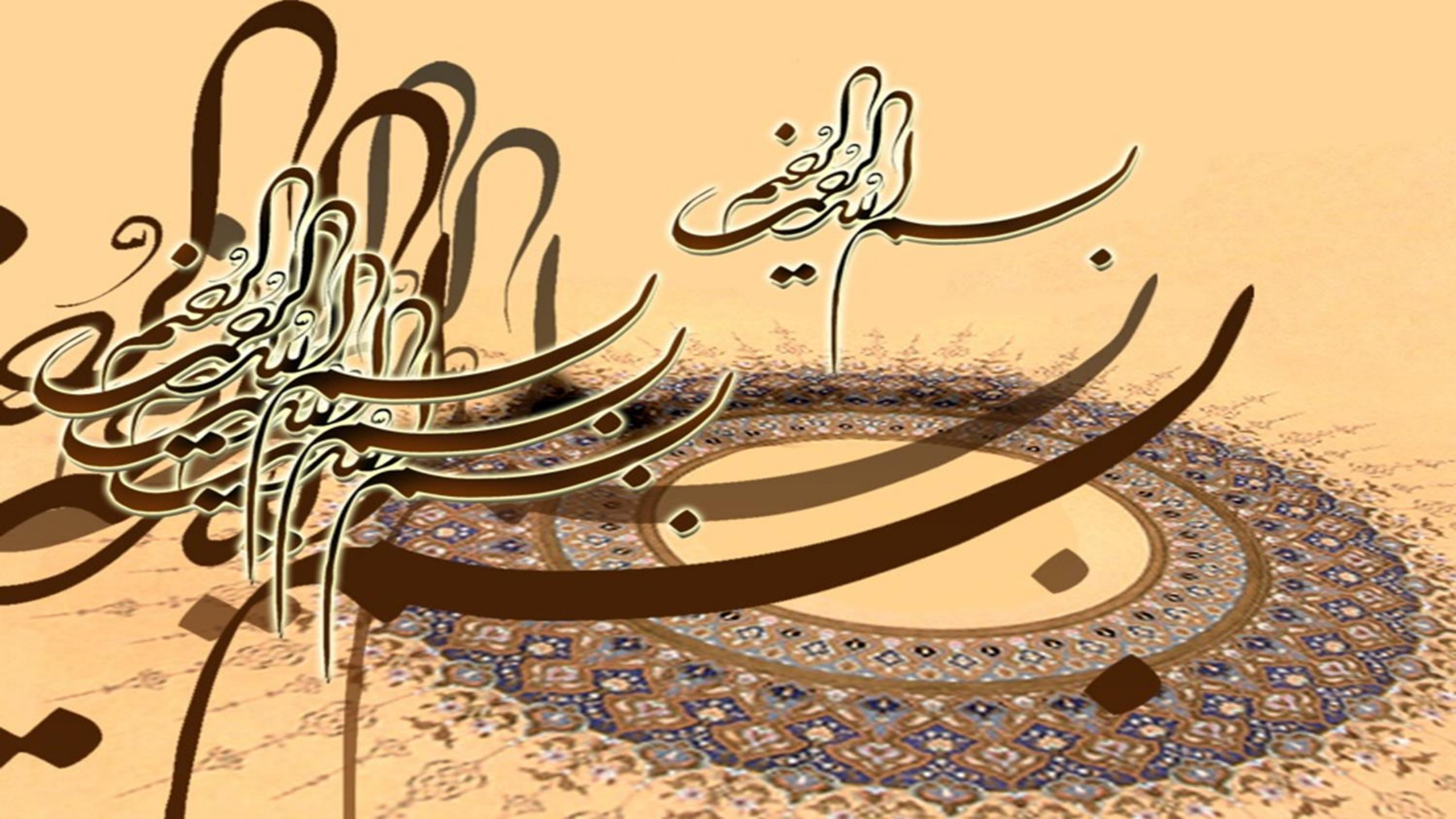
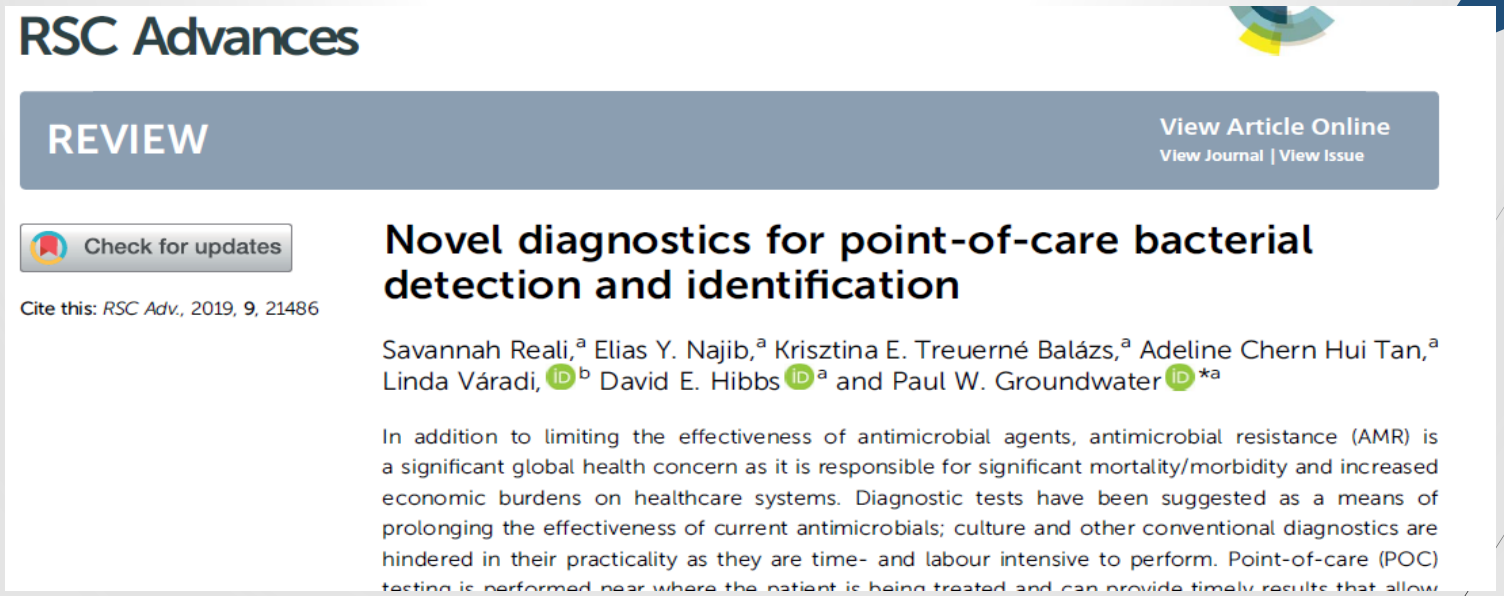

بسم الله الرحمن الرحیم

point-of-care tests:

rapid detection methods for bacterial infections



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Novel diagnostics for point-of-care bacterial detection and identification

Savannah Reali,[a] Elias Y. Najib,[a] Krisztina E. Treuerné Balázs,[a] Adeline Chern Hui Tan,[a] Linda Váradi,[b] David E. Hibbs[a] and Paul W. Groundwater*[a]

In addition to limiting the effectiveness of antimicrobial agents, antimicrobial resistance (AMR) is a significant global health concern as it is responsible for significant mortality/morbidity and increased economic burdens on healthcare systems. Diagnostic tests have been suggested as a means of prolonging the effectiveness of current antimicrobials; culture and other conventional diagnostics are hindered in their practicality as they are time- and labour intensive to perform. Point-of-care (POC) testing is performed near where the patient is being treated and can provide timely results that allow

By: razie Kamali

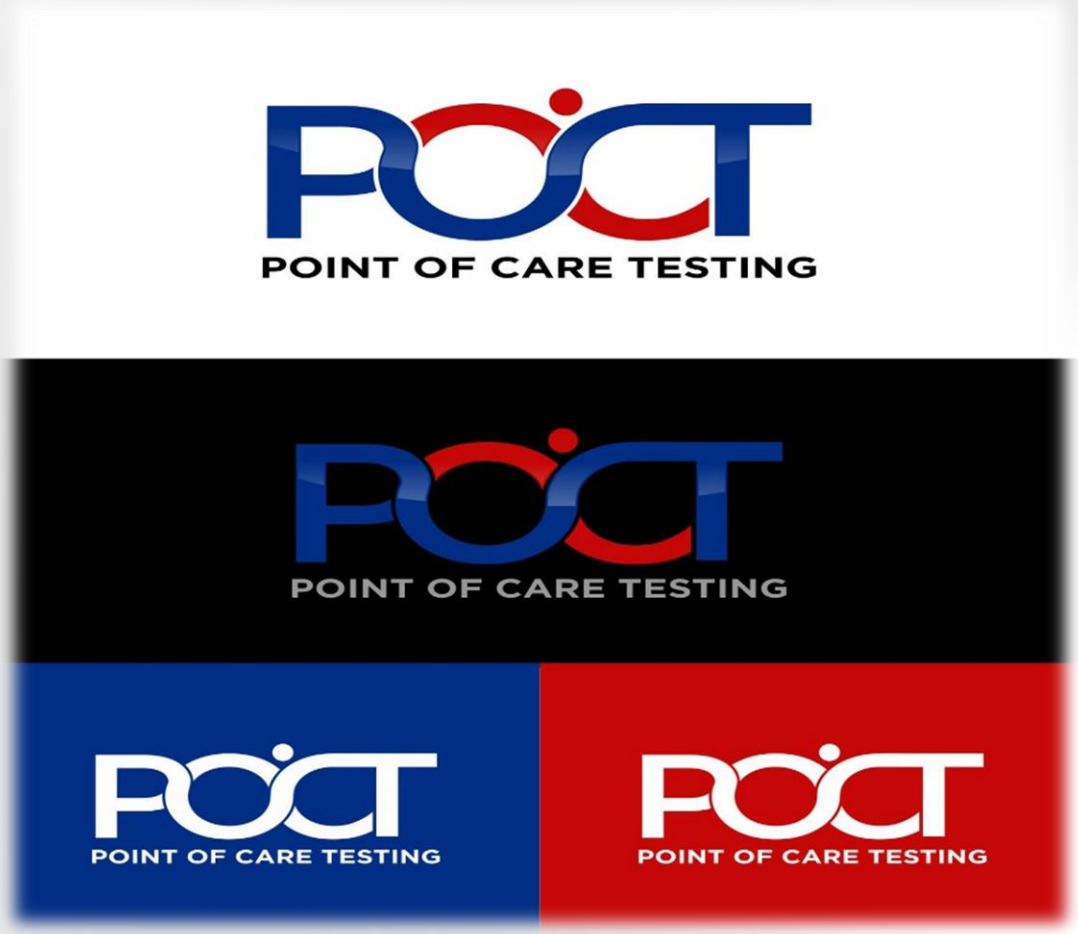
CONTENTS

1

What is POCT?

1 point-of-care (POC) tests

- performed near the patient or treatment facility
- fast turnaround time
- lead to a change in patient management
- do not require access to centralised laboratory facilities
- ideally be sufficiently rapid to allow clinically meaningful interventions



2

Why is necessary to do POCT?

2 Why is necessary to do POCT?

AMR

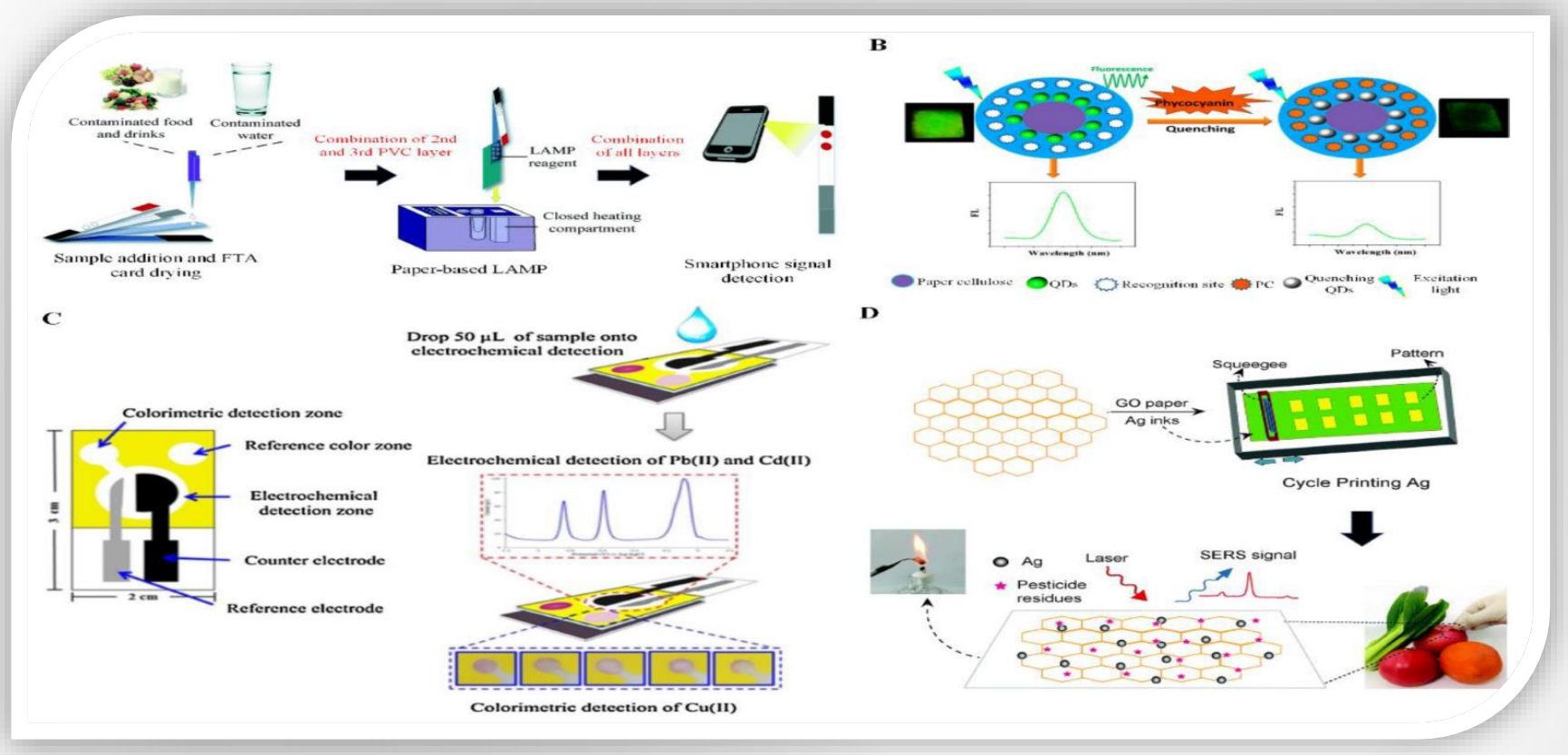
- Antimicrobial resistance is a significant global issue and can to completely alter the landscape of modern healthcare
- At the current rate, AMR will be responsible for ten million deaths each year by 2050
- The 2016 O'Neill report on 'Tackling Drug-Resistant Infections Globally' suggests that by 2020 all clinicians should

Suitable guides patient treatment

For bacterial infections, this first step guides patient treatment strategies and effective usage of antibiotics & Proper drug use is necessary to mitigate the growing emergence of antibiotic resistance

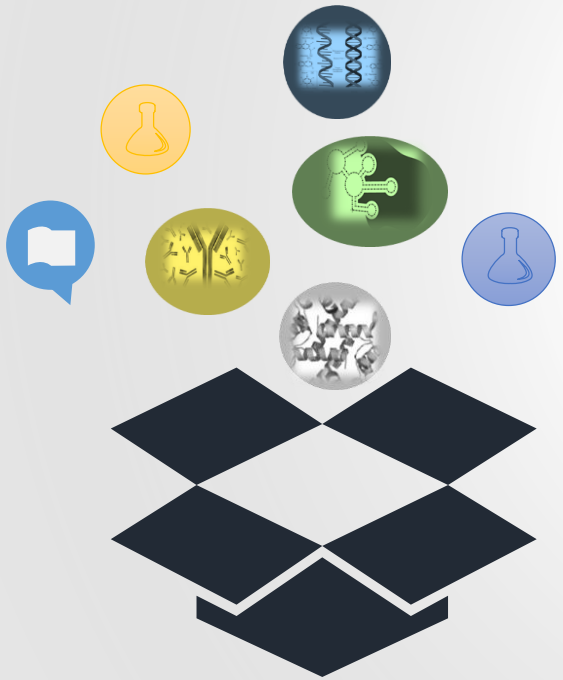
Time & accurate

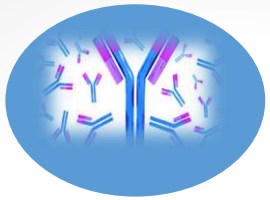
The timely and accurate identification of the causative agent responsible for an infection is the critical first step in effective patient care



Diagnostic techniques suitable for point-of-care (POC) devices

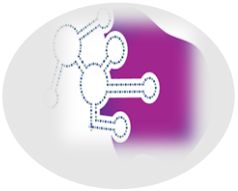
Diagnostic techniques suitable for POC devices





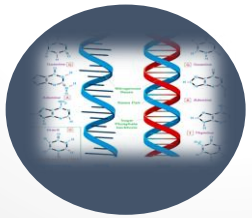
Antibodies

Electrochemical detection, Optical detection



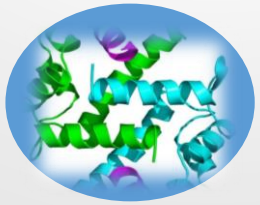
Aptamers

Electrochemical detection, Optical detection



Nucleic acids

Electrochemical detection, Optical detection, Magnetic detection

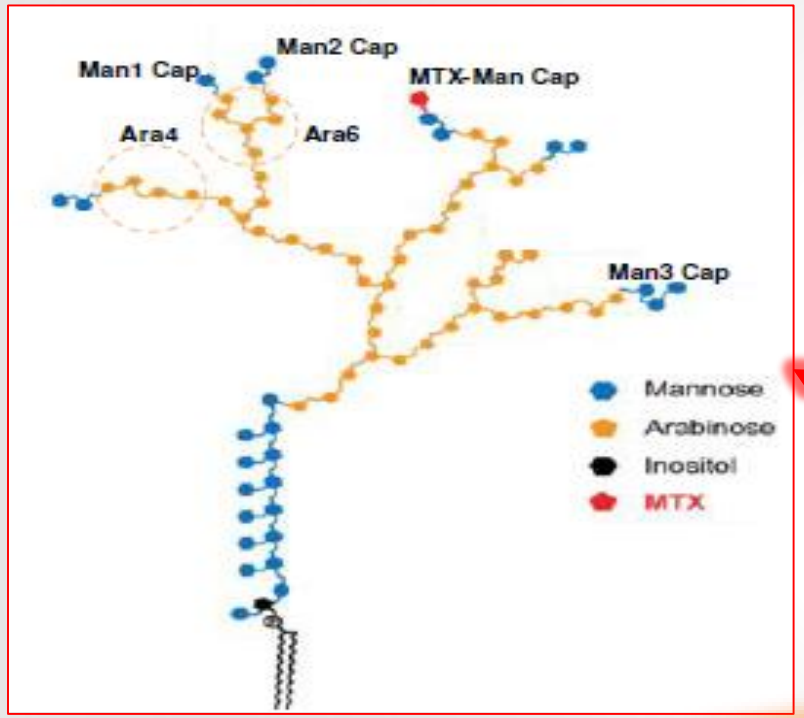


Proteins

Electrochemical detection, Optical detection

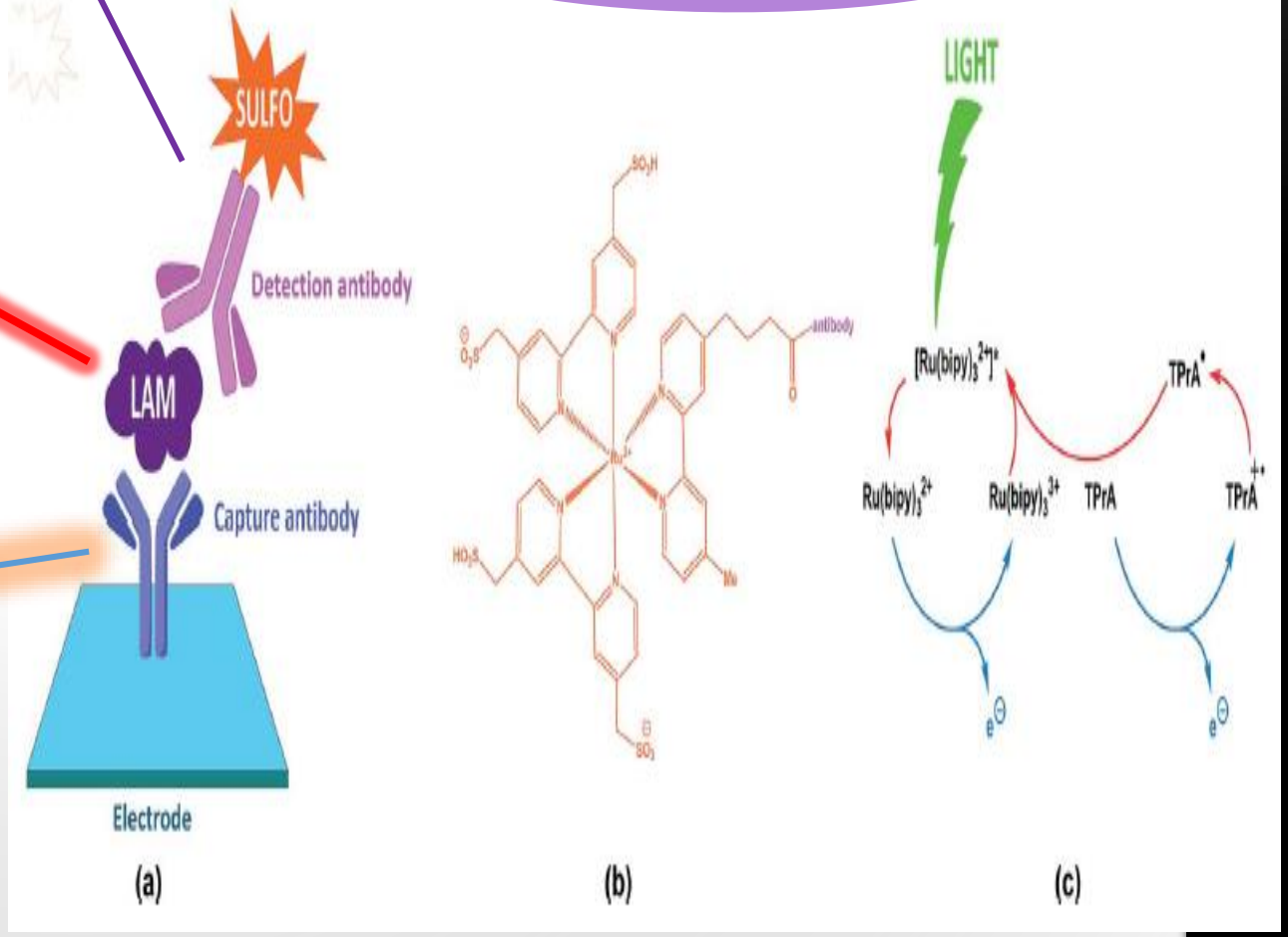
sensitivity =93%
specificity =97%

1. Antibodies(a. Electrochemical detection)



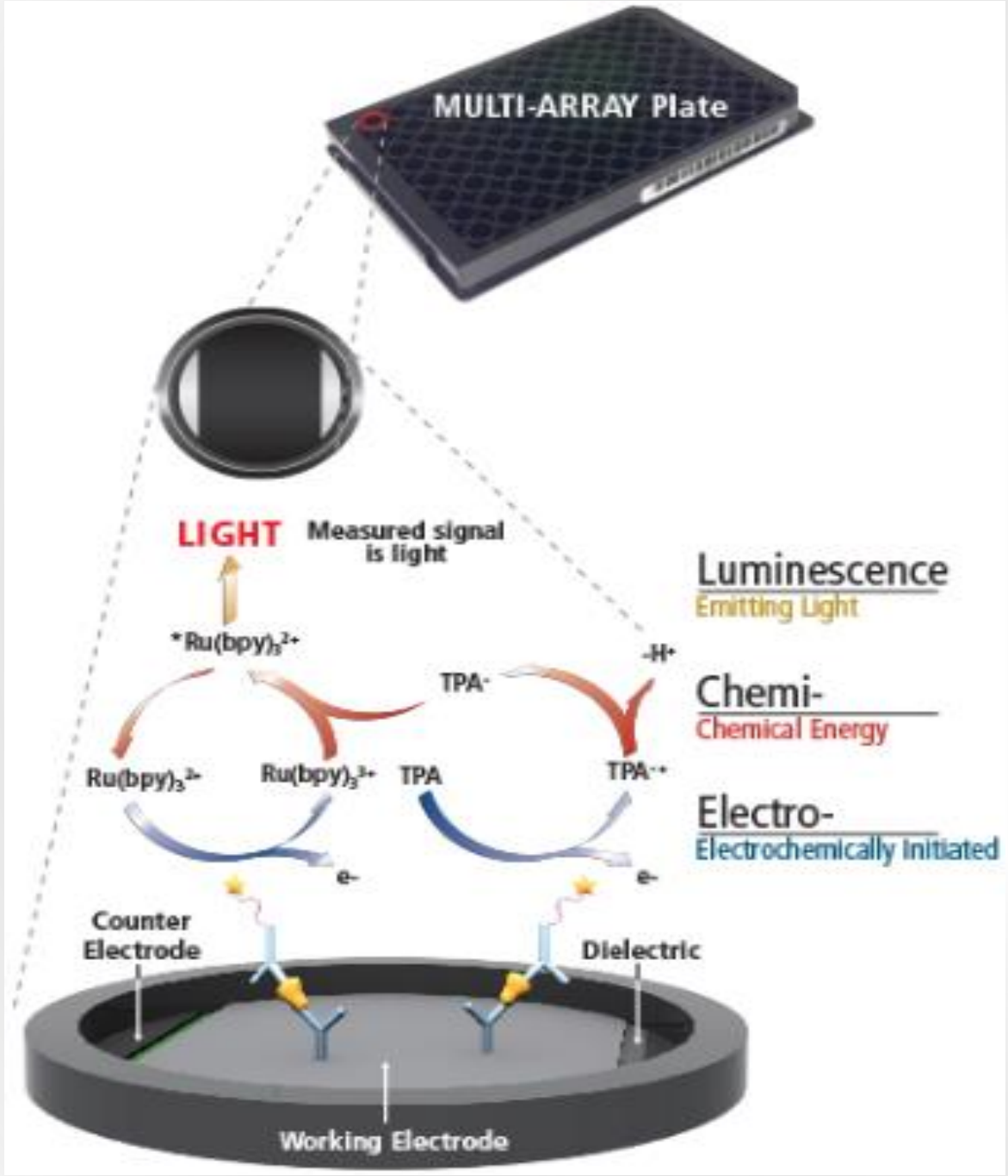
Detection mono Antibody(A194-01)

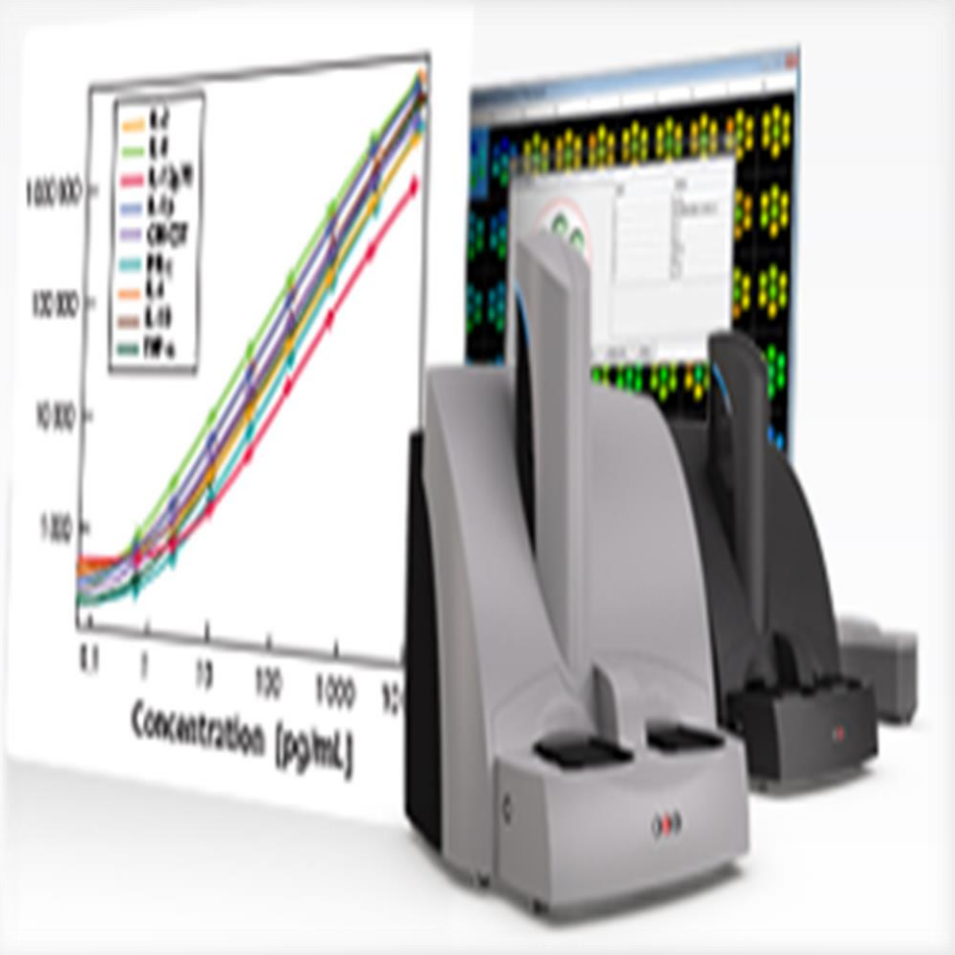
Ara4, Ara6 ± Man1 Cap



capture mono-antibody (S4-20)

MTX-Man **Cap**

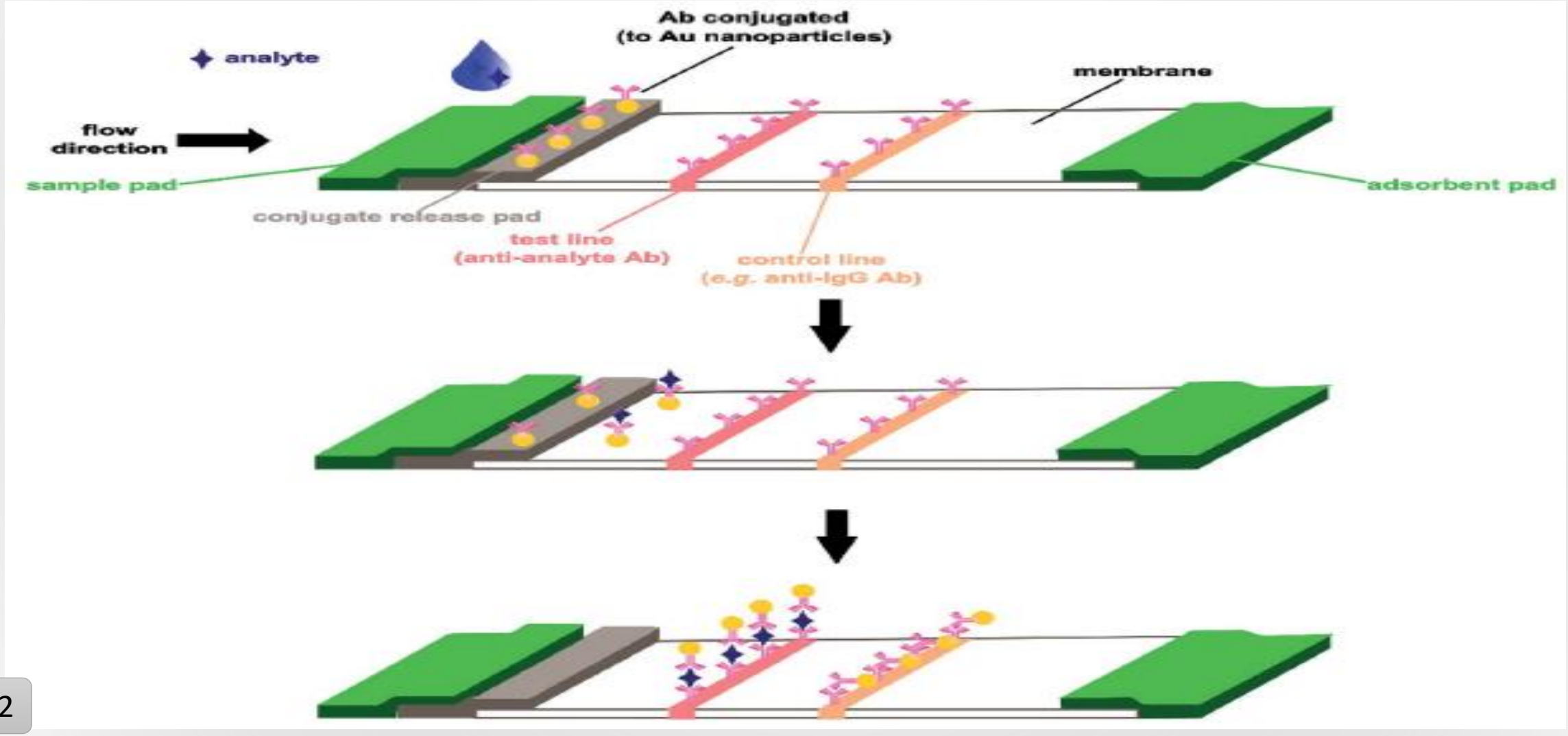

MULTI-ARRAY Plate
LIGHT
Measured signal is light
*Ru(bpy)3 2+
TPA·
-H+
Ru(bpy)3 2+
Ru(bpy)3 3+
TPA
TPA·+
e-
e-
Counter Electrode
Dielectric
Working Electrode
Luminescence
Emitting Light
Chemi-
Chemical Energy
Electro-
Electrochemically Initiated


Concentration (pg/mL)

1. Antibodies(b. Optical detection)

- Immunochromatography, also known as lateral flow immunoassay (LFIA), is simple, rapid and allows for portability
- This technique has been commercialized for several decades
- recent advancements: its sensitivity, reproducibility and detection of multiple analytes
- preparation of unique antibody pairs are often time consuming, but with high stability and an unrefrigerated shelf life of 24 months, these systems can be produced and stored on a large-scale to minimize associated costs.

1. Antibodies(b. Optical detection)



Diagnostic techniques suitable for POC devices

1. Antibodies(b. Optical detection)

100% sensitivity

>90% specificity

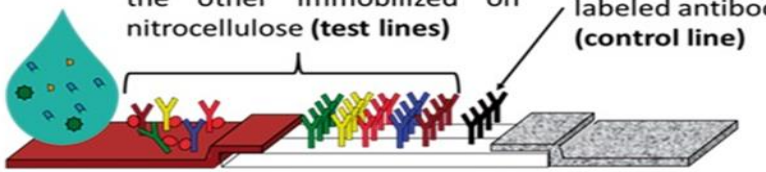
detected by the naked eye within 15 minutes

1 - Structure of the strip

Antibody pairs recognizing the 5 main carbapenemases. One antibody of the pair labelled with colloidal gold, the other immobilized on nitrocellulose **(test lines)**

Sample

Antibodies recognizing labeled antibodies **(control line)**



Sample pad | Nitrocellulose | Absorption pad

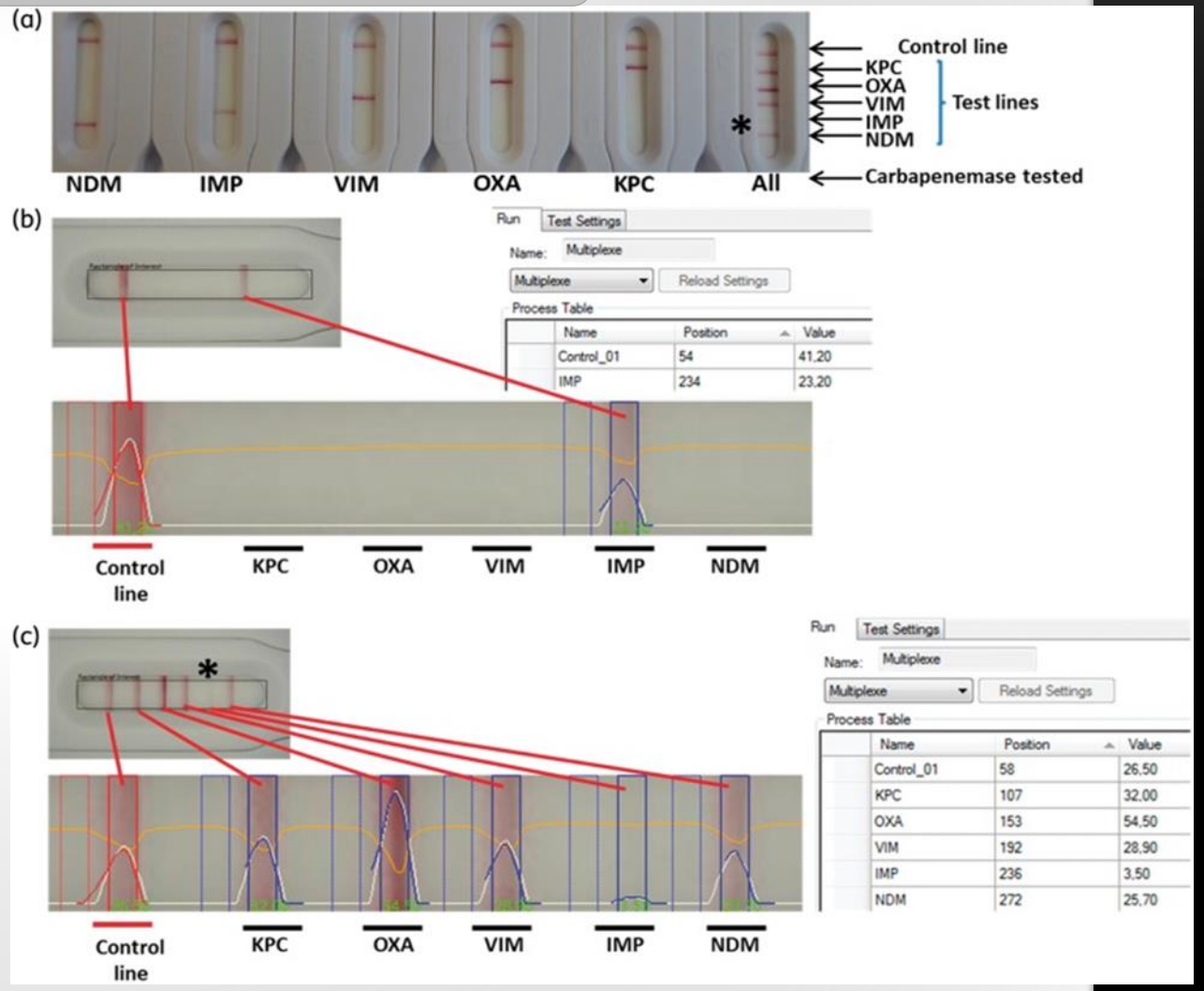
2 - Immunological detection

Sample flow: capillarity

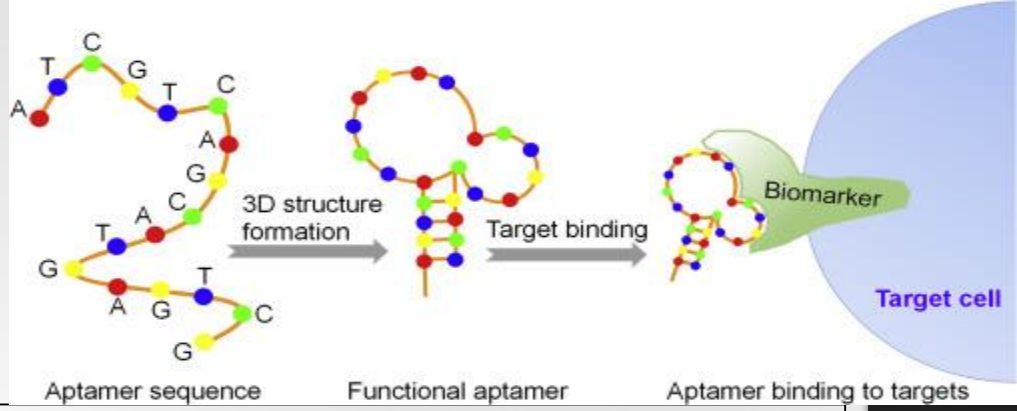
3 - Result

- ✓ The control line appears: the test is correct
- ✓ One or several test lines appear: positive test for the corresponding carbapenemase(s)
- ✓ No test line appears: negative test for the 5 carbapenemases

Carba5 (NG Biotech, France)



2. Aptamers



- short, single-stranded DNA or RNA oligonucleotide biosensors
- Upon binding to their target analyte, aptamers fold into specific three dimensional structures with many surface interactions for strong bonding
- typically with a dissociation constant in the nano- or pico-molar level
- chemically synthesized in vitro by a process known as Systematic Evolution of Ligands by Exponential enrichment (**SELEX**)
- Aptamer can capture most biomolecules, from small molecules to whole cells, by covalent bond and. Because of the strong affinity towards targets, aptamers have been extensively used as **capture probes in sensors**

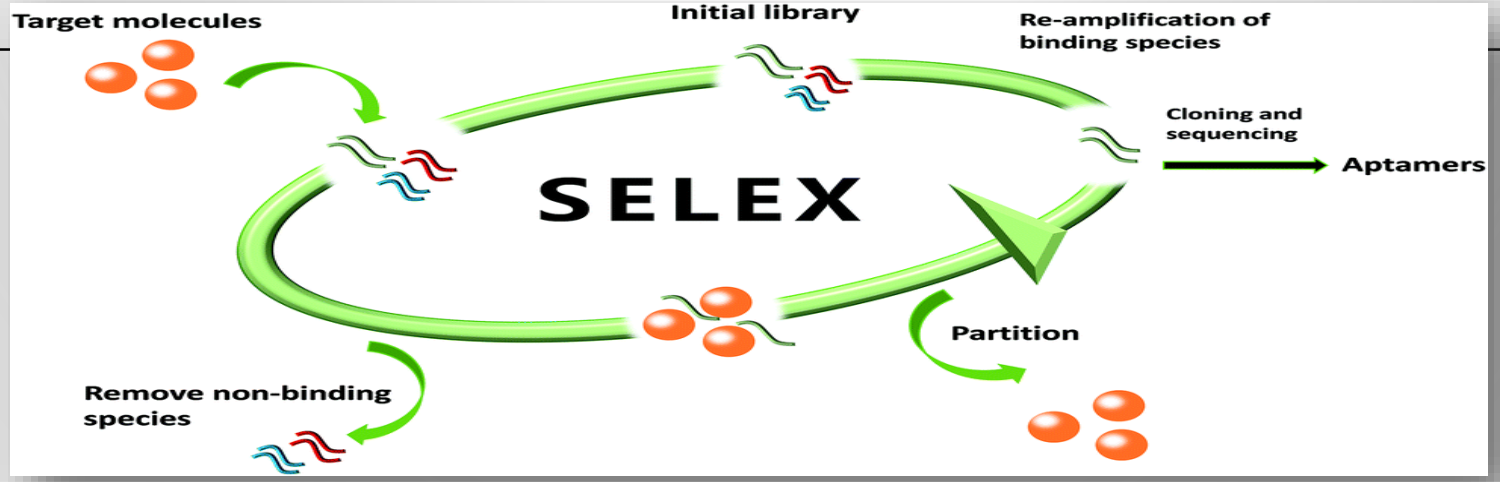
2. Aptamers

SELEX

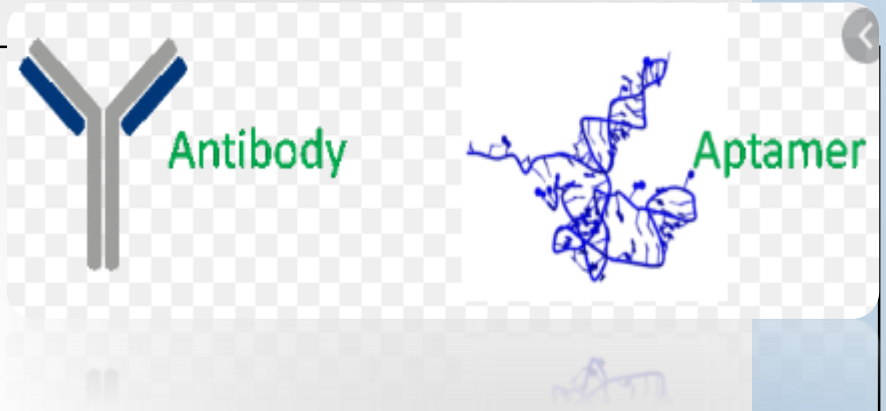
(1) the incubation of an oligonucleotide sequence library with the target analyte to assess which structures bind

(2) the elution of unbound oligonucleotides, separating them from those bound to the analyte

(3) amplification of the remaining oligonucleotide sequences by PCR



Advantages aptamer over antibodies

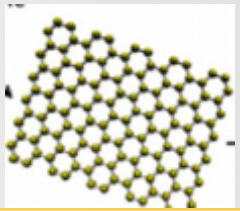


- Low toxicity
- stable over a wide temperature and pH range
- products of simple and reproducible chemical syntheses
- Although the determination of aptamer structure requires several steps, their in vitro synthesis is preferable to that of antibodies, which require synthesis in biological systems under highly specific conditions

2. Aptamers(a. Electrochemical detection)

- detection relies upon changes in electrical properties (current, impendence, potential and conductance) due to interactions between aptamer &analyte
- Electrochemical impedance spectroscopy (EIS) is an ultrasensitive technique that detects impedance variations along reaction interfaces, label-free strategies

2. Aptamers(a. Electrochemical detection)

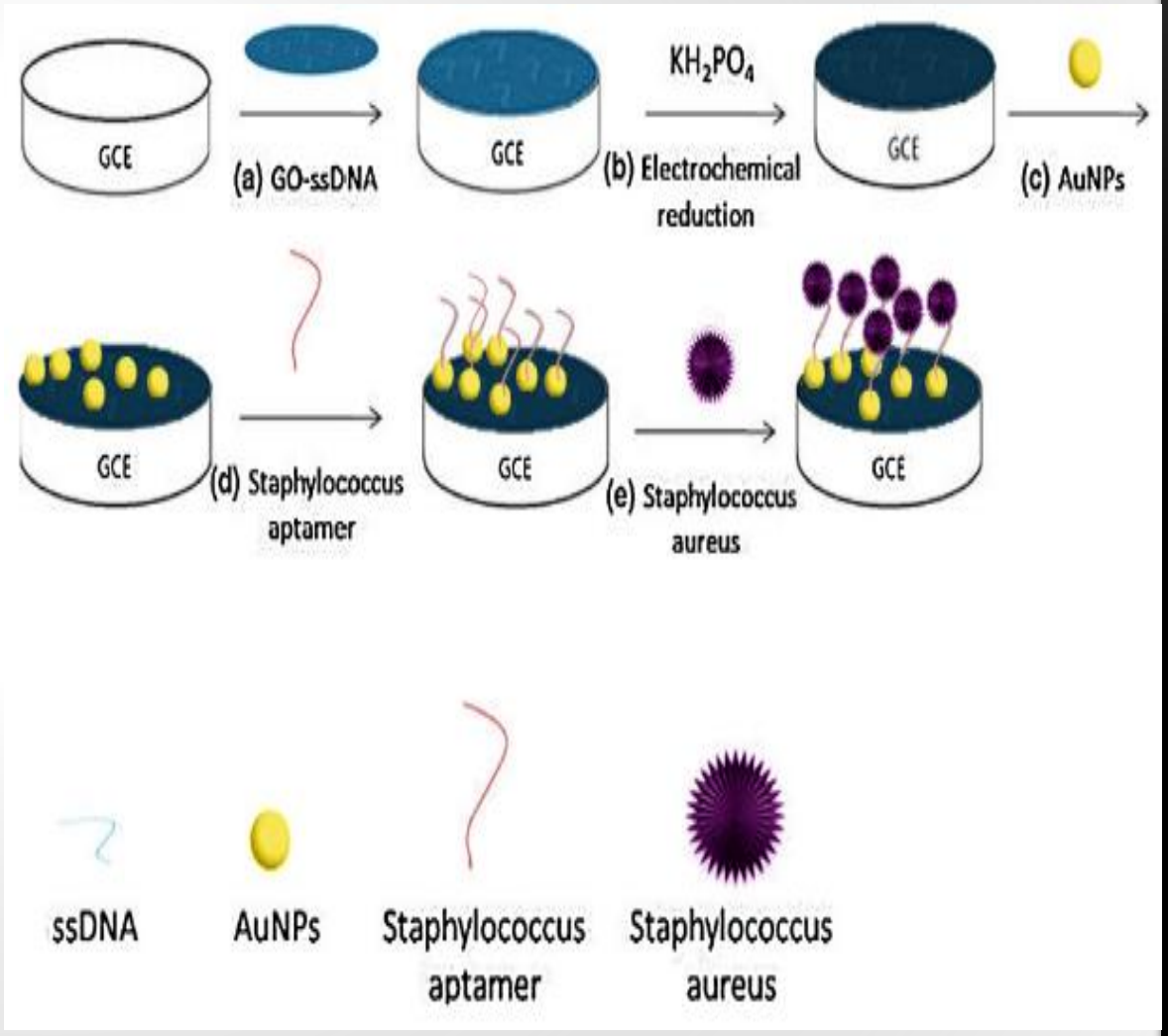


Graphen Oxide(GO)

GO+ aptamer

10 CFU/ml -1

60 minutes

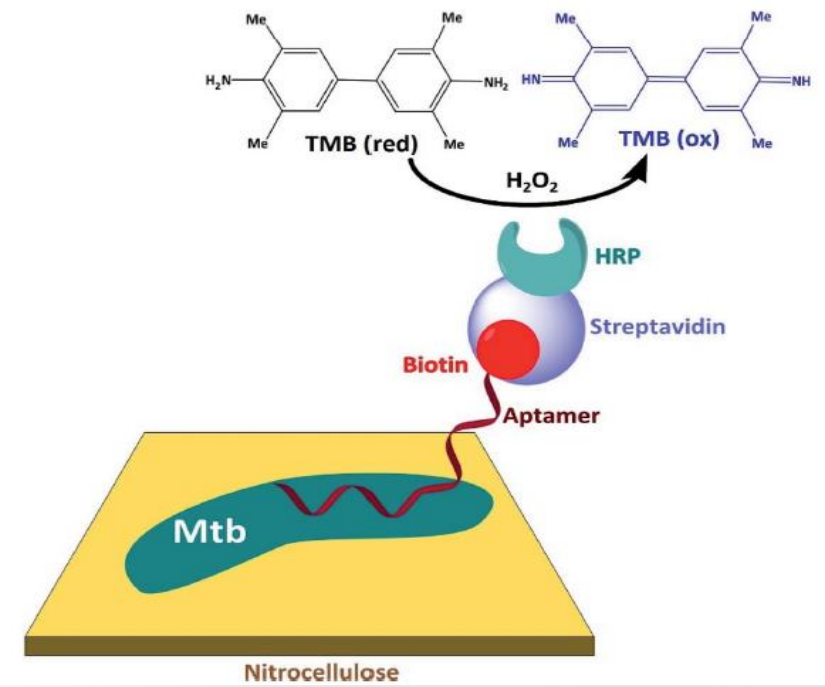


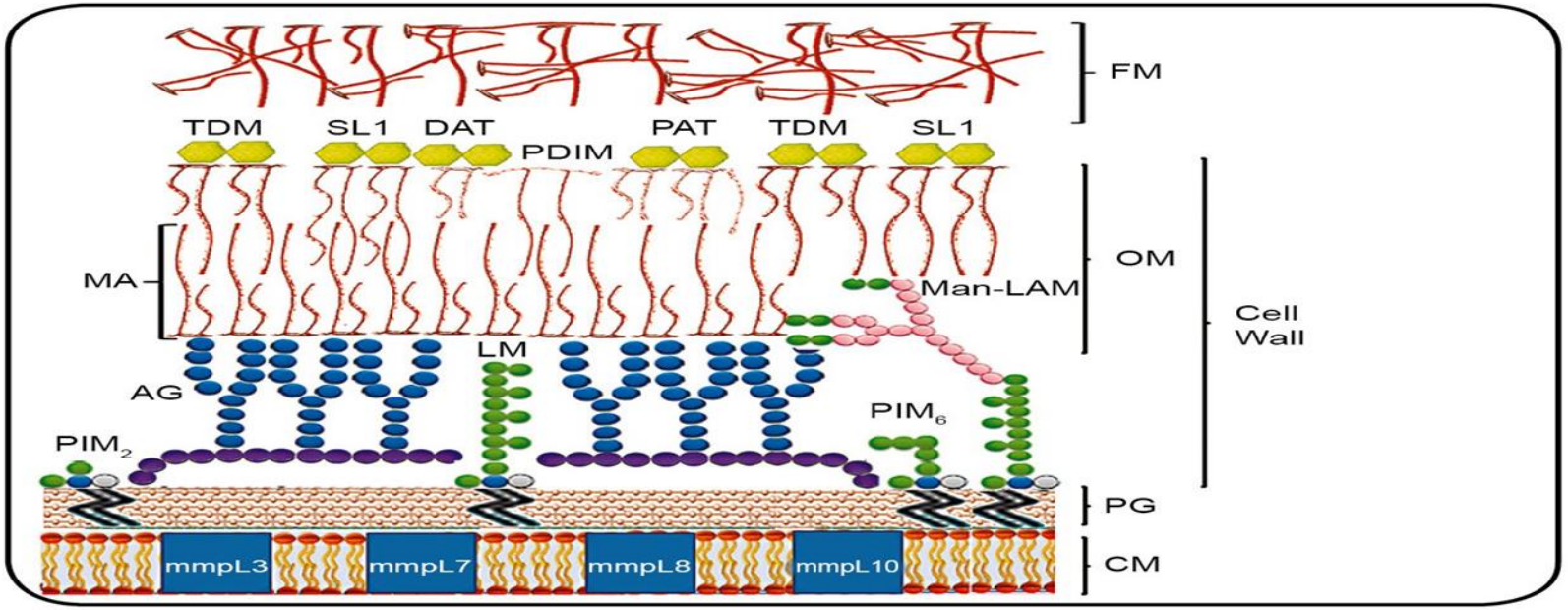
10^4 CFU/ ml $^{-1}$

5 h

2. Aptamers (b. Optical detection)

- Colorimetric detection of a biotin-labeled aptamer of tuberculosis infection (TBI)
- specifically recognize mannose-capped lipoarabinomannan (ManLAM)



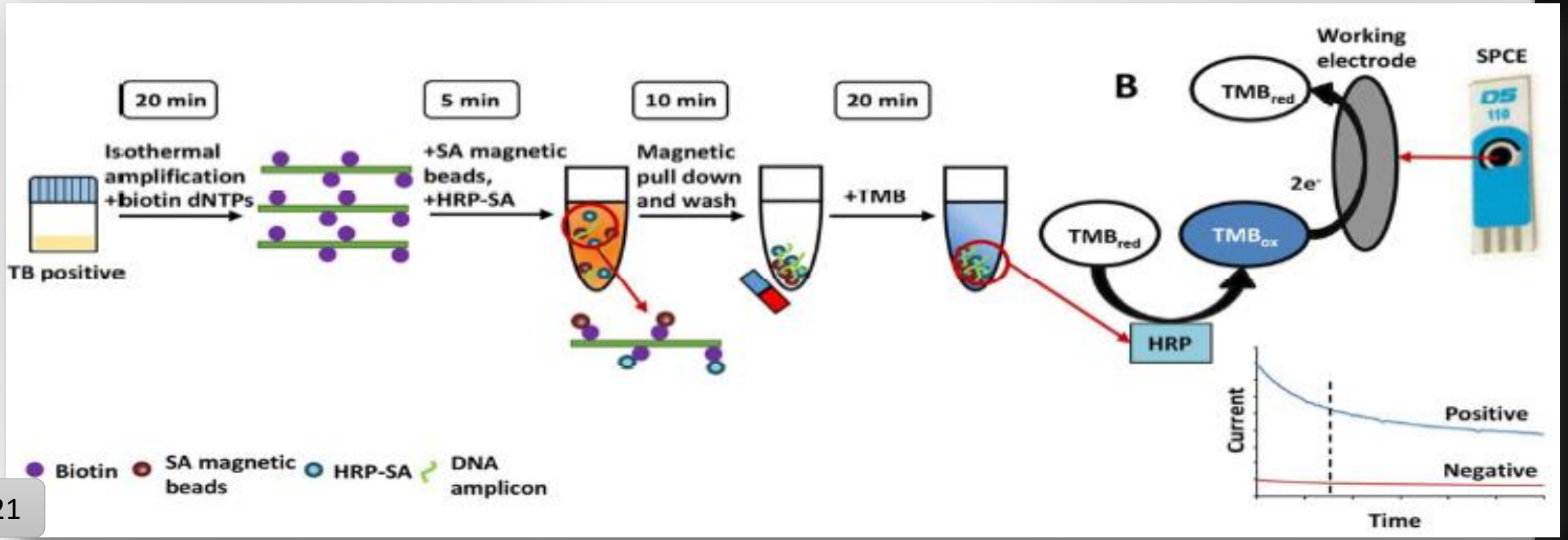


3. Nucleic acids

- PCR based techniques are the basis of many diagnostic tests
- reduced the duration of empirical- therapy
- require minimal sample manipulation(time required and the risk of contamination)
- Isothermal techniques for nucleic acid amplification and detection have circumvented key technical and resource limitations of PCR-based assays and make them feasible at the point of care

3. Nucleic acids (a. Electrochemical detection)

specific biosensor for M. tuberculosis, detection of an RPA amplified target region within the early secretory antigenic target-6 (ESAT-6) gene



3. Nucleic acids (a. Electrochemical detection)

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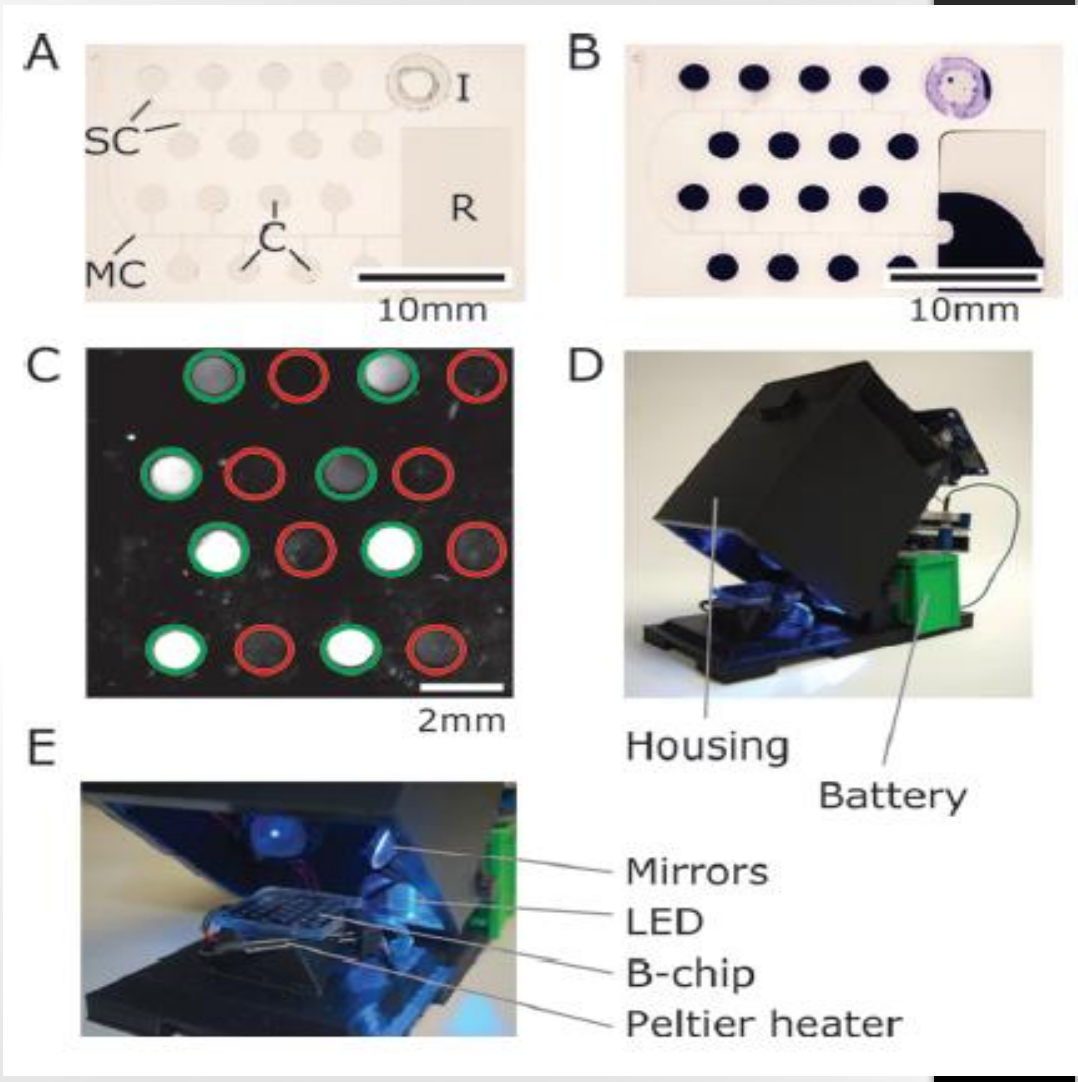
Detection of ESKAPE Bacterial Pathogens at the Point of Care Using Isothermal DNA-Based Assays in a Portable Degas-Actuated Microfluidic Diagnostic Assay Platform

Lars D. Renner,[a,d] Jindong Zan,[a] Linda I. Hu,[a] Manuel Martinez,[e] Pedro J. Resto,[a,e] Adam C. Siegel,[a] Clint Torres,[f] Sara B. Hall,[g] Tom R. Slezak,[h] Tuan H. Nguyen,[h] Douglas B. Weibel[a,b,c]



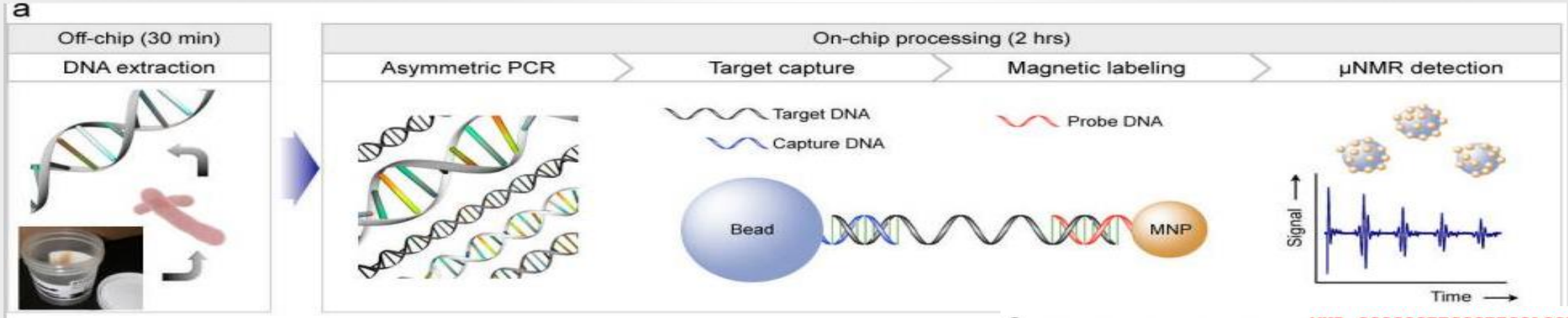
3. Nucleic acids(c. Magnetic detection)

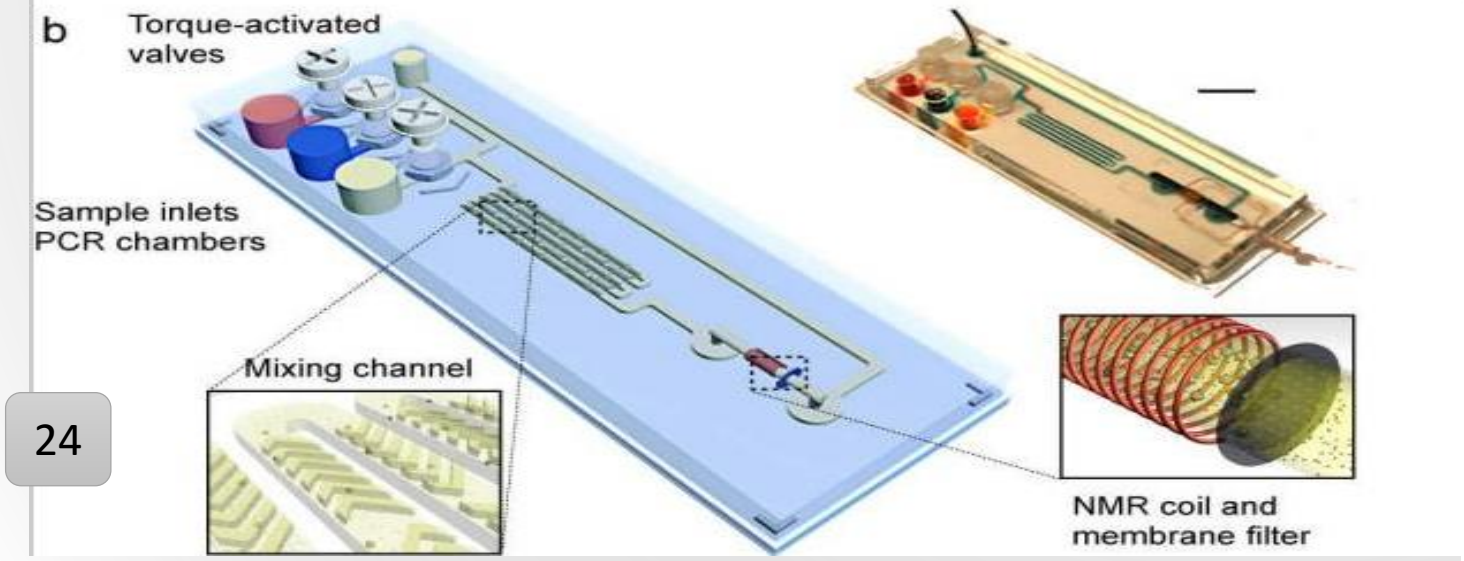
- Recent advancements in nanotechnology → the development of a range of diagnostic devices (containing nanoparticles that provide several advantages)
- Rapid detection, high sensitivity, capacity for miniaturization and portability make them suitable for application in POC diagnostic systems

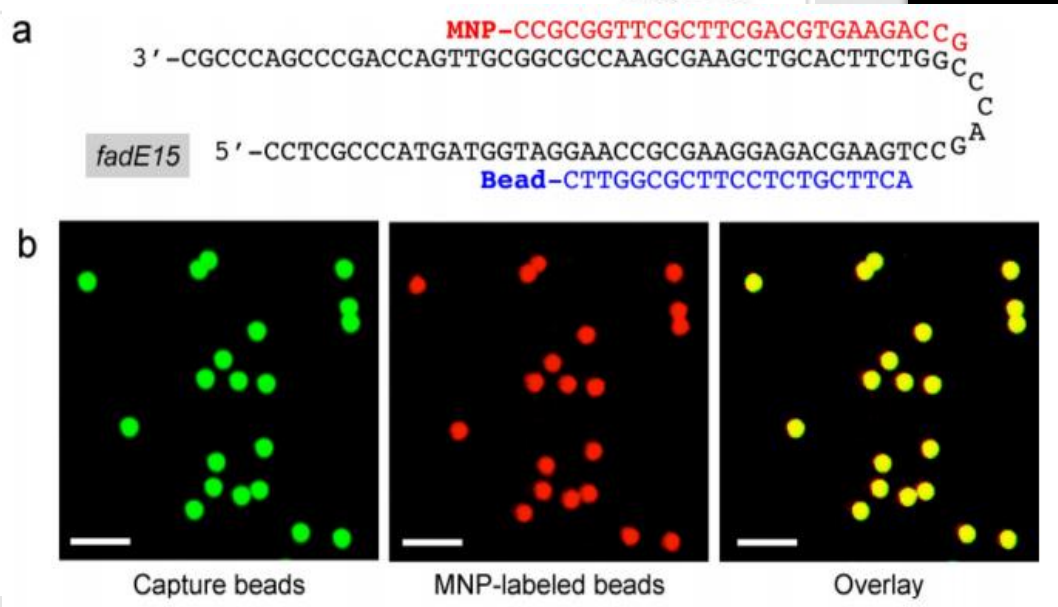
Sensivity = 10^2 - 10^3 CFU/ml $^{-1}$

2.5 h

magnetic barcode assay (detection of *M. tuberculosis*)

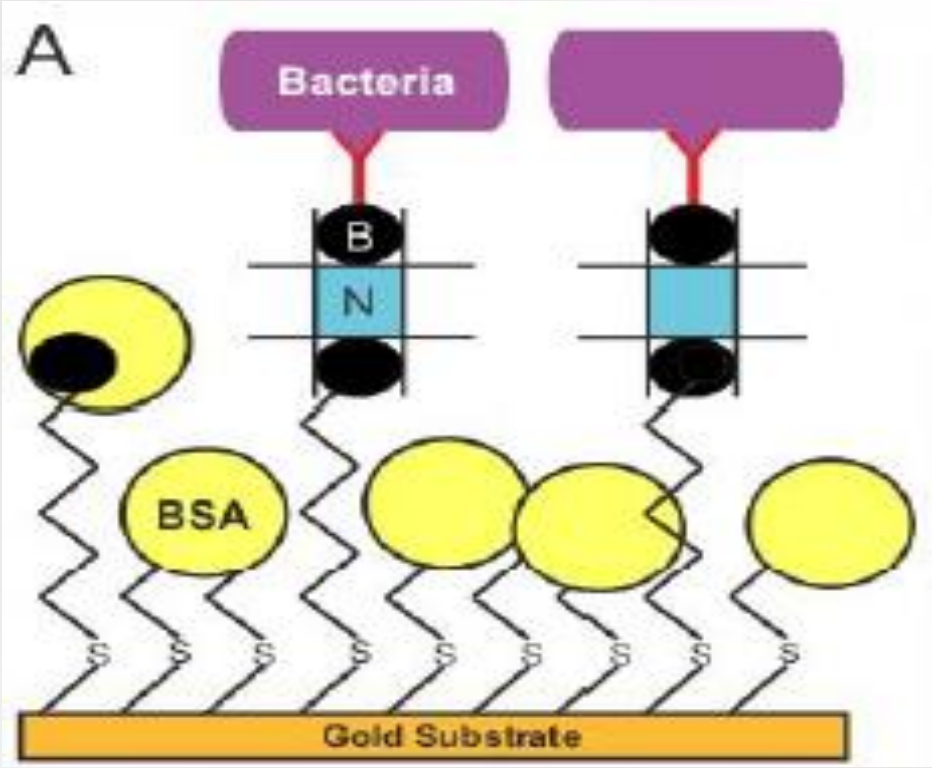


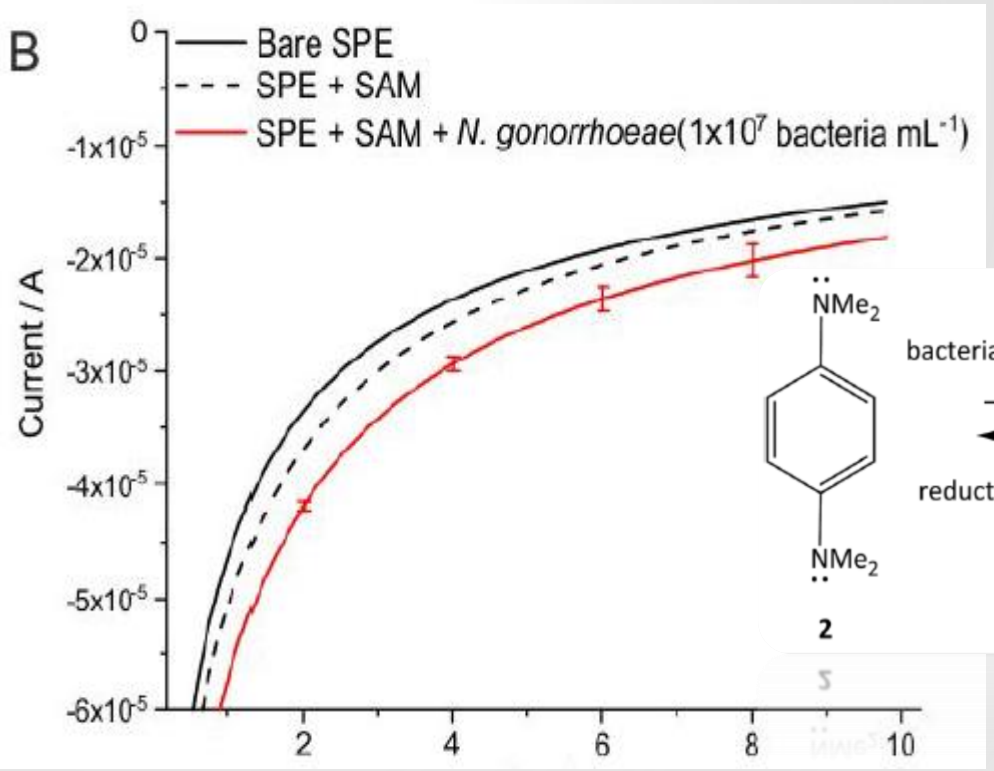


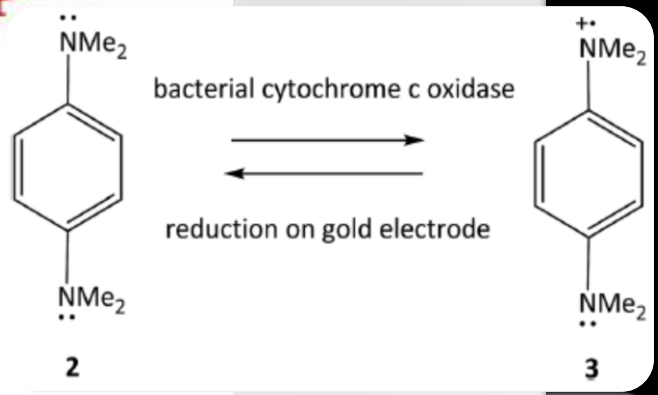


4. Proteins(a. Electrochemical detection)

Detection of *Neisseria gonorrhoeae* relies upon the bacterial expression of cytochrome c oxidase and enhanced electrochemical current produced when this enzyme oxidizes tetramethyl-p-phenylenediamine (TMPD) 2







4. Proteins(b. Optical detection)

- optical biosensors are simple diagnostic tools that offer several advantages over conventional techniques as they provide direct, rapid and label-free detection of bacterial pathogens

- A novel diagnostic platform for the detection of S. aureus :

combination of enzyme–substrate interactions, nanotechnology and colorimetric techniques on a single biosensor chip

4. Proteins(b. Optical detection)

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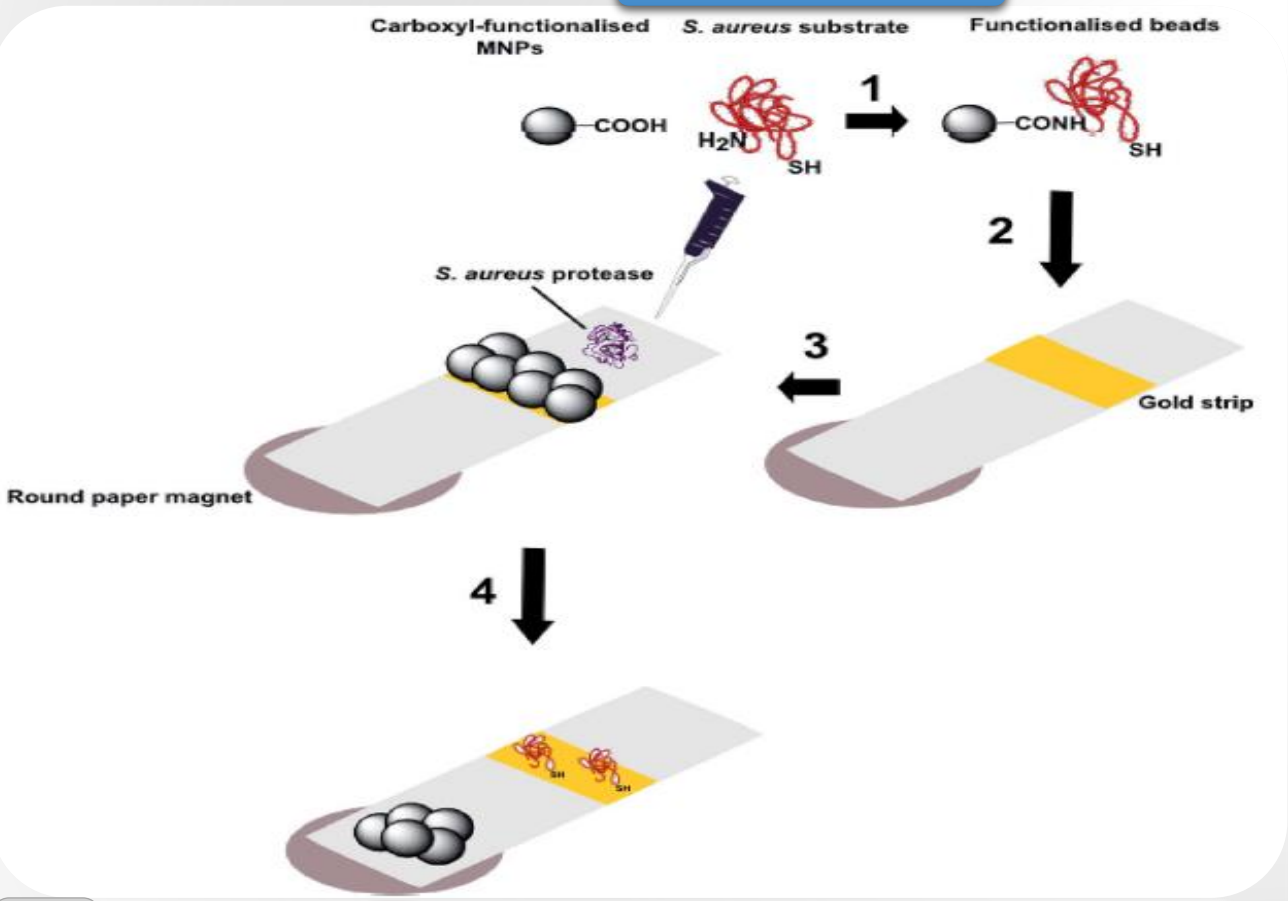
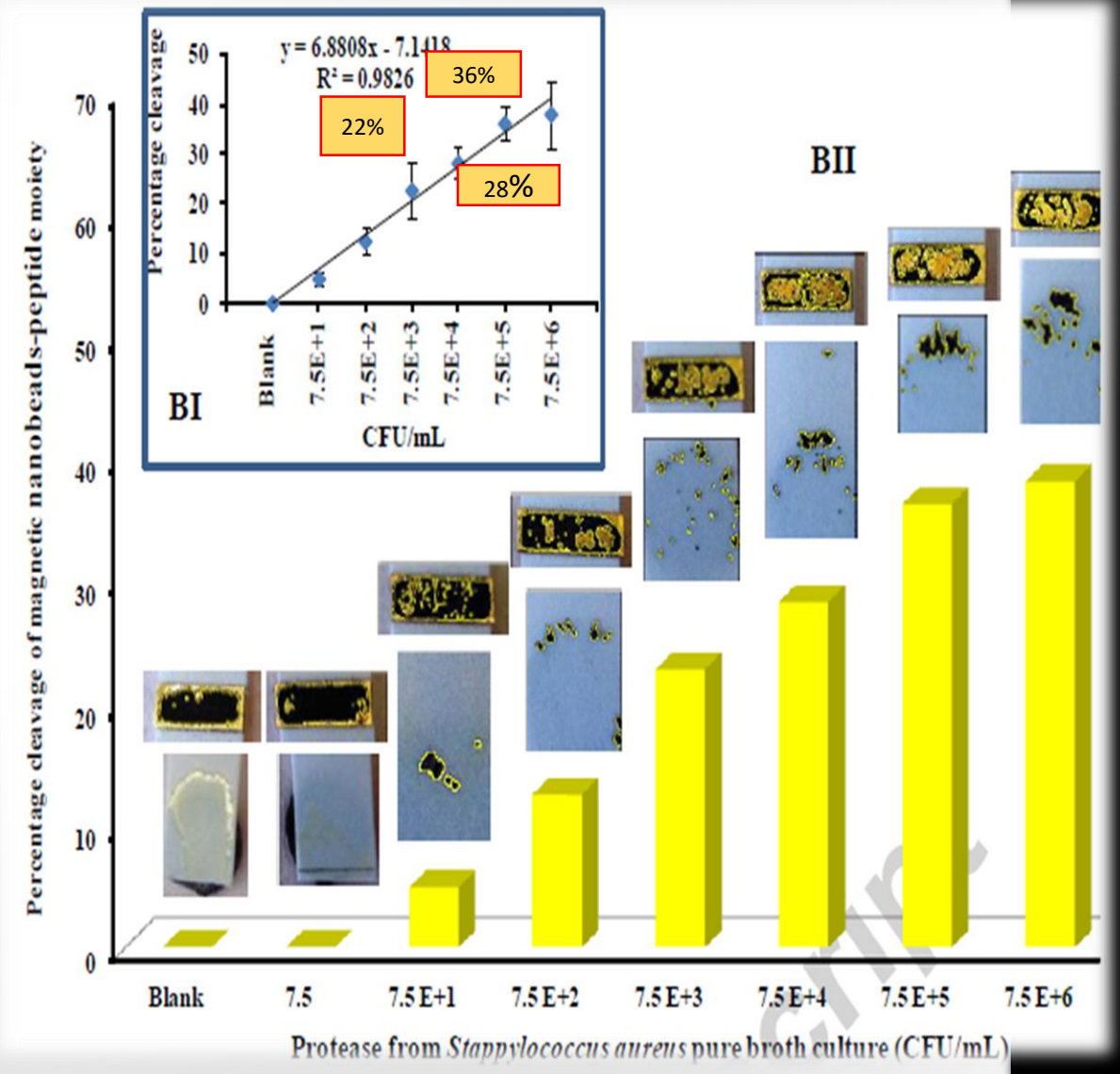
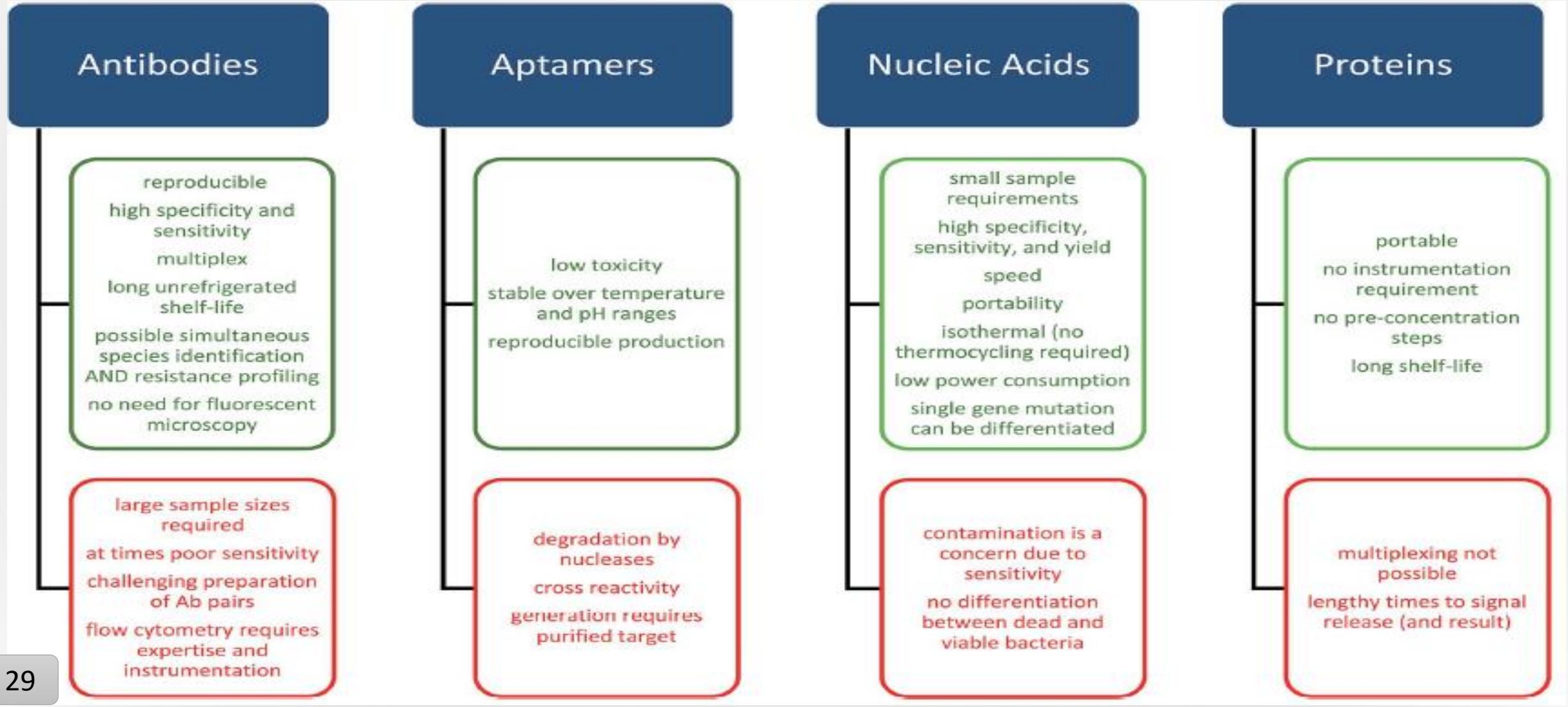




4

Advantages and disadvantages of biorecognition elements utilized in POC

4 Advantages and disadvantages of bio recognition elements utilized in POC



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THANKS

For Your attention