MAY 06, 2020
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BIOSENSORS FOR PANDEMICS

ICN2 Institut Català de Nanociència i Nanotecnologia — EXCELENCIA SEVERO OCHOA

Electrochemical Sensors For Pandemics Management: A Review of Current Diagnostic Devices and New Rapid-Deploy Systems

NANO Bioelectronics & Biosensors Group

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SARS-CoV-2 (COVID19) outbreak

Timeline of SARS-CoV-2 Outbreak

30/12/2019 Cluster of cases of pneumonia of unknown origin reported in Wuhan to China National Health Commission

01/01/2020 Huanan Seafood Wholesale market closed

07/01/2020 Novel coronavirus isolated

11/01/2020 First fatal case reported

12/01/2020 Whole genome sequence shared with WHO and public; virus designated 2019-nCoV

13/01/2020 WHO reports case in Thailand, the first outside China

20/01/2020 First reports of infection in healthcare workers caring for patients with 2019-nCoV

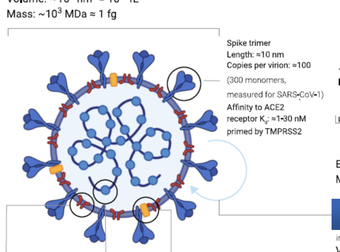
30/01/2020 WHO declares outbreak a global health emergency

11/02/2020 WHO has named the disease COVID-19, short for "coronavirus disease 2019"

https://www.who.int/emergencies/diseases/novel-coronavirus-2019

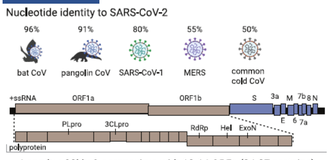
Size & Content

Diameter: ≈100 nm
Volume: ~10^6 nm^3 = 10^{-3} fL
Mass: ~10^3 MDa ≈ 1 fg



Genome

Nucleotide identity to SARS-CoV-2



Length: ≈30kb; β-coronavirus with 10-14 ORFs (24-27 proteins)

Evolution rate: ~10^{-3} nt^{-1} yr^{-1} (measured for SARS-CoV-1)
Mutation rate: ~10^{-6} nt^{-1} $cycle^{-1}$ (measured for MHV coronavirus)

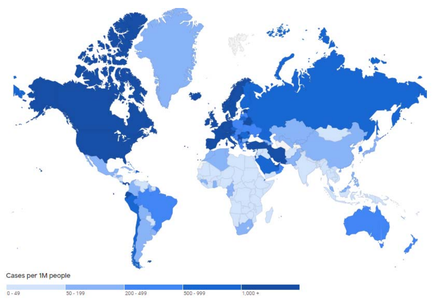
Replication Timescale

in tissue-culture
Virion entry into cell: ~10 min (measured for SARS-CoV-1)
Eclipse period: ~10 hrs (time to make intracellular virions)
Burst size: ~10^3 virions (measured for MHV coronavirus)

Y. M. Bar-On et al. *eLife* (2020); 9:e57309 doi: 10.7554/eLife.57309

Reported Coronavirus Cases Worldwide

As of April 26, 2020

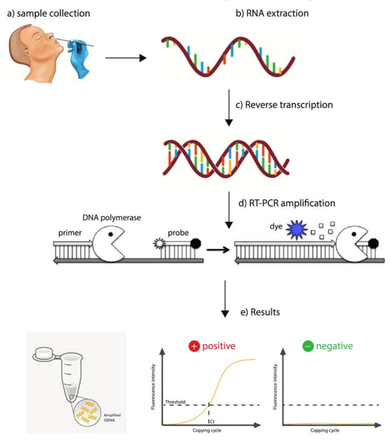


For real-time update, please scan the QR Code

https://google.com/covid19-map/?hl=en

Molecular Assays For Detection of SARS-CoV-2 Viral RNA

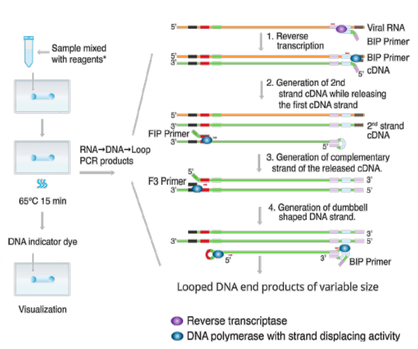
Reverse Transcription-Polymerase Chain Reaction (RT-PCR)



Limitations

- Amplification process should be repeat around 40 cycles until the viral cDNA can be detected;
- Turnaround time of 24-72 hours;
- Sensitivity of 50% - 79%, depending on the protocol used the sample type and number of clinical specimens collected;
- Expensive laboratory instrumentation;
- Requires trained operators.

Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP)



Limitations

- Limited to one sample per run;
- Expensive laboratory instrumentation;
- Requires trained operators;
- High cost.

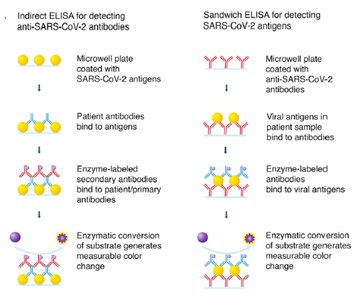
Limitations

- Typically a qualitative test (positive or negative) for SARS-CoV-2 antibodies;
- Usually less sensitive than ELISA.

L. Carter et al., *ACS Cent. Sci.* (2020) 6, 591

Serological and Immunological Assays

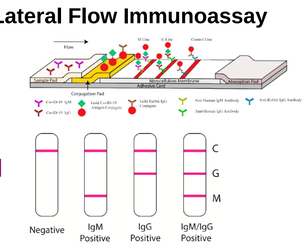
Enzyme-Linked Immunosorbent Assay ELISA



Limitations

- False seronegative due to the lag in antibody production following infection;
- False seropositive due the reflecting clearance of an earlier, milder infection;
- Limitation in sensitivity and specificity of the assays.

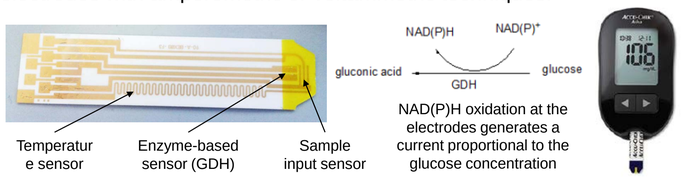
Lateral Flow Immunoassay



Electrochemical Biosensors Principles and Technologies

Electrochemical biosensors are usually divided in enzymatic or affinity biosensors

❖ The first using enzymes to catalyze the transformation of the analyte in an electrochemically active specie detectable on electrodes with amperometric or voltammetric techniques.



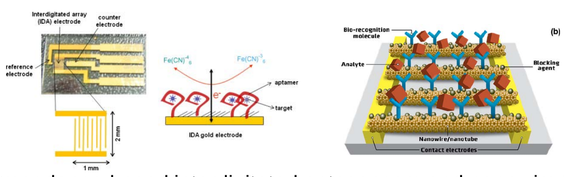
NAD(P)H oxidation at the electrodes generates a current proportional to the glucose concentration

A classical example for this kind of biosensors is the glucometer

Advantages:
- ✓ Low-cost electrodes / enzymes
- ✓ independent **from** analyte molecular weight
- ✓ Label-free
- ✓ Very specific (enantioselective)

Disadvantages:
- o Temperature/pH-dependence
- o Delicate bio-receptor
- o Redox mediator often required
- o Stable functionalization difficult

❖ Affinity biosensors transduce the binding of the analyte to specific bioreceptors on the electrodes surface using amperometric / voltammetric techniques or impedance / current variations.



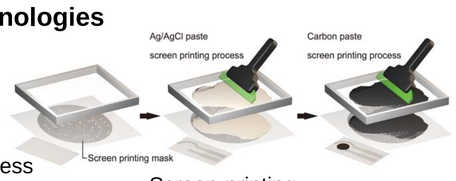
Advantages:
- ✓ Label-free or labeled approach
- ✓ Stable bioreceptors available
- ✓ Ultra-high sensitivity (FETs)
- ✓ Mediator-free possible

Disadvantages:
- o High bioreceptors cost
- o Relatively high LOD
- o Often requiring expensive electrodes

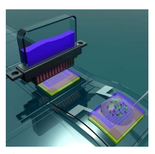
Impedance-based interdigitated aptasensors and nanowire-based immunosensors are well-known affinity biosensors.

Fabrication Technologies

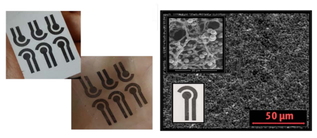
Photolithography process Clean-room



Screen-printing



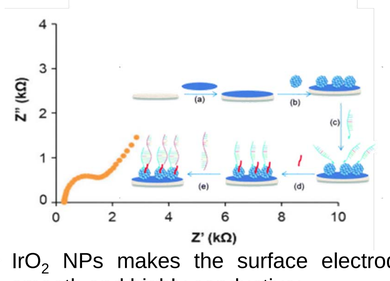
Inkjet printing



GO-transfer

Electrochemical Biosensors for Pathogen Detection

Impedimetric IrO_2 NP-Based Platform for Leishmania Detection

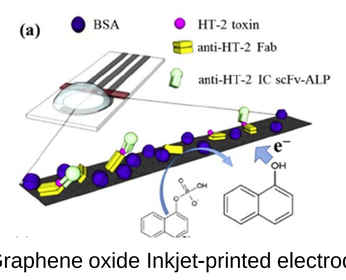


❖ IrO_2 NPs makes the surface electrode smooth and highly conductive;

❖ Detect up to 1000 times diluted PCR amplified DNA;

Mayorga et al. *J. Mater. Chem. B* (2015) 3, 5166.

Inkjet-printed rGO microelectrode for HT-2 mycotoxin biosensing



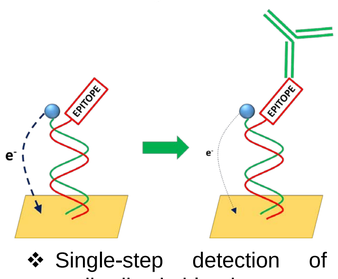
❖ Graphene oxide Inkjet-printed electrodes;

❖ Low cost and ease of scale-up biosensors;

❖ High sensitivity with detection limit of 1.6 ng/mL;

J. Kudr et al. *Biosensors and Bioelectronics* (2020), 156, 112109

E-DNA scaffold sensor for antibody detection



❖ Single-step detection of antibodies in blood;

❖ Multiplexing capabilities:

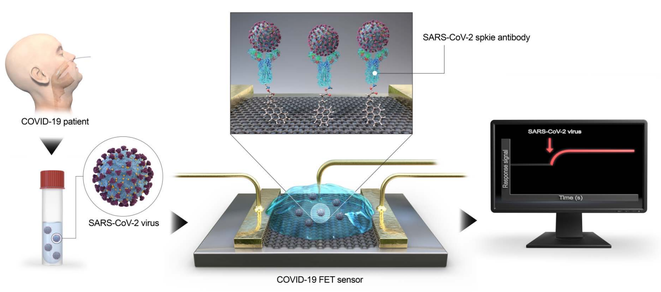
❖ MSCA - Premother

C. Parolo et al. *Microsyst. & Nanoengin. (2020),* 6 (1)

Electrochemical COVID19 Biosensors - State of the Art

Graphene Field-Effect Transistor (FET)-Based Biosensing Device for Detecting SARS-CoV-2

❖ Detection of SARS-CoV-2 spike protein and SARS-CoV-2 culture virus in clinical transport medium and clinical samples.



Advantages

- ❖ High selectivity and sensitivity;
- ❖ Low time response (processing time around 1 min.)
- ❖ Low voltage operation (V_{DS} ~10 mV)
- ❖ Miniaturized device.

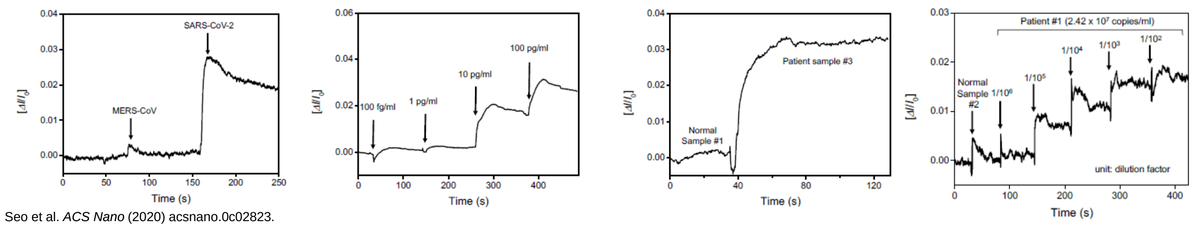
Challenges

- ❖ Clean room processing;
- ❖ High cost;
- ❖ Reduce noise signal in real samples.

❖ LOD of SARS-CoV-2 spike protein in nasopharyngeal clinical transport medium 100 fg/ mL;

❖ High selective to SARS-CoV-2;

❖ LOD of SARS-CoV- 2 virus patients 1:1×10^5 (242 copies/ml)



G. Seo et al. *ACS Nano* (2020) acsnano.0c02823.

Ultra-Low-Cost Integrated Silicon-Based Transducer for Genetic Detection of SARS-CoV-2 RNA

❖ Functions of TriSilix device:
i) electrical (Joule) heater;
ii) temperature sensor (*i.e.* thermistor);
iii) electrochemical sensor for detecting target nucleic acids (NAs);

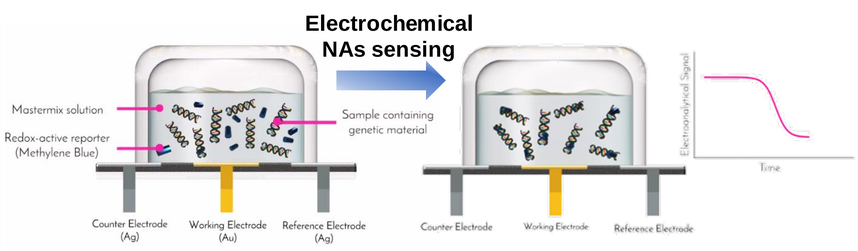
❖ Large scale fabrication: 4-inch Si wafer yields 37 TriSilix chips;

❖ Fast manufacturing time (37 TriSilix chips in 7 hours);

❖ Cleanroom-free low-cost fabrication (**~US $0.35 per device**);

Real time detection of NAs by PCR in a Silicon chip, based on electrochemical detection

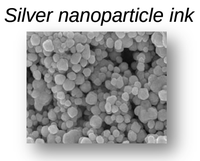
Detection of the cDNA from SARS-CoV-2 (1 pg) through PCR (lasting ~40 min)



E. Bajo et al. *bioRxiv* (2020), doi.org/10.1101/2020.03.23.002931 14

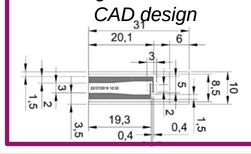
Our Approach

Silver nanoparticle ink

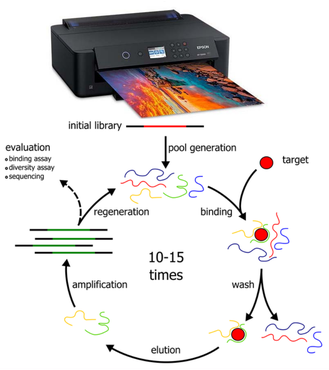


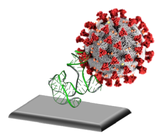
PET substrate

Interdigitated electrodes CAD design



Consumer inkjet printing and functionalization with thiolated aptamers

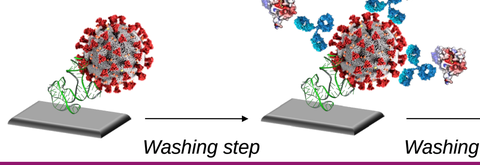


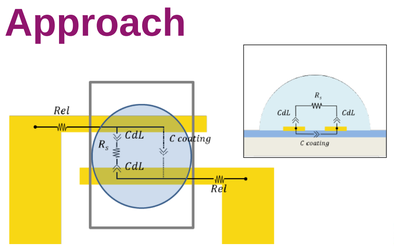


Aptamer binding of SARS-CoV-2

Variation of the impedance of the electrodes and of the accessibility to the surface by redox mediators (e.g. NADH, which can be detected by the glucometer)

GDH labeled sec. antibodies

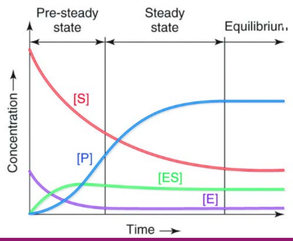


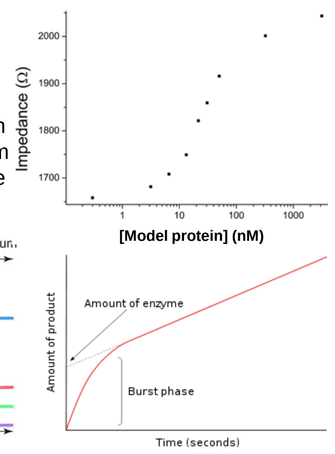


G. Rosati et al. *Sensing and Biosensing Research* (2019)

The labeling allows for signal amplification. The kinetics of the NADH formation are proportional to the GDH enzymes bound, and in turn to the virus particles trapped by the aptamers.

The modeling of the electrochemical system and impedance measurements by smartphone readout allow for the specific label-free detection of the virus binding. The system has already been tested for the detection of a model protein.





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CONCLUSIONS AND ACKNOWLEDGMENTS

Electrochemical sensors, even if not massively present on the market are an extremely useful and effective tool for rapid and low-cost diagnostics. Integrated with telemedicine easy-to-use systems their potential for real-time diagnosis and monitoring is incredible. The first electrochemical systems for detection of SARS-CoV-2 are already present in several research laboratories but from our perspective, their interoperability with already existing readout systems is fundamental for a rapid deploy.

MARIE CURIE ACTIONS — GRAPHENE FLAGSHIP — EXCELENCIA SEVERO OCHOA — EUROPEAN UNION European Regional Development Fund — BIST Barcelona Institute of Science and Technology — CAPES

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