2022 IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT)
March 10-11, 2022. Texas A&M University - EnMed, Houston, TX, USA

Sensitive and Selective Determination of multiple Diagnostic Targets using a Modular, ASSURED POC Platform called ESSENCE

Yu-Hsuan Cheng, Charmi Chande, Li Zhenglong, Sreerag Kaaliveetil, and Sagnik Basuray

2022 IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT) | 978-1-6654-9615-5/22/$31.00 ©2022 IEEE | DOI: 10.1109/HI-POCT54491.2022.9744075

***Abstract*— The sensor platform, ESSENCE, uses a Shear-Enhanced, flow-through non-planar 3D Nanoporous Electrode to overcome current electrochemical sensors limitations as a POC sensor, specifically selectivity and sensitivity limitations. ESSENCE consists of a microfluidic channel packed with a transducer like carbon nanotubes, functionalized with capture molecules surrounded by interdigitated electrodes. The porous electrode architecture enhances shear forces leading to high selectivity. The increased convective fluxes disrupt diffusive processes like the electric double layer, leading to rapid measurements. The enhanced electric field penetration due to the 3D electrode leads to a significant increase in signal from target molecule acquisition. The removal of parasitic noises from the double layer and the measurements at high frequency leads to substantial enhancement in the signal-to-noise ratio and thus high sensitivity. The unique chip architecture allows us to assemble the chip at room temperature (modular, solving cold chain issues). The transducer material can be easily exchanged to target different classes of biomolecules, thus giving making ESSENCE a universal modular platform. ESSENCE detects DNA and proteins with fM and pg/L sensitivity, respectively, against other background molecules in undiluted artificial urine. Interestingly protocol optimizations allow us to run ESSENCE in 10 minutes, making it a rapid POC test.**

***Clinical Relevance* — ESSENCE is specifically designed to be modular. It allows it to be a one-stop instrument for clinicians to screen for infectious diseases, liquid biopsy, and toxin detection to detect emerging pathogens while significantly reducing false positives and false positives negatives.**

I. Introduction

The delayed response in COVID-19 detection during the pandemic has hampered the need to develop a universal biosensor platform with adaptive nature for quick response to outbreaks[1]. Rapid diagnostics, especially RT-PCR, is considered a gold standard to detect various pathogenic viruses [2]. During the recent COVID-19 pandemic, RT-PCR was the most accurate and CDC-approved method for detecting the SARS-CoV-2 virus from the nasopharyngeal swab of patients [3]. However, RT-PCR and other rapid diagnostics tools limitations include affordability, portability, dedicated instrumentation, cool chain for primers and buffers, dedicated trained personal for the analysis, and chances of false-positive and false-negative, which delayed in quickly responding to the pandemic with a rapid point-of-care (POC) test [4]. Serology-based tests are less expensive with rapid diagnosis. However, such tests are not suitable for early-stage diagnosis, and the positive test has to be backed up by a quantitative detection tool like PCR[5]. Therefore, there is a pressing need for low-cost affordable screening tools WITH modularity to facilitate rapid detection of future outbreaks.

The biosensors used in POC devices have received significant attention from treatment to diagnosis and prevention in the medical field due to the high potential of biomolecules as biomarkers [6]. Traditionally biosensors have two essential steps one with the biorecognition molecule for a specific biochemical reaction involving enzymes [7], antibody [8], antigen [9] or a whole cell [10] for capturing targets and a signal transducer which converts the biochemical signal into measurable signal via optical, electrical or thermal detection. Several innovations in the field of biosensors could be witnessed for quick detection of biomolecules like enzymes, antigens, antibodies, proteins, and DNA in complex sample matrices [11]. However, the biorecognition binding event is prone to non-specific binding and biofouling, resulting in a false-positive test. Moreover, the capture and signal mechanism is predominantly limited by complicated readout signals, diffusion limitations result in false-negative tests, and require bulky, expensive instruments for accurate signal measurement [12].

Physicochemical signal transducers like surface plasmon resonance [13], fluorescence [14], electrochemical [15] have been majorly explored for detecting biomolecules. Electrochemical-based biosensors have been investigated widely due to advantages like multiplexed detection capabilities, short detection time, simple manufacturing process, and ease of miniaturization for portable POC applications. Furthermore, electrochemical signals have high sensitivity and selectivity, lowering the detection limit for target biomolecules [16]. However, electrochemical biosensing suffers from limitations like compromised signals from self-assembled layers of biomolecules and non-specific adsorption to the electrode surface, which significantly lowers sensitivity and selectivity [17].

Our new POC electrochemical platform technology, ESSENCE (**Figure 1**), utilizes a shear-enhanced, flow-through, non-planar, 3D, and nanoporous electrode.

*Research supported by

Sagnik Basuray, corresponding author is with the Chemical and Materials Department, New Jersey Institute of Technology, Newark, NJ 07039 USA (phone: 973-596-5706; e-mail: sbasuray@ njit.edu).

Yu-Hsuan Cheng is with the Chemical and Materials Department, New Jersey Institute of Technology, Newark, NJ 07039 USA (e-mail yc576@njit.edu)

Charmi Chande is with the Chemical and Materials Department, New Jersey Institute of Technology, Newark, NJ 07039 USA (e-mail: charmi_chande@yahoo.com)

Li Zhenglong is with the Chemical and Materials Department, New Jersey Institute of Technology, Newark, NJ 07039 USA (e-mail: zl479@njit.edu)

Sreerag Kaaliveetil is with the Chemical and Materials Department, New Jersey Institute of Technology, Newark, NJ 07039 USA (e-mail: cb.sc.i5phy16041@cb.students.amrita.edu)

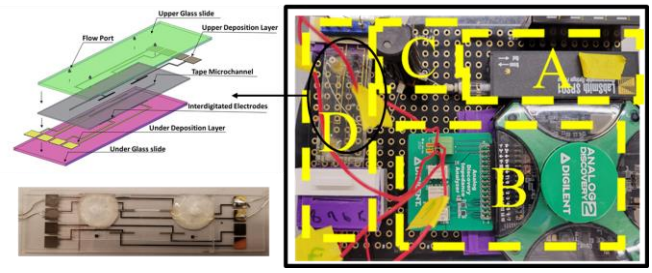

Figure 1 ESSENCE platform and PNP-µIDE chip. The overall size of the ESSENCE platform **(A)** Fluidic pump **(B)**Analog Discovery is the small electrochemical impedance spectroscopy machine being used **(C)** Valves to control the sample loading and buffer **(D)** Replaceable PNP-µIDE chip. (right) The PNP-µIDE chip is shown in more details.

ESSENCE has three layers. A top and bottom three-dimensional interdigitated micro-electrode array (NP-µIDE) sandwiches a microfluidic channel packed with nano-ordered, tunable-porosity material (like metal-organic-framework, hierarchical porous carbons, etc.) with grafted target-specific probes. The integration of ESSENCE is done at room temperature, which can solve the cold chain problem with the storage of capture probes. Furthermore, the packed layer is easily swapped to respond to the diagnostic target of interest, leading to the modularity of ESSENCE.

The packing leads to enhanced selectivity and sensitivity. Unlike planar electrodes, due to the 3D electrode, the electric field penetration across the whole channel allows every captured target molecule to contribute to the signal. A packed channel has higher Zeta potential than an open channel and significantly reduces the length of the electrical double layer (EDL). This moves the EDL relaxation frequency (D/λ^2, D is diffusion coefficient of ions) to a higher frequency, allowing sensor data to be taken at considerably higher frequencies than ambient noise. These lead to increased signal-to-noise ratio and enhanced sensitivity. Further, the diffusion limitations are removed due to the high Peclet Number in ESSENCE, allowing rapid measurements. Thus, ESSENCE's high sensitivity comes from: (i) Overcoming diffusion limitations, (ii) Increased signal-to-noise (from NP-µIDE), (iii) Electric field penetration across the whole channel allows every captured target molecule to contribute to the signal. Furthermore, analyte flow through the nanoporous layer leads to enhanced shear forces (~hydrogen bond), mitigating non-specific adsorption tremendously increasing selectivity. Hence ESSENCE, a new modular adaptive diagnostic platform technology, mitigates fouling, decreases artifacts in the measurable signal, and significantly lowers false-positive and false negatives.

II. Method and Materials

A. Chemical and Instruments

Standard glass slides (1304G) are ordered from Globe Scientific Inc. (USA). De-ionized (DI) water is obtained from a Milli Q Direct Water Purification System. Double-sided tapes (90880 and 90106AB) with SR-26 silicone and MA-69 medical-grade acrylic pressure-sensitive adhesives on both sides are obtained from ARcare, USA. The tape thickness is 142 µm (90880) and 140 µm (90106AB), including the PP layer and the two adhesives. The 4294A Precision Impedance Analyzer from Keysight Technologies is used for the EIS measurements. Carboxylic acid-functionalized short single-walled carbon nanotube (SWCNT, 98%+) are acquired from US Research Nanomaterials Inc. Fabrication of the top, and bottom microelectrode glass slide is carried out in the Nano-fabrication facility at CUNY Advanced Science Research Center. Cricut® Maker™ cuts the channel tapes. The Human Epidermal growth factor Receptor 2 (HER2) recombinant protein is acquired from Prosci-inc. The capturing antibody and tumor protein p53 are obtained from the Abcam. Probe-DNA (pDNA), target-DNA (tDNA), and mismatched-DNA (CMDNA) oligo sequence of 5'-/5AmMC6/CGTCCAAGCGGGCTGACTCATCAAG-3', 5'-CTT GATGAGTCAGCCCGCTTGGACG-3', and 5'-CGTCCAAGCGGGCTGACTCATCAAG-3', respectively, are acquired from Integrated DNA Technologies (IDT). The Artificial Urine is obtained from Fisher scientific. Analog Discovery 2 is obtained from Adafruit.

B. ESSENCE platform

A complete ESSENCE platform consists of three parts - a replaceable/reusable microfluidic chip, a fully automatic fluidic controlling system, and Electrical impedance spectroscopy (EIS) machine. The fluidic platform contains a valve and a pump to automatically manipulate the sample and buffers' flow and pretreatments. The microfluidic chip in ESSENCE (called PNP-µIDE or packed non-planar interdigitated microelectrode chip) consists of a microfluidic channel cut from a double-sided polymer tape packed with functionalized CNT sandwiched by a top and bottom microelectrode (µE). The packing transducer material CNT is coupled with a capture probe against a specific bio-target. PNP-µIDE is assembled at room temperature just before an experimental run. The frequency for EIS measurements is 1 kHz to 100 MHz and takes about $1/10^{th}$ of a second.

C. Chip Protocol

ESSENCE's detecting procedure protocol (**Figure** 2) has three steps, namely, 1) initial buffer signal, 2) target molecule capture, 3) rinsing chip using the fluidic shear force and acquiring the detection signal. EIS is used for detecting the attachment of the target molecule. The Nyquist plot from the EIS signal is fitted to a two-electrode equivalent circuit to obtain the detection signal. This platform has been used to detect different molecules, including target DNA (TDNA),

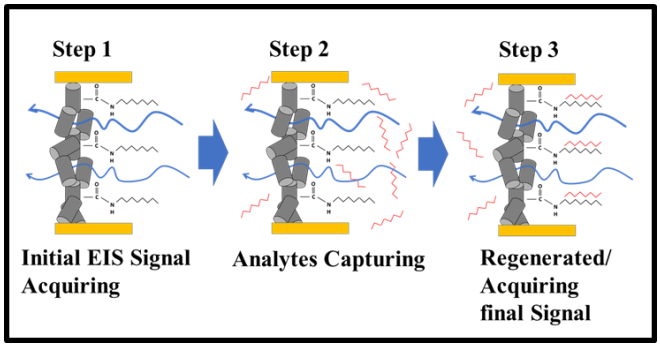

Figure 2 (A) ESSENCE detecting step. 1) Initial EIS signal is acquired after the system is stabilized 2) In Analytes Capturing is a stage that the unknown target molecule is flowed through the PNP-µIDE chip 3) Regenerated/Final signal is to acquire the change in the EIS signal due to target molecule capture. Step 1 and 3 have the same buffer solution.

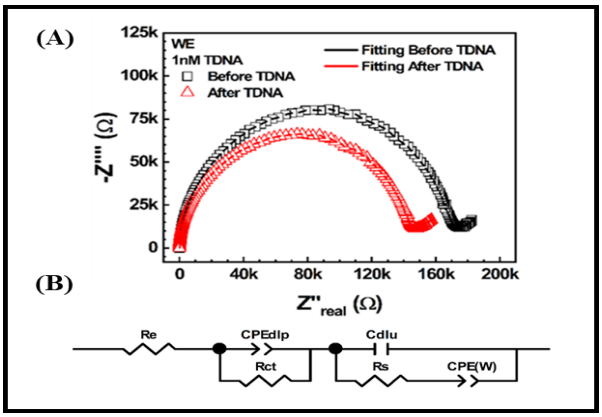

Figure 3 (A) EIS Nyquist curve showing the signal response of passing 1 nM Target DNA though the ESSENCE platform for step 1 (before TDNA) and step 3 (after TDNA) as shown in protocol in figure 2 **(B)** Equivalent circuit

Proteins like the cancer biomarker p53 and is being extended to mRNA and biomolecules like Dopamine that do not have a specific capture molecule associated with it.

III. Result and Discussion

The ESSENCE platform is a modular POC device that can be used against multiple targets. Due to its flow-through, porous, 3D electrode, and enhanced shear design, the signal from ESSENCE is highly selective and sensitive, reducing false positives and false negatives.

A. Signal acquisition and analysis

The impendence data analysis is performed by using Zview software. Both initial and post-target wash EIS data is plotted as a Nyquist Curve (**figure 3(A)**) is fitted with the equivalent circuit shown in **figure 3(B)**. Each component in the equivalent circuit represents physio-chemical processes in the device, as described in detail in our other publications [18]. In brief, **Re:** Inherent resistance in the machine, **Cdlu**: capacitance from clear electrode pair, **Rs:** charge transfer resistance or polarization resistance from empty electrode and solvent. **CPE(W)**: Warburg impedance signal from bare electrode. **CPEdlp**: a constant phase element signal for the packed electrode. **Rct** is the charge transfer resistance from the packed electrode. Among all the parameters, Rct directly reflects the change in the available electrode area due to the target molecule's binding to the capture molecule. This Rct value change is also related to the numbers of target molecules bonding on the packed electrode. Thus, the difference in the Rct is a specific measure of the number of target molecules captured.

The fitting results are shown in **figure 3(C)** and **Table 1**. The Rct value is the only parameter that changes between initial and post-target wash. This also suggests that the system is stable without contamination. The Rct is normalized to reduce the packing variability and use different chips for each DNA ad protein concentration. The normalized signal is the change in the Rct due to target binding divided by the initial Rct as defined in **equation 1:**

$$Normolized\ Rct = \left|\frac{Rct(Initial) - Rct(Post\_art)}{Rct(Initial)}\right| \quad (1)$$

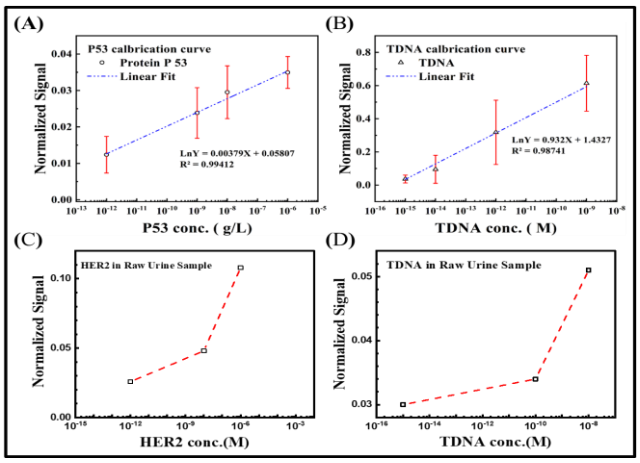

Figure 4. Calibration curve in PBS and Artificial Urine
P53 (A) and TDNA oligo (B). The calibration curves show our device under the handmade control still has a quantitative ability. The lowest P53 signal is 1 pg/L, and the TDNA is 1fM. The R squire value is 0.99412 for P53 and 0.98741 for HER 2. Detections in undiluted artificial urine for target protein (C) HER2, and target DNA (D) TDNA.

Table I Fitting parameters

Circuit Fitting	Fitting parameters									
	Chi-sqr	*Sum-sqr*	*Re*	*CPEdlp-T*	*CPEdlp-P*	*Rct*	*Cdlu*	*Rs*	*CPE(W)-T*	*CPE(W)-p*
Initial	4.92 E-04	0.195	13.51	1.15E-11	0.998	40953	9.25E-12	1.26E+05	5.11E-07	5.18E-01
Post	5.13 E-04	0.203	11.91	1.74E-11	0.998	13748	6.86E-12	1.26E+05	6.03E-07	0.486

These Normalized Rct values create a calibration curve as a standard value from different chips.

B. Selectivity and sensitivity of ESSENCE for target DNA and target protein in PBS and artificial Urine

The calibration curves are shown in **Figure 4** for target protein P53**(Figure 4A),** and target DNA TDNA **(Figure 4B)** has been published elsewhere (Reprinted here with permission from Biosensors and Bioelectronics). Our lowest detectable concentrations from the calibration charts are 10^{-15}M for TDNA and 10^{-13}g/L for P53. Normalization eliminates the variation across the different DNA and protein concentrations due to manual loading of the packing material from chip to chip. The limit of detection (LOD) and limit of quantification (LOQ) using well-established statistical tools are 10^{-7}g/L for P53 and 10^{-11} M for TDNA.

Target molecule detection in artificial urine background is shown in **Figure 4(C)** for target protein HER2 and **Figure 4(D)** for target DNA TDNA. Initial results clearly show that the LOD and LOQ have not changed significantly from PBS to artificial uring for either target protein or target DNA. The HER2 proteins still show a significant change in Rct at 10^{-12}g/L. For TDNA at 10^{-15} M., An optimized protocol that detects target molecules in artificial urine using a higher initial flow rate is investigated in the ESSENCE platform. The detecting time at the higher flow rate is significantly lowered to around 10 mins instead of 3 hours. It can be hypothesized that the time to detection can be reduced further with further optimization. **Figure 5 (A)** shows the pressure data at the start of a run and indicates that the pressure can be stabilized in just a few seconds. **Figure 5 (B)** indicates the background signal in the artificial urine. Analyte flow through the nanoporous layer leads to enhanced shear forces (~ hydrogen bond) mitigating non-specific adsorption. Hence the artificial urine samples by themselves do not have any significant signal showing the potential of ESSENCE as a POC platform.

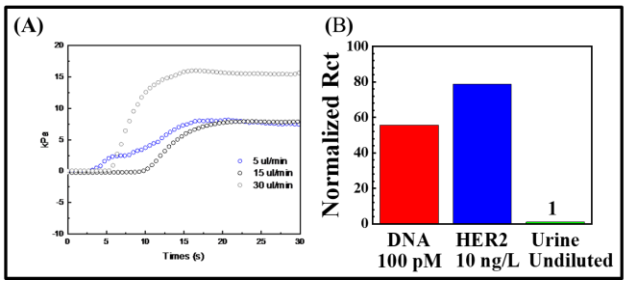

Figure 5. Chip stabilized and background Urine influence
(A) The chip has been pre-washed by different flow rate. The pressure drop indicated the chip can be stabilized just in ~10 seconds instead of 1 hours from our previous publish**. (B)** The Rct changes between each concentration. These data shows pure undiluted artificial Urine does not influence the Rct.

C. ESSENCE cost as a POC platform

The capture antibody is the most expensive cost for ESSENCE. It is $485 for 100 µl, which is enough to do 600 experimental runs, which brings down the cost of capturing antibodies ($0.8/chip). All the other chemicals used in the chip are far cheaper than the capture antibody. Hence, the ESSENCE chip costs about $20 to produce an academic lab like ours. This is significantly cheaper than ELISA ($60 with an additional $34 for supplies per test [19]), PCR ($200 per test [20]), or PCR-ELISA costs $42.30 per patient[20]. In addition, Our electrode pattern with the smallest electrode feature of 10 µm and gold electrode thickness of 100 nm is similar to those found in most point-of-care devices. Hence these electrode features are still within the benchmark resolution for sputter coating, screen-printed[21], or inject-printed[22] electrodes. Further, the ESSENCE platform is a fully automatically detecting POC platform. These give ESSENCE tremendous value in the clinical field.

IV. Conclusion

The ESSENCE, a modular platform, can detect different target molecules by switching different PNP-IdµE. It can detect target DNA and target protein in the artificial urine. The LOD and LOQ in the PBS are 10^{-7}g/L for P53 and 10^{-11} M for TDNA. In synthetic urine, the LOD and LOQ do not change. The whole detection time can be optimized to 10 minutes, and the chip running fluidic protocol to signal acquisition can be fully automated without human interference. Compared to most advanced relevant planar electrode sensing technologies, the ESSENCE platform has benefits mainly (I) the electrode nanoporosity improves selectivity by mitigating non-specific adsorption (II) the PNP-IdµE design fosters nanoconfinement effects and improves the signal to noise ratio (SNR), resulting in high sensitivity; (III) the PNP-IdµE architecture drastically reduces the distance between the adsorbed analyte and the sensing element improving the quality of the measurable signal (IV) controllable shear force enables a focused selectivity. Thus, ESSENCE shows excellent promise as a new modular POC platform in clinical settings.

V. Acknowledgment

This research is supported by Sagnik Basuray's NSF grant # 1751795, Career: "ASSURED" electrochemical platform for multiplexed detection of Cancer Biomarker Panel using Shear Enhanced Nanoporous Capacitive Electrodes and a New Jersey Health Foundation Grant, # PC 54-20, "ESSENCE - A Selective and Sensitive Electrochemical POC Platform for Liquid Biopsy."

References

1. Morales-Narvaez, E. and C. Dincer, *The impact of biosensing in a pandemic outbreak: COVID-19.* Biosens Bioelectron, 2020. **163**: p. 112274.
2. Hernández-Huerta PhD, M.T., et al., *Should RT-PCR be considered a gold standard in the diagnosis of COVID-19?* Journal of Medical Virology, 2021. **93**(1): p. 137-138.
3. *CDC diagnostic tests for COVID-19.* [cited 2021 Nov. 5]; Available from: https://stacks.cdc.gov/view/cdc/108676.
4. Syal, K., *Guidelines on newly identified limitations of diagnostic tools for COVID-19 and consequences.* J Med Virol, 2021. **93**(4): p. 1837-1842.
5. Sidiq, Z., et al., *Benefits and limitations of serological assays in COVID-19 infection.* Indian Journal of Tuberculosis, 2020. **67**(4, Supplement): p. S163-S166.
6. Meisam, O., et al., *A Label-Free Detection of Biomolecules Using Micromechanical Biosensors.* Chinese Physics Letters, 2013. **30**: p. 068701.
7. Kuswandi, B., R. Andres, and R. Narayanaswamy, *Optical fiber biosensors based on immobilized enzymes.* Analyst, 2001. **126**(8): p. 1469-1491.
8. Sharma, S., H. Byrne, and Richard J. O'Kennedy, *Antibodies and antibody-derived analytical biosensors.* Essays in Biochemistry, 2016. **60**(1): p. 9-18.
9. Antiochia, R., *Developments in biosensors for CoV detection and future trends.* Biosens Bioelectron, 2020. **173**: p. 112777.
10. *Handbook of Cell Biosensors*. Springer, Cham.
11. Purohit, B., et al., *Biosensor nanoengineering: Design, operation, and implementation for biomolecular analysis.* Sensors International, 2020. **1**.
12. Xu, J. and H. Lee, *Anti-Biofouling Strategies for Long-Term Continuous Use of Implantable Biosensors.* Chemosensors, 2020. **8**(3).
13. Falkowski, P., Z. Lukaszewski, and E. Gorodkiewicz, *Potential of surface plasmon resonance biosensors in cancer detection.* J Pharm Biomed Anal, 2021. **194**: p. 113802.
14. Sharma, A., et al., *Designed Strategies for Fluorescence-Based Biosensors for the Detection of Mycotoxins.* Toxins (Basel), 2018. **10**(5).
15. Cesewski, E. and B.N. Johnson, *Electrochemical biosensors for pathogen detection.* Biosens Bioelectron, 2020. **159**: p. 112214.
16. Kaya, H.O., et al., *Pathogen detection with electrochemical biosensors: Advantages, challenges and future perspectives.* J Electroanal Chem (Lausanne), 2021. **882**: p. 114989.
17. Menon, S., et al., *Recent advances and challenges in electrochemical biosensors for emerging and re-emerging infectious diseases.* J Electroanal Chem (Lausanne), 2020. **878**: p. 114596.
18. Cheng, Y.-H., et al., *ESSENCE – A rapid, shear-enhanced, flow-through, capacitive electrochemical platform for rapid detection of biomolecules.* Biosensors and Bioelectronics, 2021. **182**.
19. Dalvie, M.A., et al., *Cost analysis of ELISA, solid-phase extraction, and solid-phase microextraction for the monitoring of pesticides in water.* Environ Res, 2005. **98**(1): p. 143-50.
20. Sammy Saab, M., MPH, Timothy Ahn, BS, Terina McDaniel, RN, Beshoy Yanny, MD, and Myron J. Tong, PhD, MD, *Economic Comparison of Serologic and Molecular Screening Strategies for Hepatitis C Virus.* Gastroenterology & Hepatology, August 2018. **14**.
21. Antuna-Jimenez, D., et al., *Screen-Printed Electrodes Modified with Metal Nanoparticles for Small Molecule Sensing.* Biosensors (Basel), 2020. **10**(2).
22. Trudeau, C., et al., *All inkjet-printed perovskite-based bolometers.* npj Flexible Electronics, 2020. **4**(1).