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Cutting-Edge Advancements in EIS Technologies for Rapid Detection of Pathogenic Bacteria in Water

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ABSTRACT

The emerging research surrounding biosensors has seen an unprecedented rise in recent years: from innovative methods of exploiting the bio-recognition event to groundbreaking first-generation designs, work around biosensors is playing a more substantial role in all facets of modern biotechnology. This mini-review explores a type of biosensor that analyzes bio-recognition events through a technique known as electrochemical impedance spectroscopy (EIS). The EIS technique belongs to the electrochemical class of biosensors and is used to examine analyte-electrode interactions through the transfer of electrons. While the technique has proved effective in detecting several virulent bacteria, this review will primarily focus on *E. coli O157:H7*, *L.monocytogenes,* and *S. Typhimurium*. These three pathogens are all highly contagious and capable of causing severe infections and thus must be carefully managed in essential resources such as water. Though existing methods are effective, there are ways EIS biosensing can be further enhanced in terms of accuracy and precision. At the end of the manuscript, we further overview the state-of-the-art challenges and opportunities in EIS.

Introduction

The Earth's surface is 71% covered in water, an irreplaceable substance for all life on this planet [1]. Despite more than two-thirds of the terrain being covered by water, only 0.3% of all water is suitable for human consumption [2]. On top of that, one in three people lives without sanitation and access to safe drinking water [3]. For this reason, one of the 17 global goals agreed to by worldwide leaders is access to clean water for all [4]. Current data shows 771 million people around the globe receiving substandard services to water, with 1 in 4 people lacking access to safely managed drinking water [5, 6]. The *Global Burden of Disease* is a study that assessed the risk factors for diseases and deaths. The study estimates that unsafe water is responsible for 1.2 million deaths each year, which was three times the number of homicides in 2019 [7, 8]. In addition, it is reported that 85,700 under-15 children die from illnesses related to unsafe water [9]. It can, therefore, be reasoned that water should be carefully monitored and managed.

Biosensors are promising devices for maintaining water safety, and they work by detecting chemical components in a target analyte through the use of biological sensing elements. The biosensor is usually made up of three components: 1) a bioreceptor, 2) a transducer, and 3) an interface. The bioreceptor is a macromolecule that attaches to the target analyte, causing a compositional change and generation of electrical signals. The transducer then receives these signals, which are amplified before being processed by the interface. The interface is the final component of a biosensor that collects electrical signals and converts them to data for further analysis [10].

Many existing biosensing techniques have been previously researched (e. g. cyclic voltammetry, integrated microfluidics, PCR, electrochemical impedance spectroscopy, etc.) [11, 12, 13, 14]. Electrochemical

impedance spectroscopy (EIS) is a sensitive technique that utilizes a two-electrode format to measure the impedance in a circuit. EIS offers many advantages over other electrochemical techniques, as it is a steady-state technique that uses small signal analysis, allowing ultra-sensitive detections. Additionally, most EIS designs are simplistic, cost-effective, and highly portable. This technique will be focused on throughout the review when discussing the detection of specific waterborne pathogens.

Multiple reviews have been previously published around EIS techniques to detect pathogens, but none has focused on the detection of pathogenic waterborne bacteria [15, 16, 17. 18. 19]. This brief review attempts to offer a cross-disciplinary outlook on recent studies for detecting three pathogenic waterborne bacteria, namely *Escherichia coli 0157:H7 (E. coli O157:H7)*, *Listeria monocytogenes (L.monocytogenes),* and *Salmonella enterica serovar Typhimurium (S. Typhimurium)* using EIS. In the following section, we present the importance of detection, experimental methods, and resulting limits of detection (LOD) of studies.

Detection of E. coli O157:H7, L.monocytogenes, and S. Typhimurium using EIS

E. coli O157:H7

E. coli O157:H7 is a pathogenic bacteria most commonly contracted by the consumption of contaminated food or water. The gram-negative bacterium is a Shiga toxin-producing *Escherichia coli* (STEC); it produces highly potent toxins that threaten the lining of the intestines and kidneys, causing diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome (HUS) [20]. *E. coli O157:H7* is responsible for up to 73,000 illnesses, 2,200 hospitalizations, and 60 deaths alone in the United States annually [21]. The EIS technique, which has seen much success in previous *E. coli* detection studies, holds great potential for its portability, efficiency, and effectiveness as an early-warning detector of the pathogen in water [22-24].

A study published in 2018 tested multiple immobilization techniques, one of which used ferrocene as an electro-transfer mediator (see Figure 1). The use of ferrocene as an electron-transfer mediator between the *E. coli O157:H7* and a gold electrode yielded very LOD, with results equalling 3 CFU/ml. Though ferrocene is highly conductive, it is still considered a large molecule with a considerable surface area. By treating a standard gold-cysteamine-ferrocene (Au-Cys-Ferrocene-Ab) electrode surface with cysteamine, the energy lost due to its surface area decreased, and LOD was brought even lower. This was shown by changes in the faradaic charge (Q), increasing from 3.98×10^{-5} Coulombs (C) to 3.19×10^{-4} C. When the immunosensors were tested in spiked milk, the system produced highly sensitive measurements [25]. Overall, the study demonstrates the effectiveness of using ferrocene as an electro-transfer mediator and the importance of optimizing the surface chemistry of the electrode to improve LOD.

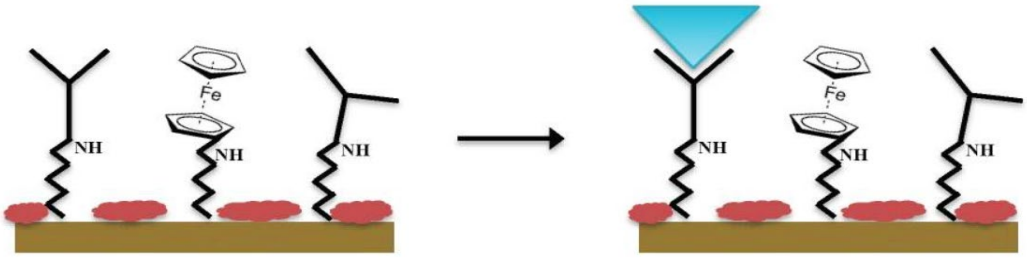

Figure 1. Diagram illustrating the biosensor fabrication of anti-*E. coli* O157:H7 on Au-Cys-Ferrocene-Ab surface. Adapted from Malvano et al. (2018), with permission from PMC.

In a different research, anti-*E. coli* antibodies were immobilized onto a gold electrode through the use of a self-assembled monolayer (SAM) and produced a LOD of 2 CFU/ml. The SAM contained mercaptohexadecanoic acid (MHDA), which created a robust gold-thiolate interaction for the system with an oxidized gold surface [26]. Fluorescence microscopy was used to assess various detection capabilities of antibodies and bacteria. A sandwich technique was used to detect bacteria. In this method, *E. coli* O157:H7 bacteria were placed onto an antibody pattern and then incubated with a secondary FITC-conjugated anti-*E. coli* polyclonal antibody (refer to Figure 2). The antibody microarray only captured *E. coli* O157:H7 bacteria, thereby proving its specificity and binding potency. Furthermore, the absence of bacteria detected outside of the microarray proved that the non-specific adsorption of proteins or bacteria was inhibited. With the combination of the SAM interaction and the efficiency of the sensing platform, the design reported low LODs under laboratory conditions [27].

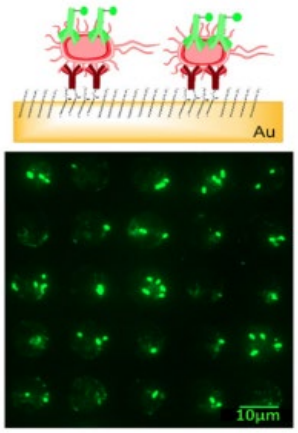

Figure 2. Fluorescence microscopy images of *E. coli* O157:H7, selectively grafted by anti-*E. coli*, presenting low grafting capacity towards a non-specific binding bacteria such as S. Typhimurium. Adapted from Barreiros dos Santos et al. (2013), with permission from Elsevier.

Listeria Monocytogenes

Listeria monocytogenes (L. monocytogenes) is another pathogenic gram-positive bacteria that enters the body through contaminated food and untreated water. *L. monocytogenes* causes an infection known as listeriosis and is most severe in pregnant women, newborns, elderly, and immunocompromised individuals [28]. There are approximately 1,600 people diagnosed with listeriosis annually, and about 260 people die from the infection [29]. Various clinical syndromes have been associated with *L. monocytogenes*, including sepsis, infections in the central nervous system, gastroenteritis, and more [30]. Many techniques (*e. g.* cyclic voltammetry, PCR, RNA) have been innovated to detect *L.monocytogenes*, with EIS having seen increased usage and success rates in recent years [31-33].

A study investigated an immobilization-free EIS biosensor, which combined immunomagnetic separated nanoparticles and urease to detect *Listeria.* The cells were first placed in a urease-AuNP-*Listeria*-MNP complex, which is composed of urease, gold nanoparticles, and magnetic nanoparticles (see Figure 3). Then, the apparatus would be rinsed with polybutylene terephthalate (PBT) and deionized water in a high-gradient magnetic field to remove excess materials and ions. Following this separation, the processed solution is reinjected into the solution as ammonium and carbonate ions to increase the overall ionic strength. Finally, the solution would be measured for impedance changes by an electrochemical workstation with a microfluidic detection chip and an interdigitated microelectrode. Testing on lettuce samples yielded a detection of *Listeria* ranging from 1.6×10 to 6×10^2 CFU/ml. Furthermore, the results from the trials also showed an increase in efficiency, as the time taken for immunomagnetic separation decreased from a conventional 90 minutes down to 30 minutes [34].

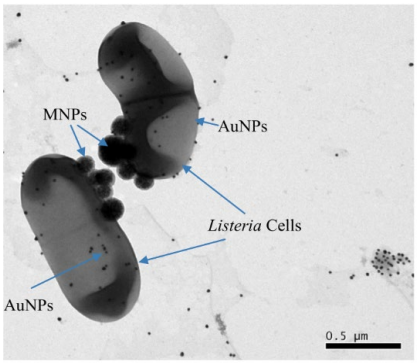

Figure 3. TEM image of the interaction between *L. monocytogenes* cells and MNP-*Listeria*-AuNP complexes. Adapted from Chen et al. (2016), with permission from Elsevier.

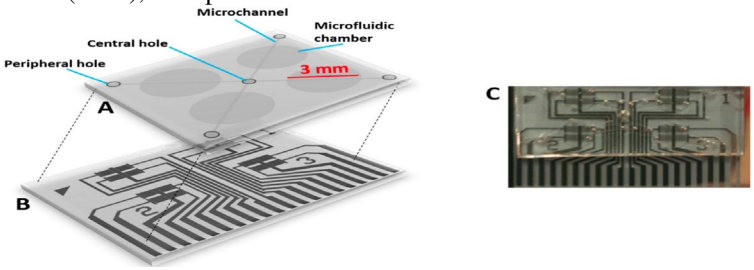

Figure 4. Schematic diagram that shows a) the platform's microfluidic module, including microchambers, microchannels, central and peripheral holes. Beneath this surface contains the b) sensing module with four arrays of microelectrodes, each composed of four pairs of interdigitated electrodes. c) The assembled device with the microfluidic module aligned with the electrode array at the bottom. Adapted from Chiriacò et al. (2018), with permission from MDPI.

Another study developed a miniaturized two-layered EIS biochip for the detection of *L. monocytogenes*. The upper layer of the biochip consists of a microfluidic platform while the lower contained an array of sensing microelectrodes. In this system, the analyte enters through one of the four chambers located near the surface of the microfluidic platform. Then, microchannels connected to the chambers carry the solution down toward the array of gold-interdigitated microelectrodes for impedance detection (refer to Figure 4). The apparatus was designed to be capable of analyzing multiple solutions at once, providing greater flexibility and efficiency. When tested with a contaminated milk sample, the biochip was able to detect *L. monocytogenes* from a LOD of 2.2×10^3 CFU/ml to 1×10^2 CFU/ml [31]. The use of the microfluidic platform enabled researchers to reduce the volume of the sample required for analysis, making the technique suitable for the analysis of specific water samples. Further, the miniaturized design of the biochip allows the biosensor to be highly portable and easy to use.

Salmonella Typhimurium

The other pathogenic bacteria of interest, *Salmonella enterica serovar Typhimurium (S. Typhimurium)*, also enters the body through the consumption of contaminated food and water. This gram-negative bacterium is a common serotype that leads to nontyphoidal salmonellosis (NTS) [35], causing gastroenteritis, bacteremia, and subsequent focal infection. Annually, the bacteria causes 153 million cases of gastroenteritis and up to 57,000 deaths around the globe [36]. Portable and sensitive biosensors like EIS have seen many novel applications in the detection of *S. Typhimurium* in food and water samples [37-40].

A study based on EIS assessed the effects of different sample treatments, including combinations of heat and sonification, to increase detection sensitivity. An *S. Typhimurium* antibody was first immobilized to a screen-printed electrode (SPE) through a cysteamine monolayer (see Figure 5). The activation of this monolayer through glutaraldehyde provided a robust protein immobilization for the antibody [41], thus increasing the measurement sensitivity. Various sample treatments found that heat-treated and sonicated *Salmonella* produced the lowest LOD of 1 × 10^3 CFU/ml with a 20-minute total analysis time. A similar method was later tested with milk but slightly decreased in sensitivity, resulting in a LOD of 9×10^3 CFU/ml [38].

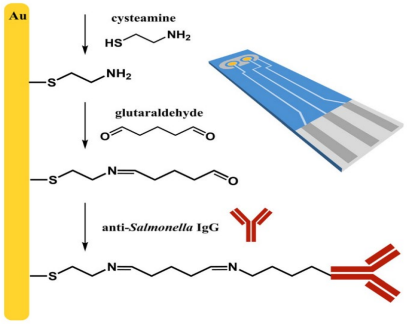

Figure 5. Illustration of antibody immobilization (left); design of SPE electrode (right). Adapted from Fark et al. (2016), with permission from Wiley.

Another study looked into interdigitated microelectrodes using glutathione (H_2O_2) to break down SiO2@MnO2 nanocomposites into Mn2+ for enhanced sensitivity (see Figure 6). The model immobilized monoclonal *S. Typhimurium* antibodies to magnetic beads for separation processes. Subsequently, a sandwich complex was developed by conjugating monoclonal antibodies with SiO2@MnO2 nanocomposites. The samples were then immersed in the H2O2 solution, causing MnO2 to decompose and release Mn2+, which was used as a way to increase the overall conductance of the system. The reduction of MnO2 to Mn2+ by reducing agent glutathione to monitor glucose abundance in biological organisms has been previously studied [40-41]. MnO2-modified SiO2 nanocomposites release Mn2+, which is detected as impedance changes by interdigitated array microelectrodes. Testing this method in spiked milk samples yielded sensitive and efficient detection of *S. Typhimurium*, with a reported LOD of 2.1 × 10 CFU/ml in 45 minutes [38].

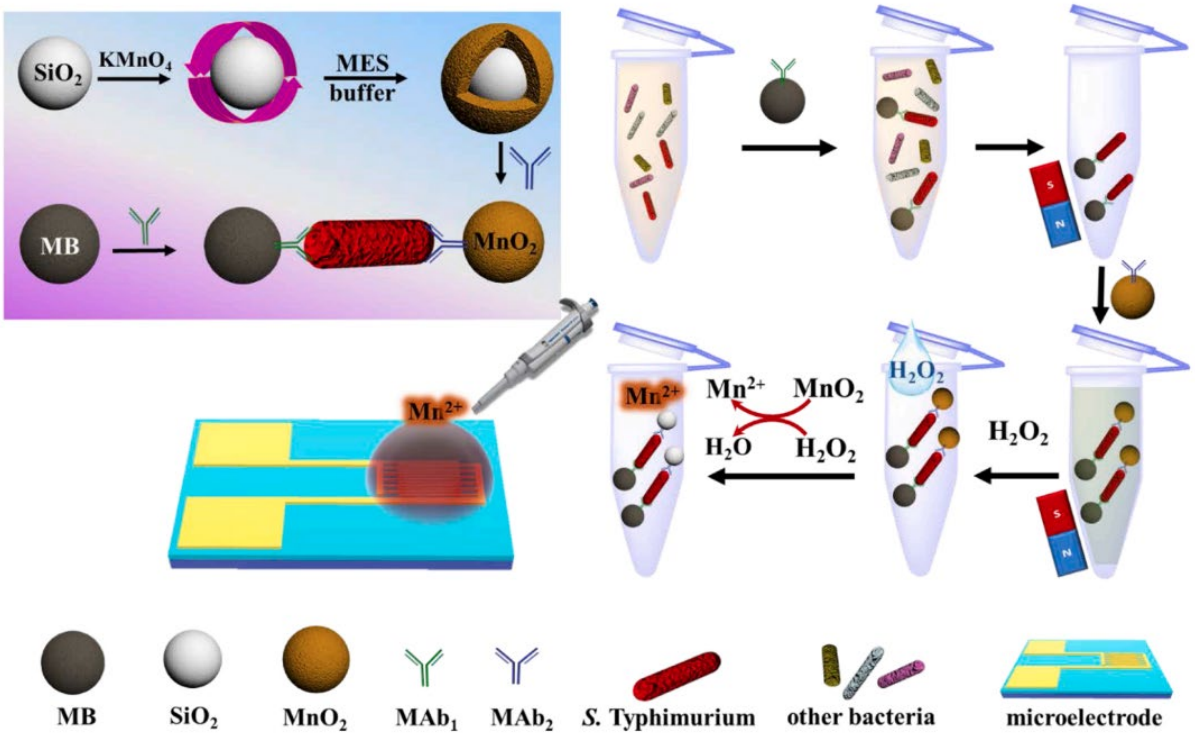

Figure 6. Design of the impedance biosensor for detecting *S. Typhimurium* through immunomagnetic separation and MnO_2 reduction by H_2O_2. Adapted from Wang et al. (2020), with permission from Elsevier.

Discussion and Future Directions

Though a powerful technique for detecting waterborne pathogenic bacteria, applications of EIS face some limitations. The main drawback of the technique is the amount of time and labor required for reliable data. Due to the low signal-to-noise ratio, EIS must be precautionary in label-free assays to avoid false positive results [44]. It is, therefore, necessary to implement extensive experimental controls to avoid errors caused by non-specific binding. Furthermore, it is an expensive technique with many necessary components to perform adequately.

Two future steps for EIS biosensors innovation include 1) increasing surface area for higher detection sensitivity and 2) using intermediate steps to conduct a more successful experiment. The idea behind applying nanocomposite materials to EIS biosensors is to further minimize the possibility of non-specific binding between the analyte and bioreceptor. The implementation of nanocomposites (e.g. Au@Pt-TiO2, Pt@TiO2, Au@TiO2) into EIS increases the surface area of the electrode while improving the conductance of the sensing interface [45]. This allows greater chemical accessibility to the analyte and improves analyte-receptor binding. Additionally, using atomic force microscopy (AFM) imaging to observe binding reactions will improve the accuracy of experimental results. AFM imaging should be an intermediate step in observing binding reactions. It serves as a powerful validation tool to greatly reduce experimental errors.

Conclusion

In this review study, we have highlighted the potential that various EIS techniques have on the detection of waterborne pathogenic bacteria, specifically *E. coli O157:H7, L.monocytogenes,* and *S. Typhimurium*. EIS bi-

osensors are potent tools for on-site quantitative detection of bacteria, and thus serve important roles in improving water quality through sensitive detection in a global setting. Recent advancements in EIS biosensors for the detection of these bacteria have all aimed to lower LOD by increasing electrode surface area and improving electrical conductivity. Further advancements in EIS design are envisaged to enhance detections in similar ways, specifically through the addition of nanocomposite materials and AFM as intermediate steps.

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References

1. *How much water is there on earth?* U.S. Geological Survey. (2019, November 13). Retrieved August 26, 2022, from https://www.usgs.gov/special-topics/water-science-school/science/how-much-water-there-earth

2. *Groundwater: Information on Earth's water*. Default. (n.d.). Retrieved August 26, 2022, from https://www.ngwa.org/what-is-groundwater/About-groundwater/information-on-earths-water

3. World Health Organization. (n.d.). *1 in 3 people globally do not have access to safe drinking water – UNICEF, who*. World Health Organization. Retrieved August 26, 2022, from https://www.who.int/news/item/18-06-2019-1-in-3-people-globally-do-not-have-access-to-safe-drinking-water-unicef-who

4. *Goal 6: Clean water and sanitation*. The Global Goals. (2022, March 15). Retrieved August 26, 2022, from https://www.globalgoals.org/goals/6-clean-water-and-sanitation/

5. Sitecontrol. (2021, July 1). *Access to drinking water*. UNICEF DATA. Retrieved August 26, 2022, from https://data.unicef.org/topic/water-and-sanitation/drinking-water/

6. *Billions of people will lack access to safe water, sanitation and hygiene in 2030 unless progress quadruples – WARN WHO, UNICEF*. UNICEF. (2021, July 1). Retrieved August 26, 2022, from https://www.unicef.org/press-releases/billions-people-will-lack-access-safe-water-sanitation-and-hygiene-2030-unless

7. Stanaway, J. D., Afshin, A., Gakidou, E., Lim, S. S., Abate, D., Abate, K. H., ... & Bleyer, A. (2018). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, *392*(10159), 1923-1994.

8. *Global Burden of Disease*. The Lancet. (n.d.). Retrieved August 26, 2022, from https://www.thelancet.com/gbd

9. United Nations. (2019, March 21). *More children killed by unsafe water, than bullets, says UNICEF chief | | UN news*. United Nations. Retrieved August 26, 2022, from https://news.un.org/en/story/2019/03/1035171

10. Cesewski, E., & Johnson, B. N. (2020). Electrochemical biosensors for pathogen detection. *Biosensors and Bioelectronics*, *159*, 112214.

11. Barghouthi, S. A. (2011). A universal method for the identification of bacteria based on general PCR primers. *Indian journal of microbiology*, *51*(4), 430-444.

12. Sharma, M. K., Goel, A. K., Singh, L., & Rao, V. K. (2006). Immunological biosensor for detection of Vibrio cholerae O1in environmental water samples. *World Journal of Microbiology and Biotechnology*, *22*(11), 1155-1159.

13. Luka, G., Ahmadi, A., Najjaran, H., Alocilja, E., DeRosa, M., Wolthers, K., ... & Hoorfar, M. (2015). Microfluidics integrated biosensors: A leading technology towards lab-on-a-chip and sensing applications. *Sensors*, *15*(12), 30011-30031.

14. Alatraktchi, F. A. A., Breum Andersen, S., Krogh Johansen, H., Molin, S., & Svendsen, W. E. (2016). Fast selective detection of pyocyanin using cyclic voltammetry. *Sensors*, *16*(3), 408.

15. K'Owino, I. O., & Sadik, O. A. (2005). Impedance spectroscopy: a powerful tool for rapid biomolecular screening and cell culture monitoring. *Electroanalysis: An International Journal Devoted to Fundamental and Practical Aspects of Electroanalysis*, *17*(23), 2101-2113.

16. Amiri, M., Bezaatpour, A., Jafari, H., Boukherroub, R., & Szunerits, S. (2018). Electrochemical methodologies for the detection of pathogens. *ACS sensors*, *3*(6), 1069-1086.

17. Yang, L., & Bashir, R. (2008). Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria. *Biotechnology advances*, *26*(2), 135-150.

18. Strong, M. E., Richards, J. R., Torres, M., Beck, C. M., & La Belle, J. T. (2021). Faradaic electrochemical impedance spectroscopy for enhanced analyte detection in diagnostics. *Biosensors and Bioelectronics*, *177*, 112949.

19. Magar, H. S., Hassan, R. Y., & Mulchandani, A. (2021). Electrochemical impedance spectroscopy (EIS): Principles, construction, and biosensing applications. *Sensors*, *21*(19), 6578.

20. Ameer, M. A., Wasey, A., & Salen, P. (2018). Escherichia Coli (E Coli 0157 H7).

21. Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., ... & Tauxe, R. V. (1999). Food-related illness and death in the United States. *Emerging infectious diseases*, *5*(5), 607.

22. Yang, H., Zhou, H., Hao, H., Gong, Q., & Nie, K. (2016). Detection of Escherichia coli with a label-free impedimetric biosensor based on lectin functionalized mixed self-assembled monolayer. *Sensors and Actuators B: Chemical*, *229*, 297-304.

23. Chowdhury, A. D., De, A., Chaudhuri, C. R., Bandyopadhyay, K., & Sen, P. (2012). Label free polyaniline based impedimetric biosensor for detection of E. coli O157: H7 Bacteria. *Sensors and Actuators B: Chemical*, *171*, 916-923.

24. Shi, F., Gan, L., Wang, Y., & Wang, P. (2020). Impedimetric biosensor fabricated with affinity peptides for sensitive detection of Escherichia coli O157: H7. *Biotechnology letters*, *42*(5), 825-832.

25. Malvano, F., Pilloton, R., & Albanese, D. (2018). Sensitive detection of Escherichia coli O157: H7 in food products by impedimetric immunosensors. *Sensors*, *18*(7), 2168.

26. Xue, Y., Li, X., Li, H., & Zhang, W. (2014). Quantifying thiol–gold interactions towards the efficient strength control. *Nature communications*, *5*(1), 1-9.

27. Dos Santos, M. B., Agusil, J. P., Prieto-Simón, B., Sporer, C., Teixeira, V., & Samitier, J. (2013). Highly sensitive detection of pathogen Escherichia coli O157: H7 by electrochemical impedance spectroscopy. *Biosensors and Bioelectronics*, *45*, 174-180.

28. Swaminathan, B., & Gerner-Smidt, P. (2007). The epidemiology of human listeriosis. *Microbes and infection*, *9*(10), 1236-1243.

29. Centers for Disease Control and Prevention. (2022, May 3). *People at risk.* Centers for Disease Control and Prevention. Retrieved August 26, 2022, from https://www.cdc.gov/listeria/risk.html

30. Doganay, M. (2003). Listeriosis: clinical presentation. *FEMS Immunology & Medical Microbiology*, *35*(3), 173-175.

31. Chiriacò, M. S., Parlangeli, I., Sirsi, F., Poltronieri, P., & Primiceri, E. (2018). Impedance sensing platform for detection of the food pathogen listeria monocytogenes. *Electronics*, *7*(12), 347.

32. Radhakrishnan, R., & Poltronieri, P. (2017). Fluorescence-free biosensor methods in detection of food pathogens with a special focus on Listeria monocytogenes. *Biosensors*, *7*(4), 63.

33. Poltronieri, P., Primiceri, E., & Radhakrishnan, R. (2019). EIS-based biosensors in foodborne pathogen detection with a special focus on Listeria monocytogenes. In *Foodborne Bacterial Pathogens* (pp. 87-101). Humana, New York, NY.

34. Chen, Q., Wang, D., Cai, G., Xiong, Y., Li, Y., Wang, M., ... & Lin, J. (2016). Fast and sensitive detection of foodborne pathogen using electrochemical impedance analysis, urease catalysis and microfluidics. *Biosensors and Bioelectronics*, *86*, 770-776.

35. Won, G., & Lee, J. H. (2017). Salmonella Typhimurium, the major causative agent of foodborne illness inactivated by a phage lysis system provides effective protection against lethal challenge by induction of robust cell-mediated immune responses and activation of dendritic cells. *Veterinary research*, *48*(1), 1-12.

36. Healy, J. M., & Bruce, B. B. (n.d.). *Salmonellosis (Nontyphoidal)*. Centers for Disease Control and Prevention. Retrieved August 26, 2022, from https://wwwnc.cdc.gov/travel/yellowbook/2020/travel-related-infectious-diseases/salmonellosis-nontyphoidal

37. Nandakumar, V., La Belle, J. T., Reed, J., Shah, M., Cochran, D., Joshi, L., & Alford, T. L. (2008). A methodology for rapid detection of Salmonella typhimurium using label-free electrochemical impedance spectroscopy. *Biosensors and Bioelectronics*, *24*(4), 1039-1042.

38. Farka, Z., Juřík, T., Pastucha, M., Kovář, D., Lacina, K., & Skládal, P. (2016). Rapid immunosensing of Salmonella typhimurium using electrochemical impedance spectroscopy: the effect of sample treatment. *Electroanalysis*, *28*(8), 1803-1809.

39. Dong, J., Zhao, H., Xu, M., Ma, Q., & Ai, S. (2013). A label-free electrochemical impedance immunosensor based on AuNPs/PAMAM-MWCNT-Chi nanocomposite modified glassy carbon electrode for detection of Salmonella typhimurium in milk. *Food chemistry*, *141*(3), 1980-1986.

40. Wang, S., Peng, T., Meng, Q., Zhu, X., Guo, L., Yao, K., ... & Jiang, H. (2020). Rapid and ultrasensitive detection of Salmonella typhimurium using a novel impedance biosensor based on SiO2@ MnO2 nanocomposites and interdigitated array microelectrodes. *Sensors and Actuators B: Chemical*, *324*, 128654.

41. Ionescu, R. E. (2022). Use of Cysteamine and Glutaraldehyde Chemicals for Robust Functionalization of Substrates with Protein Biomarkers—An Overview on the Construction of Biosensors with Different Transductions. *Biosensors*, *12*(8), 581.

42. Yuan, J., Cen, Y., Kong, X. J., Wu, S., Liu, C. L., Yu, R. Q., & Chu, X. (2015). MnO2-nanosheet-modified upconversion nanosystem for sensitive turn-on fluorescence detection of H2O2 and glucose in blood. *ACS applied materials & interfaces*, *7*(19), 10548-10555.

43. Han, X., Zhou, L., Zhuang, H., Wei, P., Li, F., Jiang, L., & Yi, T. (2022). Hybrid Mesoporous MnO2-Upconversion Nanoparticles for Image-Guided Lung Cancer Spinal Metastasis Therapy. *ACS Applied Materials & Interfaces*, *14*(16), 18031-18042.

44. Garrote, B. L., Santos, A., & Bueno, P. R. (2019). Perspectives on and precautions for the uses of electric spectroscopic methods in label-free biosensing applications. *ACS sensors*, *4*(9), 2216-2227.

45. Mezni, A. (2020). Ternary hybrid Au@ Pt–TiO2 nanocomposites: highly thermally stable photocatalyst with highly efficient visible-light photocatalytic activity. *Journal of Materials Research and Technology*, *9*(6), 15263-15272.