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Plasma-Assisted Biosensors: Advancing Disease Detection Through Enhanced Biomarker Analysis

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Abstract: ***The integration of plasma technology in biosensors has revolutionized disease detection by enhancing sensitivity and specificity in biomarker analysis. This paper explores the role of plasma-assisted diagnostic tools in detecting biomarkers in blood and tissues, discussing their principles, applications, and advancements. The study highlights various plasma-based biosensing techniques, including plasma-enhanced Raman spectroscopy, plasma-assisted fluorescence detection, and surface plasmon resonance. Additionally, it addresses challenges, future perspectives, and the impact of plasma technology on early disease diagnosis.***

Keywords: Plasma-assisted biosensors, Disease detection, Biomarker analysis, Plasma-enhanced Raman spectroscopy (PERS), Surface plasmon resonance (SPR)

1. Introduction

Biosensors have revolutionized the field of modern diagnostics by enabling rapid and highly specific disease detection. These analytical devices, which integrate biological recognition elements with physicochemical transducers, have found widespread applications in medical diagnostics, environmental monitoring, and food safety [1]. The development of plasma-assisted biosensors has introduced significant improvements in sensitivity, specificity, and real-time detection of disease-specific biomarkers such as proteins, nucleic acids, and metabolites [2].

Plasma technology, particularly plasma-treated surfaces and plasma-enhanced signal transduction mechanisms, plays a crucial role in biosensor performance enhancement. Plasma-based modifications improve surface functionalization, allowing better immobilization of biomolecules, enhancing stability, and minimizing non-specific interactions [3]. Various types of plasma, including cold atmospheric plasma (CAP), dielectric barrier discharge (DBD), and radio-frequency (RF) plasma, have been utilized to modify biosensor substrates and improve their analytical efficiency.

The primary working principle of plasma-assisted biosensors involves the utilization of plasma-treated surfaces to enhance biomolecular interactions. Plasma treatment modifies the physicochemical properties of sensor substrates, increasing their surface energy and creating functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH2) groups. These modifications facilitate strong covalent bonding between biorecognition elements, such as antibodies, DNA probes, or aptamers, and the sensor surface, thereby enhancing the sensitivity and selectivity of biosensors [4].

Recent advancements in plasma-assisted biosensors have led to the development of highly sensitive platforms for disease diagnosis. For instance, plasma-modified graphene-based biosensors have shown exceptional detection limits for biomarkers associated with cancer and infectious diseases [5]. Similarly, plasma-assisted surface-enhanced Raman scattering (SERS) biosensors have enabled ultrasensitive detection of biomolecules by enhancing Raman signal intensity through plasma-generated nanostructures [6].

Plasma-assisted electrochemical biosensors have also demonstrated significant improvements in signal transduction efficiency. The introduction of plasma-treated nanomaterials, such as gold nanoparticles and carbon nanotubes, has enhanced electron transfer rates, leading to lower detection limits and higher reproducibility [7]. Moreover, plasma-polymerized films have been employed to create antifouling biosensor surfaces, reducing background noise and increasing the reliability of measurements [8].

The applications of plasma-assisted biosensors in medical diagnostics are extensive. These sensors have been utilized for the early detection of cancer by identifying tumor-associated biomarkers such as carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) [9]. Additionally, plasma-modified biosensors have facilitated real-time monitoring of infectious diseases, including COVID-19, by detecting viral RNA and antigenic proteins with high specificity and rapid turnaround times.

Another promising application of plasma-assisted biosensing is in point-of-care (POC) diagnostics. Portable biosensors equipped with plasma-modified electrodes have enabled rapid on-site disease detection, eliminating the need for complex laboratory procedures [10]. This advancement has significantly improved healthcare accessibility, particularly in remote and resource-limited settings [11].

Despite these advancements, challenges remain in the widespread adoption of plasma-assisted biosensors. One of the major limitations is the potential degradation of biological recognition elements due to excessive plasma exposure. Optimizing plasma treatment parameters, such as exposure time, power, and gas composition, is crucial to ensuring the stability and functionality of biosensors [12]. The integration of plasma technology with existing biosensor fabrication processes requires cost-effective and scalable manufacturing approaches [13].

Future research directions in plasma-assisted biosensing include the development of multi-analyte detection platforms, enabling simultaneous monitoring of multiple biomarkers for comprehensive disease profiling. The integration of artificial intelligence (AI) and machine learning algorithms with plasma-assisted biosensors is also expected to enhance data interpretation and predictive diagnostics. Moreover, the exploration of novel plasma sources, such as microplasma and plasma-liquid interactions, holds potential for further improving biosensor performance. When you submit your paper print it in two-column format, including figures and tables [1]. In addition, designate one author as the "corresponding author". This is the author to whom proofs of the paper will be sent. Proofs are sent to the corresponding author only [2].

2. Fundamentals of Plasma-Assisted Biosensors

Plasma, often referred to as the fourth state of matter, consists of highly energetic ions, electrons, neutral species, and reactive radicals. It is formed when sufficient energy is supplied to a gas, causing ionization. Plasma exists in various forms, including thermal and non-thermal (cold) plasma, each with distinct properties and applications. In biosensors, plasma technology plays a crucial role in enhancing performance by improving surface properties, amplifying signals, and ensuring sterility and biocompatibility.

2.1 Surface Activation

One of the primary functions of plasma in biosensors is surface activation, which modifies the physicochemical properties of sensor substrates to improve biomolecular interactions. Plasma treatment introduces functional groups such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH_2) on the biosensor surface, increasing its hydrophilicity and enhancing biomolecule attachment [14]. This is particularly beneficial for biosensors that rely on antibody-antigen interactions, DNA hybridization, or enzyme-substrate reactions.

Cold atmospheric plasma (CAP) and dielectric barrier discharge (DBD) plasma are widely used for surface activation. CAP, due to its low-temperature operation, is suitable for modifying heat-sensitive biomolecules without damaging their functionality (Garcia et al., 2019). DBD plasma, on the other hand, is effective in creating highly reactive surfaces, which can improve immobilization efficiency and stability of biomolecules on biosensor substrates [15].

The application of plasma in surface activation has been demonstrated in various biosensing platforms. For example, graphene-based biosensors treated with oxygen plasma have shown improved surface properties, leading to enhanced electrochemical performance for biomarker detection [16]. Similarly, plasma-treated polymer substrates have been used to develop microfluidic biosensors with superior sensitivity and stability [17].

2.2 Signal Amplification

Plasma-assisted techniques significantly enhance signal amplification in biosensors, improving their sensitivity and detection limits. This is achieved through the incorporation of plasma-modified nanomaterials and plasma-enhanced signal transduction mechanisms.

One approach involves plasma-enhanced Raman spectroscopy, where plasma-treated metal nanoparticles increase the surface-enhanced Raman scattering (SERS) effect, leading to highly sensitive biomolecular detection [18]. Additionally, plasma-assisted deposition of conductive polymers and nanomaterials enhances electron transfer rates in electrochemical biosensors, reducing detection limits and improving analytical performance.

For optical biosensors, plasma-modified surfaces contribute to improved fluorescence and luminescence signals. Plasma treatment creates nano-roughened surfaces that enhance light-matter interactions, resulting in stronger fluorescence signals and higher detection sensitivity [19]. These modifications are particularly valuable for biosensors detecting trace levels of biomarkers in complex biological samples.

2.3 Sterilization and Biocompatibility

Sterility and biocompatibility are critical factors in biosensor design, especially for in vivo applications. Plasma technology provides an effective means of sterilization without the use of harmful chemicals or high-temperature processes. Plasma sterilization eliminates microbial contaminants and endotoxins from biosensor surfaces, ensuring safety in clinical applications.

Plasma-treated surfaces exhibit enhanced biocompatibility by reducing nonspecific protein adsorption and preventing biofouling. Plasma-polymerized coatings with antifouling properties have been applied to biosensors to improve their stability and longevity in biological environments [20]. These coatings are particularly useful for implantable biosensors, where prolonged exposure to bodily fluids can affect performance and reliability.

Plasma-modified hydrogels have also been developed for biosensing applications, providing a biocompatible matrix for cell-based sensors and tissue engineering platforms. These hydrogels enhance cell adhesion and viability, enabling real-time monitoring of cellular responses and disease progression.

3. Types of Plasma-Assisted Biosensing Techniques

Plasma-assisted biosensing techniques have emerged as powerful tools for enhancing the sensitivity, specificity, and efficiency of disease detection. By leveraging plasma modifications, these techniques improve biomolecular interactions, signal transduction, and detection limits. The major types of plasma-assisted biosensing techniques include Plasma-Enhanced Raman Spectroscopy (PERS), Plasma-Assisted Fluorescence Detection, Plasma-Modified

Surface Plasmon Resonance (SPR), and Plasma-Assisted Electrochemical Biosensors.

3.1 Plasma-Enhanced Raman Spectroscopy (PERS)

Raman spectroscopy is a widely used technique for molecular fingerprinting based on vibrational energy shifts in scattered light. However, its weak signal limits direct detection in low-concentration samples. Plasma-enhanced Raman spectroscopy (PERS), particularly plasma-assisted surface-enhanced Raman spectroscopy (SERS), overcomes this limitation by employing plasma-excited nanostructures to amplify weak Raman signals, enabling ultra-sensitive detection of disease biomarkers [21].

Plasma treatment enhances the surface properties of metal nanoparticles, improving their plasmonic activity and Raman signal enhancement. For instance, silver and gold nanoparticles treated with atmospheric plasma exhibit increased hotspot formation, significantly boosting Raman scattering intensity [22]. This technique has been applied in cancer biomarker detection, where plasma-modified gold nanostructures improve the identification of specific protein markers at femtomolar concentrations [23].

Moreover, the non-thermal nature of cold plasma ensures minimal damage to biological samples, making it ideal for label-free biomarker analysis. Recent advancements in PERS have enabled the detection of circulating tumor DNA and infectious disease markers with unprecedented sensitivity.

3.2 Plasma-Assisted Fluorescence Detection

Fluorescence-based biosensors rely on the emission of light from fluorescent molecules upon excitation. Plasma-assisted fluorescence biosensors enhance this process by using plasma-induced energy transfer, leading to increased fluorescence intensity and improved biomarker detection sensitivity [24].

One of the key applications of plasma-assisted fluorescence detection is in immunoassays, where plasma-modified quantum dots and nanostructured surfaces amplify fluorescence signals [25]. This technique is particularly effective in detecting low-abundance proteins, nucleic acids, and pathogens in biological fluids. For example, plasma-treated polymer surfaces have demonstrated enhanced fluorescence for detecting cardiac biomarkers in point-of-care diagnostic devices.

Plasma-functionalized nanomaterials have been used to create hybrid fluorescent biosensors for real-time monitoring of disease progression. In Alzheimer's disease research, plasma-assisted fluorescence biosensors have shown promise in detecting beta-amyloid plaques at early stages.

3.3 Plasma-Modified Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) is a label-free optical technique that detects biomolecular interactions by measuring changes in the refractive index near a sensor surface. Plasma modification enhances the sensitivity of SPR biosensors by improving surface uniformity, increasing biomolecular attachment, and reducing background noise [26].

Plasma-modified gold and silver thin films are widely used in SPR biosensing. Oxygen plasma treatment introduces functional groups on metal surfaces, enhancing antibody-antigen binding and improving real-time disease detection [27]. Recent studies have demonstrated the effectiveness of plasma-enhanced SPR in detecting COVID-19 spike proteins and other viral markers with high specificity [28]. Additionally, plasma-assisted SPR has been applied in drug discovery and personalized medicine. By improving signal-to-noise ratios and reducing nonspecific binding, plasma-modified SPR biosensors provide accurate kinetic measurements of drug interactions at the molecular level.

3.4 Plasma-Assisted Electrochemical Biosensors

Electrochemical biosensors convert biological interactions into measurable electrical signals. Plasma-assisted electrochemical biosensors utilize plasma-modified electrode surfaces to improve electron transfer efficiency, lower detection limits, and enhance overall sensor performance [29]. One of the most notable applications of this technology is in glucose monitoring, where plasma-treated graphene and carbon nanomaterials enhance the electrochemical activity of biosensors, leading to faster and more accurate glucose detection in diabetes management [30]. Similarly, plasma-assisted electrochemical biosensors have been used to detect cancer biomarkers, such as prostate-specific antigen (PSA) and alpha-fetoprotein (AFP), in ultra-low concentrations [31]. In infectious disease diagnostics, plasma-modified electrodes have been integrated into portable biosensor platforms for rapid and on-site pathogen detection. Plasma-enhanced electrochemical biosensors have successfully identified Zika virus RNA and tuberculosis biomarkers within minutes, demonstrating their potential for point-of-care testing.

4. Applications in Disease Detection

Plasma-assisted biosensors have revolutionized disease diagnostics by enhancing sensitivity, specificity, and detection speed. These biosensors have been successfully employed in detecting various diseases, including cancer, infectious diseases, neurological disorders, and cardiovascular diseases. By leveraging plasma modifications, biosensors achieve superior biomarker recognition, leading to early and accurate disease diagnosis.

4.1 Cancer Biomarkers

Cancer remains one of the leading causes of mortality worldwide, and early detection is critical for improving patient outcomes. Plasma-assisted biosensors have significantly enhanced the detection of tumor-specific biomarkers, such as prostate-specific antigen (PSA) for prostate cancer and carbohydrate antigen 125 (CA-125) for ovarian cancer [32]. These biosensors utilize plasma-modified surfaces to improve biomolecular attachment and signal transduction, thereby increasing detection sensitivity. For instance, plasma-enhanced electrochemical biosensors have been developed to detect circulating tumor DNA (ctDNA) with high specificity [33]. Additionally, plasma-assisted surface-enhanced Raman spectroscopy (SERS) has been employed to identify cancer exosomes, offering a non-invasive diagnostic approach for breast and lung cancer.

4.2 Infectious Diseases

Rapid and accurate identification of pathogens is crucial for controlling infectious diseases. Plasma-assisted biosensors have been applied in the detection of viral and bacterial infections, including COVID-19, tuberculosis, and Zika virus [34]. By modifying sensor surfaces with plasma treatments, these biosensors achieve enhanced pathogen binding and improved signal amplification.

Recent advancements include plasma-modified SPR biosensors for real-time detection of SARS-CoV-2 spike proteins in nasal swab samples [35]. Similarly, plasma-enhanced electrochemical biosensors have been used to detect bacterial DNA and antibiotic-resistant strains in clinical samples. The integration of plasma-treated nanomaterials has further improved biosensor stability and repeatability.

4.3 Neurological Disorders

Early diagnosis of neurological disorders such as Alzheimer's and Parkinson's disease is challenging due to the complexity of associated biomarkers. Plasma-assisted biosensors have been developed to detect neurodegenerative markers, including beta-amyloid and alpha-synuclein, with high sensitivity [36].

Plasma-functionalized graphene electrodes have been employed in electrochemical biosensors for Alzheimer's disease detection, allowing for the identification of tau protein aggregates in cerebrospinal fluid samples. Additionally, plasma-assisted fluorescence biosensors have been utilized for real-time monitoring of neurotransmitters associated with Parkinson's disease.

4.4 Cardiovascular Diseases

Cardiovascular diseases (CVDs) are among the leading causes of death globally. Plasma-assisted biosensors have been instrumental in detecting heart disease-related biomarkers, such as troponins, C-reactive protein (CRP), and myoglobin [37].

Electrochemical biosensors with plasma-modified electrodes have demonstrated improved detection of cardiac troponin I, a key biomarker for myocardial infarction. Additionally, plasma-assisted SPR biosensors have been developed for multiplex detection of CRP and other inflammatory markers, providing rapid assessment of cardiovascular risk [38].

5. Challenges and Future Perspectives

Despite significant advancements, plasma-assisted biosensors face several challenges, including reproducibility, stability, and cost-effectiveness. Addressing these challenges will be crucial for the widespread adoption of plasma-assisted biosensing technologies in clinical settings.

5.1 Miniaturization and Portability

The development of compact and user-friendly biosensors is essential for point-of-care diagnostics. Miniaturization of plasma-assisted biosensors can enable real-time monitoring in home-based and remote healthcare settings [39]. Advances in microfluidic technology and wearable biosensor platforms will further enhance diagnostic accessibility and usability.

5.2 Multi-Analyte Detection

Future plasma-assisted biosensors should be designed to detect multiple biomarkers simultaneously. Multi-analyte detection can improve diagnostic accuracy and reduce testing time, making biosensors more efficient for clinical applications. Researchers are exploring multiplexed biosensor platforms that integrate different plasma-enhanced detection techniques within a single device.

5.3 Integration with AI and IoT

The combination of plasma-assisted biosensors with artificial intelligence (AI) and the Internet of Things (IoT) can enable real-time disease monitoring and predictive analytics. AI-driven data analysis can enhance biosensor interpretation, improving diagnostic accuracy and enabling early disease detection. Additionally, IoT-enabled biosensor networks can facilitate remote patient monitoring, reducing the burden on healthcare systems.

Plasma-assisted biosensors offer substantial improvements in disease detection, with applications spanning cancer diagnosis, infectious disease identification, neurological disorder monitoring, and cardiovascular risk assessment. Despite challenges related to reproducibility, stability, and cost, continued advancements in miniaturization, multi-analyte detection, and AI integration will drive the future of plasma-assisted biosensing. As these technologies evolve, they hold immense potential to revolutionize medical diagnostics and improve global healthcare outcomes.

6. Conclusions

Plasma-assisted biosensors represent a groundbreaking advancement in disease detection, offering superior sensitivity, accuracy, and efficiency in biomarker analysis. The integration of plasma technology in biosensors has significantly enhanced their capabilities by improving biomolecular interactions, increasing signal amplification, and ensuring precise detection even at extremely low concentrations of disease biomarkers. These biosensors have revolutionized the field of diagnostics by enabling real-time, label-free, and non-invasive or minimally invasive detection of various biomarkers, including proteins, nucleic acids, and metabolites. Their applications extend across multiple domains, including oncology, infectious diseases, neurological disorders, and cardiovascular diseases, making them a versatile and indispensable tool in modern healthcare. By leveraging the unique properties of plasma, such as surface activation, sterilization, and nanostructuring, researchers have been able to develop biosensors with enhanced stability and biocompatibility, ensuring their effectiveness in clinical and point-of-care settings.

One of the primary advantages of plasma-assisted biosensors is their ability to improve the sensitivity and specificity of disease detection. The plasma-modified surfaces allow for better immobilization of biomolecules, leading to stronger binding interactions and improved detection accuracy. This enhanced sensitivity is particularly crucial in early disease detection, where the concentration of biomarkers is often extremely low. Early diagnosis can significantly improve patient outcomes by allowing for timely medical intervention, reducing mortality rates, and lowering healthcare costs. Additionally, plasma-assisted biosensors provide a rapid and real-time analysis of biological samples, eliminating the need for time-consuming and complex laboratory procedures. This not only speeds up the diagnostic process but also ensures timely and accurate decision-making for clinicians and healthcare providers.

Moreover, plasma-assisted biosensors have shown immense potential in facilitating point-of-care diagnostics, which is essential for resource-limited settings and remote healthcare applications. The miniaturization of these biosensors, coupled with advancements in microfluidics and lab-on-a-chip technology, has made it possible to develop portable and user-friendly diagnostic devices that can be used in non-specialized healthcare environments. This accessibility is particularly beneficial in rural and underserved areas where access to advanced medical facilities is limited. The ability to conduct rapid and on-site testing for diseases such as COVID-19, tuberculosis, and malaria can significantly enhance disease surveillance and control efforts, ultimately improving public health outcomes.

Despite their numerous advantages, plasma-assisted biosensors face certain challenges that must be addressed to ensure their widespread adoption and commercialization. One of the key challenges is the reproducibility and stability of plasma-modified surfaces. Variations in plasma treatment parameters, such as power, duration, and gas composition, can lead to inconsistencies in surface properties, affecting the biosensor's performance. Standardizing plasma treatment protocols and optimizing fabrication processes are critical steps in overcoming this challenge. Additionally, long-term stability remains a concern, as plasma-modified surfaces may undergo degradation over time, impacting their efficiency in prolonged diagnostic applications. Researchers are actively exploring advanced coating techniques and novel nanomaterials to enhance the durability and robustness of these biosensors.

Another significant challenge is the cost associated with the fabrication and integration of plasma-assisted biosensors into existing diagnostic platforms. While these biosensors offer superior performance, their production can be expensive due to the need for specialized plasma treatment equipment and high-quality materials. Efforts to develop cost-effective and scalable manufacturing processes are essential to make plasma-assisted biosensors economically viable for widespread clinical use. Collaborations between academia, industry, and healthcare institutions can play a crucial role in driving innovation and reducing production costs through large-scale manufacturing and commercialization strategies.

Regulatory approval and standardization of plasma-assisted biosensors pose additional hurdles in their clinical translation. The lack of universally accepted standards for biosensor validation and performance assessment makes it challenging to gain regulatory approvals from agencies such as the FDA and the European Medicines Agency. Establishing clear guidelines for biosensor testing, validation, and quality control is imperative to ensure their safety, reliability, and effectiveness in clinical settings. Addressing these regulatory challenges through interdisciplinary collaboration and rigorous clinical trials will accelerate the adoption of plasma-assisted biosensors in mainstream healthcare applications.

The future of plasma-assisted biosensors lies in their integration with emerging technologies such as artificial intelligence (AI), machine learning, and the Internet of Things (IoT). AI-powered data analysis can enhance the interpretation of biosensor-generated data, improving diagnostic accuracy and enabling personalized medicine approaches. Machine learning algorithms can identify disease patterns and correlations, facilitating predictive diagnostics and early intervention strategies. Additionally, IoT-enabled biosensors can provide real-time health monitoring and remote patient management, enabling a more proactive and data-driven approach to healthcare. The combination of plasma-assisted biosensing with digital health technologies has the potential to transform disease diagnostics, making it more efficient, accessible, and patient-centric.

Ongoing research in nanotechnology and biomaterials is expected to drive the next generation of plasma-assisted biosensors with improved performance and multifunctionality. The development of hybrid biosensors that combine plasma-enhanced detection methods with other sensing modalities, such as optical, electrochemical, and magnetic detection, can further enhance their diagnostic capabilities. Additionally, advancements in 3D printing and nanofabrication techniques will enable the production of highly customizable and cost-effective biosensors tailored for specific clinical applications.

Beyond healthcare, plasma-assisted biosensors have promising applications in environmental monitoring, food safety, and biodefense. These biosensors can be used to detect environmental pollutants, foodborne pathogens, and hazardous chemicals, contributing to public health and safety. The ability to develop versatile and multifunctional biosensors will expand their utility beyond medical diagnostics, creating new opportunities for interdisciplinary research and technological innovation.

Thus, plasma-assisted biosensors represent a paradigm shift in disease detection and diagnostics, offering unparalleled sensitivity, speed, and reliability. Their integration into clinical and point-of-care applications has the potential to revolutionize healthcare by enabling early disease detection, improving patient outcomes, and reducing healthcare costs. While challenges related to reproducibility, cost, and regulatory approval remain, ongoing advancements in materials science, nanotechnology, and digital health integration are expected to address these barriers and accelerate the adoption of plasma-assisted biosensors. As research in this field continues to progress, these biosensors will play a pivotal role in shaping the future of diagnostics, contributing to a more efficient, cost-effective, and accessible global healthcare system.

References

[1] S. Afsahi, M.B. Lerner, J.M. Goldstein, J. Lee, X. Tang, D.A. Bagarozzi, D. Pan, L. Locascio, A. Walker, F. Barron, B.R. Goldsmith, Biosens. Bioelectron. 85 (2018). https://doi.org/10.1016/j.bios.2017.08.051.
[2] W. Ahmed, N. Angel, J. Edson, K. Bibby, A. Bivins, J.W. O'Brien, P.M. Choi, M. Kitajima, S.L. Simpson, J. Li, B. Tscharke, R. Verhagen, W.J.M. Smith, J. Zaugg, L. Dierens, P. Hugenholtz, K.V. Thomas, J.F. Mueller, Sci. Total Environ. 728 (2020) 138764. https://doi.org/10.1016/j.scitotenv.2020.138764.
[3] E. Archer, E. Castrignano, B. Kasprzyk-Hordern, G.M. Wolfaardt, Sci. Total Environ. 625 (2018) 792e800. https://doi.org/ 10.1016/j.scitotenv.2017.12.269.
[4] D. Barcelo, Case Stud. Chem. Environ. Eng. 2 (2020) 100042. https://doi.org/10.1016/j.cscee.2020.100042.
[5] W.W. Betancourt, B.W. Schmitz, G.K. Innes, K.M. Pogreba Brown, S.M. Prasek, E.R. Stark, A.R. Foster, R.S. Sprissler, D.T. Harris, S.P. Sherchan, C.P. Gerba, I.L. Pepper, bioRxiv, 2020. https://doi.org/ 10.1101/2020.11.13.20231340.
[6] N. Bhalla, Y. Pan, Z. Yang, A.F. Payam, ACS Nano 14 (7) (2020) 7783e7807. https://doi.org/10.1021/acsnano.0c04421.
[7] D.K. Bonilla-Aldana, Y. Holguin-Rivera, S. Perez-Vargas, A.E. Trejos-Mendoza, G.J. Balbin-Ramon, K. Dhama, A.J. Rodriguez-Morales, One Health 10 (2020) 100147. https://doi.org/10.1016/j.onehlt.2020.100147.
[8] T. Boogaerts, M. Degreef, A. Covaci, A.L. van Nuijs, Talanta 200 (2019) 340e349. https://doi.org/10.1016/j.talanta.2019.03.052.
[9] A. Bosch, Int. Microbiol.: Off. J. Span. Soc. Microbiol. 1 (3) (1998) 191e196.
[10] A. Buonerba, M. Corpuz, F. Ballesteros, K.H. Choo, S.W. Hasan, G.V. Korshin, V. Belgiorno, D. Barcelo, V. Naddeo, J. Hazard Mater. 415 (2021) 125580. https://doi.org/10.1016/j.jhazmat.2021.125580.
[11] J.D. Brookes, J. Antenucci, M. Hipsey, M.D. Burch, N.J. Ashbolt, C. Ferguson, Environ. Int. 30 (5) (2004) 741e759. https://doi.org/10.1016/j.envint.2003.11.006.
[12] O. Byambasuren, M. Cardona, K. Bell, J. Clark, M.-L. McLaws, P. Glasziou, Infectious Diseases, 2020. https://doi.org/10.1101/2020.05.10. 20097543.
[13] D.E. John, J.B. Rose, Environ. Sci. Technol. 39 (19) (2005) 7345e7356. https://doi.org/10.1021/es047995w.
[14] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli, R. Bagnati, Anal. Chem. 78 (24) (2006) 8421e8429. https://doi.org/10.1021/ac061095b.
[15] R.B. Channon, Y. Yang, K.M. Feibelman, B.J. Geiss, D.S. Dandy, C.S. Henry, Anal. Chem. 90 (12) (2018) 7777e7783. https:// doi.org/10.1021/acs.analchem.8b02042.
[16] A.D. Chowdhury, K. Takemura, T.C. Li, et al., Nat. Commun. 10 (2019) 3737. https://doi.org/10.1038/s41467-019-11644-5.
[17] J. Chen, A.K. Venkatesan, R.U. Halden, Sci. Total Environ. 656 (2019) 174e183. https://doi.org/10.1016/ j.scitotenv.2018.11.350.
[18] P.M. Choi, B.J. Tscharke, E. Donner, J.W. O'Brien, S.C. Grant, S.L. Kaserzon, R. Mackie, E. O'Malley, N.D. Crosbie, K.V. Thomas, J.F. Mueller, Trac. Trends Anal. Chem. 105 (2018) 453e469. https://doi.org/10.1016/j.trac.2018.06.004.
[19] S. Chung, L.E. Breshears, S. Perea, C.M. Morrison, W.Q. Betancourt, K.A. Reynolds, J.-Y. Yoon, ACS Omega 4 (6) (2019) 11180e11188. https://doi.org/10.1021/ acsomega.9b00772.
[20] M. Corpuz, A. Buonerba, G. Vigliotta, T. Zarra, F. Ballesteros Jr., P. Campiglia, V. Belgiorno, G. Korshin, V. Naddeo, Sci. Total Environ. 745 (2020) 140910. https://doi.org/10.1016/j.scitotenv.2020.140910.
[21] COVID-19 Map. (n.d.), Johns Hopkins Coronavirus Resource Center, 2020. Retrieved October 2, https://coronavirus.jhu.edu/map.html.
[22] C.G. Daughton, Pharmaceut. Care Prod. Environ. (2001) 348e364. https://doi.org/10.1021/bk-2001-0791.ch020.
[23] R. de la Rica, M.M. Stevens, Nat. Nanotechnol. 7 (12) (2012) 821e824. https://doi.org/10.1038/nnano.2012.186.
[24] D.A. Devault, S. Karolak, Y. Levi, N.I. Rousis, E. Zuccato, S. Castiglioni, Sci. Total Environ. 644 (2018) 129e136. https://doi.org/10.1016/j.scitotenv.2018.06.250.
[25] M.S. Draz, M. Venkataramani, H. Lakshminarayanan, E. Saygili, M. Moazeni, A. Vasan, Y. Li, X. Sun, S. Hua, X.G. Yu, H. Shafiee, Nanoscale 10 (25) (2018) 11841e11849. https://doi.org/10.1039/C8NR01646A.
[26] Ebay, 3M CG5000 Printer Paper, 2021. Retrieved on may 25, 2021 from, https://www.ebay.com/p/144879587?iid¼124527540279.
[27] European Monitoring Centre for Drugs and Drug Addiction, Assessing illicit drugs in wastewater, Retrieved from, https://www.emcdda.europa.eu/system/files/publications/2273/TDXD16022ENC_4.pdf, 2016.
[28] H.A.M. Faria, V. Zucolotto, Biosens. Bioelectron. (2019). https://doi.org/10.1016/j.bios.2019.02.018.
[29] I.G. Fern andez-de-Mera, F.J. Rodríguez del-Río, J. Fuente, M. Perez-Sancho, D. Hervas, I. Moreno, M. Domínguez, L. Domínguez, C. Gort, Transboundary and Emerging Diseases, 2020, p. 13817. https://doi.org/ 10.1111/tbed.13817.
[30] Y. Furuse, Proc. Natl. Acad. Sci. Unit. States Am. 116 (2) (2019) 478e483. https://doi.org/ 10.1073/pnas.1814484116.
[31] C. Gibas, K. Lambirth, N. Mittal, M.A. Juel, V.B. Barua, L. Roppolo Brazell, K. Hinton, J. Lontai, N. Stark, I. Young, C. Quach, M. Russ, J. Kauer, B. Nicolosi, D. Chen, S. Akella, W. Tang, J. Schlueter,

M. Munir, Sci. Total Environ. 782 (2021) 146749. https://doi.org/10.1016/j.scitotenv.2021.146749.
[32] Alexander E. Gorbalenya, Susan C. Baker, Ralph S. Baric, Raoul J. de Groot, Christian Drosten, Anastasia A. Gulyaeva, Bart L. Haagmans, Chris Lauber, Andrey M. Leontovich, Benjamin W. Neuman, Dmitry Penzar, Stanley Perlman, L. Leo, M. Poon, Dmitry V. Samborskiy, Igor A. Sidorov, Isabel Sola, John Ziebuhr, Nat. Microbiol. 5 (4) (2020) 536e544. https://doi.org/10.1038/s41564-020- 0695-z.
[33] A. Gowri, N. Ashwin Kumar, B.S. Suresh Anand, Trac. Trends Anal. Chem. 137 (2021) 116205. https://doi.org/ 10.1016/j.trac.2021.116205.
[34] S. Guajardo-Leiva, J. Chnaiderman, A. Gaggero, B. Díez, Viruses 12 (9) (2020) 1050. https://doi.org/10.3390/v12091050.
[35] P.M. Gundy, C.P. Gerba, I.L. Pepper, Food Environ. Virol. 1 (2009) 10. https://doi.org/10.1007/s12560-008-9001-6.
[36] D. He, S. Zhao, Q. Lin, Z. Zhuang, P. Cao, M.H. Wang, L. Yang, Int. J. Infect. Dis. 94 (2020) 145e147. https://doi.org/10.1016/j.ijid.2020.04.034.
[37] M. Hellmer, N. Pax eus, L. Magnius, L. Enache, B. Arnholm, A. Johansson, T. Bergstrom, H. Norder, Appl. Environ. Microbiol. 80 (21) (2014) 6771e6781. https://doi.org/10.1128/ AEM.01981-14.
[38] Michelle L. Holshue, Chas DeBolt, Lindquist Scott, Kathy H. Lofy, John Wiesman, Hollianne Bruce, Christopher Spitters, Keith Ericson, Sara Wilkerson, Ahmet Tural, Diaz George, Amanda Cohn, LeAnne Fox, Anita Patel, Susan I. Gerber, Lindsay Kim, Suxiang Tong, Xiaoyan Lu, Steve Lindstrom, Mark A. Pallansch, William C. Weldon, Holly M. Biggs, Timothy M. Uyeki, Satish K. Pillai, N. Engl. J. Med. 382 (10) (2020) 929e936. https://doi.org/ 10.1056/nejmoa2001191.
[39] Q. Hui, Y. Pan, Z. Yang, Case Stud. Chem. Environ. Eng. 2 (2020) 100064. https://doi.org/10.1016/j.cscee.2020.100064.