International Journal of Engineering Works
https://www.ijew.io/
ISSN-p: 2521-2419
ISSN-e: 2409-2770
Vol. 13, Issue 07, PP. 49-60 July 2026
https://doi.org/10.5281/zenodo.21551530

Recent Advantages of Chemiluminescence-Based Fully Automated Microfluidic Devices in the Detection of Pathogenic Microorganisms

Shah Muhammad Adnan[1], Md Mudassir Chowdhury[1], Daloar Hossan[2], Mohammed Imran Hossain[3], Muhammad Tahir Zaman[4]
[1]College of Mechanical Engineering, Yangzhou University, Yangzhou 225127, Jiangsu, China
[2]College of Information and Artificial Intelligence, Yangzhou University, Yangzhou, 225012, China
[3]College og Mechanical E|ngineering, Yangzhou University, Yangzhou, 225012, China
[4]School of Mechanical Engineering, Southeast University, Nanjing 210096, PR China
Received: 02 April, Revised:21 June, Accepted: 22 July

***Abstract*—** The integration of chemiluminescence and microfluidics has led to significant advances in pathogen detection and represents an excellent platform to build fast, sensitive and miniaturized diagnostic tools. This review provides a comprehensive overview of recent advances in chemiluminescent microfluidic biosensors, describing device integration, signal amplification approaches, and automation for assay performance. With developments including CRISPR-Cas detection, enzyme catalysis, and amplification approaches for isothermal amplification and AI-integrated fluid control, the enhancements offered through next-generation detection systems support improvements in detection sensitivity and throughput. Recent products for pathogens, including E. coli, Listeria monocytogenes, and respiratory viruses, are also discussed with a focus on lab-on-a-disc and smartphone-based diagnostics. Detected limits and/or reproducibility are analyzed, where relevant, to understand performance and considerations for subsequent implementation at scale and real-world applications. Finally, it considers future perspectives in the field with a discussion on standardization of assays, device fabrication costs and regulation, routine (point-of-care) use of chemiluminescent microfluidic systems, and write-up optimization of modules or diagnostic approaches. Considering the above, chemiluminescent microfluidic systems are positioned and act as game-changers or valuable tools for global health surveillance, food safety, and the practice of personalized medicine, providing transformative diagnostics in centralized laboratory settings and low-resource settings.

Keywords: Chemiluminescence, Microfluidics, Pathogen Detection, Point-of-Care Testing (POCT), CRISPR Diagnostics, Nanozymes, Biosensor Automation.

I. Introduction

Microfluidic technology has been a disruptive technology in the diagnostic sector that encompasses manipulating small liquid volumes (most often ranging from microliters to nanoliters) within a network of microchannels. The technology can bring multiple laboratory processes within a single chip and provide a fast and efficient analysis of biological samples. The miniaturization of analytical processes reduces the consumption of reagents and waste and enhances the assay's analysis speed and sensitivity. The emergence of the latest generation of microfluidic devices has set the ground for the technology to be widely applied in clinical diagnostics, environment monitoring, and food analysis. The growing importance of the application of microfluidic technology in the diagnostic sector lies in providing the solution of Point-of-Care Testing (POCT), also termed diagnostic testing at or near the patient site of care that encompasses instant decision-making at the clinician site. The merging of the application of microfluidics with POCT can alter the course of healthcare provision by providing fast, correct, and economical diagnoses, particularly in resource-constrained environments.[1], [2].
Chemiluminescence is a highly sensitive detection technology that involves light emission during a chemical reaction. The technology has been of tremendous interest in biosensing with the promise of highly sensitive detection with minimal noise and the feasibility of real-time detection. The strengths of chemiluminescence technology in biosensing include providing highly sensitive detection with an extensive dynamic and minimal sample preparation required. The promise of chemiluminescence has also been addressed in developing microfluidic biosensors, with a particular emphasis on detecting pathogens. For instance, a study demonstrated the use of a microfluidic chip and chemiluminescence in the detection of the pathogenic strain of Escherichia coli O157:H7 with highly sensitive and specific detection.[3], [4]. A study showcased the use of chemiluminescent microfluidic devices in detecting SARS-CoV-2 with a short detection time, highlighting the promise of such tools in solving public health issues.[5], [6]. The feasibility of analyzing multiple paths in a multiplex format in a microfluidic format further increases the application of such tools in detecting paths with the simultaneous analysis of various paths of particular use in the clinical environment with the need for fast and correct identification of the paths.[7], [8].
Chemiluminescence with the aid of microfluidic technology has been highly promising in detecting disease-causing agents. Using chemiluminescence detection systems with the assistance of microfluidic devices permits the detection of pathogens quickly and sensitively. It forms an integral part of the prompt detection and treatment of the disease. The construction of chemiluminescent reactions-based microfluidic platforms that detect various disease-causing agents such as bacteria and viruses has recently advanced in this study area. One study demonstrated using a microfluidic paper analytical device (μPAD) that uses chemiluminescence detection of rabbit IgG with a detection limit of 3.58 pM.[1]Another study showed

a fully integrated automatic microfluidic PCR-array system that detected 21 pathogens in a fully integrated format within 1.5 hours, demonstrating the efficiency and reliability of such systems in clinical diagnostics.[3]. The use of the capabilities of the microfluidic platforms in the execution of multiplexed assays further increases the use of such systems in the detection of pathogens such that they allow the analysis of multiple pathogens at the same time that is of particular use in the field of clinical diagnostics where prompt and correct identification of the disease is critical.[4], [5], [6]. Microfluidic system automation is vital in making POCT more efficient and reliable. Automated microfluidic instruments can carry out complex assays with minimal human intervention, a lower likelihood of errors, and higher reproducibility than non-automated systems. Automation integration permits workflow simplification, higher throughput, and ease of use, making such systems accessible to non-expert users with minimal training. Recent advances in automated microfluidic systems have proven them useful in various applications, such as detecting Group B Streptococcus and other disease-causing organisms and demonstrate the promise of such systems in changing the field of POCT and healthcare delivery.[6], [7]. An example of such a system includes developing a smartphone-aided microfluidic paper-based analytical device that combines deep learning-assisted offline smartphone detection with the detection of Alzheimer's disease biomarkers and does not need extensive computing tools and cloud computing capabilities.[2]. This development emphasizes the need for automation of the capabilities of the microfluidic device, especially in resource-constrained environments where the need for speedy and correct diagnoses is critical.[8], [9].

In brief, the synergy of chemiluminescence and fully automatic microfluidic device technology has a bright future in detecting disease organisms at a sensitive and speedy scale. The microfluidic device's technology and chemiluminescence's detection strengths put such systems at the cutting edge of diagnostic technology. The demand for efficient and effective POCT will continue to grow, and the development of automatic microfluidic device technology will be critical to enhancing patient care and patient outcomes. The future of this field of study and development will further strengthen the technologies, making them accessible and valuable in the fight against infectious diseases.[10], [11], [12].

II. Chemiluminescence in Microfluidic Detection of Pathogenic Microorganisms

i. Self-Prepared Chemiluminescence-Based Microfluidic Devices

Self-prepared microfluidic devices have been well-accepted in recent years due to their cost-efficiency, high flexibility, and ability to be designed for individualized purposes in pathogen discovery. Microfluid technology employs chemiluminescence, producing light in a chemical process that is highly selective and sensitive in distinguishing various pathogens. The new advances in microfluid technology have enabled various microfluid and lab-made devices to be constructed for individualized purposes in pathogen discovery. For instance, a study by Shang et al. designed a cloth-based microfluidic device for the ultrasensitive detection of Listeria monocytogenes via chemiluminescence. By employing a hemin/G-quadruplex DNAzyme system, in parallel with hybridization chain reaction signal amplification, scientists increased the sensitivity of their detection technique. Data were obtained indicating that the device could detect target DNA from spiked milk samples, supporting their contention that the device could be used with relatively low complexity for food safety applications (Figure 1A)[13].

Kumar et al. disclosed a microfluidic chemiluminescence device fabricated using 3D printing with an integrated and self-sustaining incubation system. The device was validated on microfluidic paper-based analytical devices (μPADs) employing alkaline phosphatase (Figure 1B)[14]. Figure 1C shows the performance analysis of CL intensity versus AAP concentration and the reproducibility assessment across five replicates. For example, Wang et al. made a microfluidic device that measures chemiluminescence detection to identify pathogens rapidly. The chemiluminescence detection device utilizes a reaction chamber that allows a chemiluminescent reaction to occur to detect pathogens sensitively by measuring light emission. The authors also demonstrated that their device could detect low concentrations of pathogens, supporting their point-of-care applications[15]. The device structure of chemiluminescence-based microfluidic systems contains various critical factors, including microfluidic channels, reaction chambers, and optical sensing mechanisms.

Microfluidic Channels

Microfluid channels are vital in controlling the fluid flow in a device. The geometrical shape of these channels is a significant determinant of the mixing efficiency, reaction time, and device operation. The latest microfabrication technologies, for example, 3D printing and soft lithography, have enabled complex channel geometries, enhancing assay functionality and fluid dynamics. For example, roll-to-roll polydimethylsiloxane (PDMS) diagnostic platforms for nucleic acid sensing of multiplex pathogens have been developed by Rannaste et al.[16].

Reaction Chambers

The chemiluminescence reactions occur in microfluidic device reaction chambers. The chambers must be designed in such a manner to create an ideal scenario for the chemical reactions in temperature, pH, and reagent concentrations. The introduction of materials such as nanoparticles of gold in microfluidic chambers has been shown to increase sensitivity in chemiluminescence assays due to improved localized reagent concentrations. The contribution of chamber design in achieving a highly sensitive E. coli O157:H7 microfluidic immunosensor based on SERS is exemplified in a study by Mao et al.[17].

Light Detectors Systems

Light sensing mechanisms are critical in measuring the chemiluminescence signal emitted during reactions. PMTs and CCDs find widespread usage for this, as they can detect traces of emitted light with exquisite sensitivity. Smartphone-enabled systems have been introduced recently to deliver portability and convenience for point-of-care diagnosis. For instance, Dong et al. introduced a point-of-care nucleic acid amplification test based on a smartphone interface for online monitoring of chemiluminescence signals[18]. The progress of microfluidic devices based on chemiluminescence is an excellent advancement in pathogen detection technologies. With new materials and designs, these microfluidic devices have been made highly flexible, specific, and sensitive for various purposes, especially in resource-limited settings. Future studies and advancements in this technology can make these devices more powerful, opening doors for enhanced diagnosis in clinical and environmental contexts.

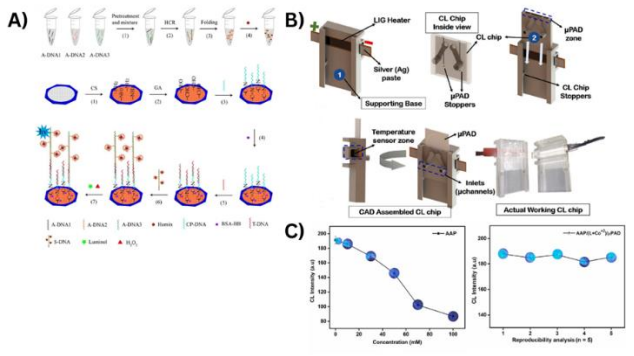

Figure 1. Chemiluminescence-Based Microfluidic Platforms for Pathogen Detection

The Figure 1.shows that Chemiluminescence-Based Microfluidic Platforms for Pathogen Detection Using μPAD and DNAzyme Strategies A) Schematic representation of a hemin/G-quadruplex DNAzyme-based CL detection strategy utilizing HCR amplification and luminol–H_2O_2 chemiluminescent reaction[13]. B) A CL microfluidic chip's structural and functional design integrates laser-induced graphene (LIG) heaters and μPAD zones for temperature control and fluid flow[14]. C) Performance analysis showing CL intensity versus AAP concentration and reproducibility assessment across five replicates[14].

Some studies have proved that self-prepared chemiluminescence-based microdevices effectively detect a broad spectrum of pathogenic microbes. The technological advancement in microfluidics has successfully implemented microfluidic molecular pathogen detection by integrating chemiluminescence and nucleic-acid-based detection schemes, allowing detection at increased sensitivity and decreased time. Abbas et al. reported on several point-of-care diagnostics developed with microfluidics for E. coli O157:H7 detection. They listed that incorporating chemiluminescence into microfluidic designs could improve detection sensitivity while reducing the time to complete an assay[19]. Moreover, Xing et al. introduced a finger-actuated microfluidic biosensor for multiplexed detection of foodborne pathogens by utilizing CRISPR/Cas12a with recombinase-aided amplification (RAA). Foodborne pathogen detection was confirmed under a limit of 500 CFU/mL, indicating that this device could be adapted to provide rapid diagnostics in the field[20]. In food safety, Shang et al. (2020) created a cloth-based microfluidic chip with a chemiluminescent assay for ultra-sensitive detection of Listeria monocytogenes, demonstrating a limit of detection of 1.5 copies/μL[13]. Similarly, Li et al. (2023) developed an automated microfluidic nucleic acid detection platform that integrated rich recombinase polymerase amplification (RPA) with CRISPRCas13a for rapid and accurate detection of pathogens with a reported sensitivity of 8 copies/reaction[21]. The examples mentioned illustrate the development of microfluidic diagnostics and the possibilities for rapid detection of infectious agents in a timely, accurate, and portable manner across various settings.

The potential for self-prepared chemiluminescence-based microfluidic devices in rapidly and sensitively detecting pathogenic microorganisms has garnered significant attention. Customizing these tools lets researchers change design, materials, and detection techniques to maximize performance for certain infections, providing many benefits. Emphasizing the flexibility of self-prepared systems, Kumar et al. showed a 3D-printed microfluidic device tailored for alkaline phosphatase detection[22]. Cost-effectiveness is another significant benefit since low-cost materials and manufacturing techniques like 3D printing and soft lithography lower manufacturing costs and enable these devices to be practical in environments with limited resources. Zheng et al. underlined the financial advantages of an automated microfluidic platform that combines several purposes into one device, reducing costly equipment requirements [23]. Fast iteration of new diagnostic designs made possible by rapid development and prototyping is also essential for addressing new infectious illnesses. Developing a wave-shaped microfluidic chip that permitted simultaneous detection of several biomarkers, Yin et al. showed how rapidly novel diagnostic tools may be generated[24]. These systems also offer sensitivity and specificity through improved test settings and sophisticated detection methods. Ghosh et al. presented a microchannel capillary flow assay platform with a malaria detection limit of 8 ng/mL, highlighting the possibility for high-performance diagnostics[25].

Despite these advantages, respectability, user-friendliness, environmental consciousness, and automation continue to pose challenges. Variability in manufacturing techniques raises questions about reproducibility, which can cause device performance variations. Although their gadget yielded encouraging results, Kumar et al. pointed out that differences in construction could influence assay results[22]. Another consideration is user-friendliness; specific devices limit their use in low-resource environments by requiring technical knowledge. Greater acceptance depends on the simplified interface and operating protocols. Dong et al. underlined user-friendly design requirements in their portable nucleic acid amplification testing system[26]. Furthermore, environmental variables influence assay performance, including temperature and humidity. Researchers must consider these factors during planning and testing to guarantee consistent findings under several circumstances. Another difficulty is integrating automation, which calls for careful fluid dynamics control, reagent handling, and systems detection. Although automation increases efficiency, using automated procedures in self-prepared microfluidic devices can be difficult. Emphasizing the need for strong design strategies, Hu et al. addressed the challenges of including automation in their magnetic digital microfluidic technology. Expanding the usage of self-prepared microfluidic devices in clinical and field environments depends on addressing these difficulties to satisfy the needs of contemporary diagnostics[27].

ii. Integration of Chemiluminescence with Microfluidic Platforms

Integrating chemiluminescence with microfluidic technologies has proven highly efficient for rapidly and sensitively detecting pathogenic bacteria. Essential parts of microfluidic systems and chemiluminescent chemicals can create quantitatively measurable light signals. Usually undergoing a chemical reaction, these substances generate light that several optical techniques can observe. Recent research has underlined numerous forms of chemiluminescent reagents applied in microfluidic devices.

Table.1 Chemiluminescent reagents and their applications in microfluidic pathogen detection.

Reagent	Type	Detection Mechanism	Target Pathogen	Sensitivity	Ref.

Luminol	Chemiluminescent	Oxidation reaction with H_2O_2	Escherichia coli	0.0012 ± 0.0001% parasitemia	[28]
Iridium (III) complex	Electrogenerated ECL	ECL reaction with specific biomolecules	Matrix Metalloproteinase 2 (MMP-2)	0.3 ng/mL	[29]
Copper-cysteine nanoparticles	Chemiluminescent	Catalytic reaction with luminol and H_2O_2	Hydrogen peroxide	Real-time	[30]
$Ru(bpy)_3^{2+}$	Chemiluminescent	ECL with DNA recognition elements	Methylated RNA	0.0003 nM	[31]
Glucose oxidase (GOx) + MOF	Enzyme-mimicking	Enzymatic cascade reaction	Extracellular vehicles (EVs)	1×10^5 particles/mL	[32]
Ozone	Chemiluminescent	Reaction with NO to produce light	Nitric oxide detection	High sensitivity	[33]
Nanobody-based biosensor	Chemiluminescent	ECL with nanobody recognition	C-reactive protein (CRP)	0.037 ng/mL	[34]
Siderophore-Dioxetane	Enzyme-activated probe	Iron transport-mediated signal amplification	Staphylococcus aureus, Pseudomonas aeruginos	9.1×10^3 CFU/mL (S. aureus) 5.0 × 104 CFU mL (P. aeruginosa)	[35]
CRISPR/Cas13a	Nucleic acid detection	Targeted RNA detection with ECL	Group B Streptococci	8 copies/reaction	[21]

The Table. 1 presents target pathogens, sensitivity levels, and distinct detection methodologies of several chemiluminescent and electrochemiluminescent (ECL) reagents employed for pathogen detection. Effective for detecting Escherichia coli, hydrogen peroxide, and nitric oxide with high sensitivity, chemiluminescent reagents include luminol, copper-cysteine nanoparticles, and ozone function by oxidation processes and produce light upon engagement with H_2O_2 or nitric oxide. ECL-based reagents, such as Iridium(III) complex and $Ru(bpy)_3^{2+}$, utilize biomolecular interactions for the detection of Matrix Metalloproteinase 2 (MMP-2) and methylated RNA at extremely low detection threshold. Enzyme-assisted chemiluminescence, illustrated by glucose oxidase (GOx) in conjunction with metal-organic frameworks (MOFs) and siderophore-dioxetane probes, augments detection signals through cascade reactions and iron-mediated amplification, demonstrating efficacy in the detection of extracellular vesicles (EVs), Staphylococcus aureus, and Pseudomonas aeruginosa. Innovative biosensing methodologies, such as nanobody-based biosensors and CRISPR-Cas13a platforms, present advanced strategies for detecting C-reactive protein (CRP) and Group B Streptococci, attaining remarkable sensitivity to a few copies per reaction. Notwithstanding their benefits, obstacles such as equipment reliance, enzymatic stability, and restricted multiplexing capabilities persist as impediments to the extensive implementation of point-of-care testing (POCT). Future innovations must improve reagent stability, create smaller detection platforms, and incorporate multi-target detection to augment chemiluminescence-based microfluidic devices in clinical diagnostics, food safety, and environmental monitoring.

iii. Techniques for enhancing sensitivity and selectivity

The merging of chemiluminescence with microfluidic technology has changed the landscape of pathogen detection by enhancing the sensitivity and specificity of detection, which is facilitated using nanomaterials. Zhou et al. created europium-functionalized graphitic carbon nitride (Eu-CN) nanoparticles that enhanced chemiluminescence due to nitrogen defects to detect singlet oxygen in complex samples selectively (Figure 2A)[36]. There are also enzyme-based approaches to amplify the chemiluminescent signals, like Chang et al., who created a phosphatase-like enzyme to catalyze the dephosphorylation of a chemiluminescent substrate, emitting bright light for glyphosate detection during dephosphorylation[37]. Another considerable method is to combine isothermal amplification methods with chemiluminescence amplification. Chen et al. provided an example with a microfluidic device based on loop-mediated isothermal amplification combined with chemiluminescence detection, which enabled a rapid method for identifying Salmonella enterica with sensitivity at a detection limit of 260 CFU/mL and high specificity reports (Figure 2B)[38]. CRISPR/Cas systems combined with chemiluminescence are also being explored. Li et al. created a dynamic DNA nanosystem applied to a ratiometric electrochemical sensing strategy to detect HIV-DNA and achieved a detection limit of 36.71 aM detection in HIV-DNA[39]. The combination of CRISPR/Cas systems and chemiluminescence directly attributes and enhances specificity and sensitivity for diagnostics. Furthermore, Surface-Enhanced Raman Spectroscopy (SERS) may be integrated with chemiluminescence to enhance detection capabilities. Oliveira et al. developed a polystyrene microfluidic system that integrates SERS with chemiluminescence detection for malaria diagnosis, achieving a

sensitivity of 0.0012% parasitemia detection compared to conventional approaches.[28]. These new innovative approaches use factor-based improvements (nanomaterials), amplified chemiluminescent signal vs. a non-amplified signal (enzyme-based amplification), isothermal amplification, and CRISPR/Cas technology. SERS have improved chemiluminescence-based assays in sensitivity and selectivity. The potential for multiplex responses will also enhance future microfluidic platforms as new chemistry for more rapid, user-friendly diagnoses at the Point of Care us to directly improve public health (Figure 2C).

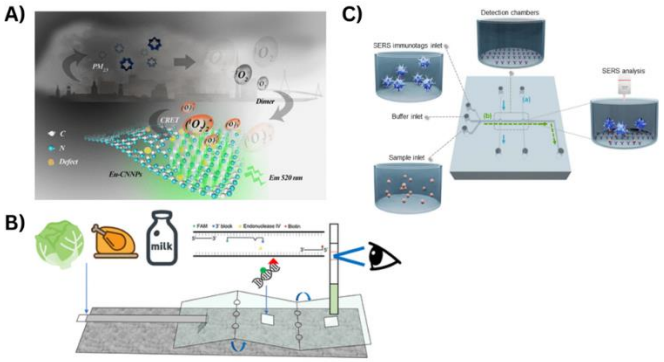

Figure 2. Microfluidic platforms utilizing advanced optical and molecular detection strategies for food and environmental safety

The Figure 2. shows that Microfluidic platforms utilizing advanced optical and molecular detection strategies for food and environmental safety A) Illustration of Eu-CNNP-based detection of $PM_{2.5}$ via singlet oxygen generation and Chemiluminescence Resonance Energy Transfer (CRET)[36]. B) Schematic of a microfluidic device for nucleic acid-based detection of foodborne pathogens, integrating enzymatic signal amplification and visual readout[38]. C) Schematic diagram of the microfluidic device setup, illustrating the SERS detection mechanism and the flow of samples through the device[28].

Recently, Jirawannaporn and colleagues utilized a microfluidic platform with single-step Recombinase Polymerase Amplification (RPA) and CRISPR/Cas12a for a rapid Leptospira detection strategy. The assay limit of detection was 100 cells/mL and exhibited 85.2% sensitivity and 100% specificity as a point-of-care system[40]. Soares et al. developed a microfluidic immunosensor using an impedance spectroscopy platform combined with chemiluminescence to detect Staphylococcus aureus. The microfluidic device designed by Soares achieved a limit of contamination detection in food and clinical samples of 2.83 CFU/mL while demonstrating excellent specificity and sensitivity[41]. Huang and colleagues reported that an automated microfluidic PCR device allowed nucleic acid detection of several pathogens in clean area temperature gradient zones. The PCR amplification took 9 minutes and displayed sensitivity and specificity to detect bacterial and viral pathogens[42]. As well as the Huang study, Daoud et al. conducted a study of dual lateral flow immunoassay detection of COVID-19 combining chemiluminescence, using more sensitive chemiluminescence to detect IgM improved sensitivity to 87.7%. The studies suggest that promising testing strategies are essential in addressing a more reliable laboratory testing approach incorporating chemiluminescence[43]. The longer-term significance of the case studies discussed is that they represent chemiluminescence-based microfluidic platforms supporting the detection concerning sensitivity and specificity with pathogen detection as combined with sensors supporting SERS detection, CRISPR/Cas12, electrical platforms, or rapid PCR detection devices among other technologies. These kinds of research and engagement with these kinds of design platforms can improve the timeliness of point-of-care diagnostics, the rapid public health response to disease threats presented with infectious disease potential, and likely improve public health response in cases, especially in resource-limited settings.

iv. Automation and efficiency in fully automated systems

Combining chemiluminescence with fully automated microfluidic systems has transformed the rapid detection of pathogenic microorganisms and increased efficiency and accuracy. Huang et al. developed the Onestart system, a fully automated microfluidic PCR-array platform capable of detecting 21 respiratory pathogens in 1.5 hours that incorporates sample preparation, amplification, and detection on a single microchip. The Onestart system exhibited high sensitivity and specificity comparable to a real-time PCR method, and it minimized human error while increasing throughput, making it a powerful option for clinical diagnostics (Figure 3A)[3]. Li et al. developed an automated RPA-T7-Cas13a platform with rotary valve-assisted sample pretreatment. The system combined a recombinase polymerase amplification (RPA) assay and CRISPR/Cas13a to detect Group B Streptococci (GBS) rapidly. The platform provided eight copies/reaction detection sensitivity, significantly surpassed traditional PCR detection limits, and completed detection in less than 30 minutes, making it a viable option for rapid point-of-care testing[21]. In a similar application, Rombach et al. developed RespiDisk, a centrifugal microfluidic platform for the fully automated detection of 19 respiratory pathogens using RT-PCR. The complete assay was automated via total spinning duration and frequency while maintaining high analytical performance and reducing hands-on time to 5 minutes (Figure 3B)[12]. For a viral detection application, Tian et al. developed a fully automated centrifugal microfluidic system for sample-in-answer-out nucleic acid testing and validation using SARS-CoV-2 oropharyngeal swabs; the system provided high sensitivity and specificity, reduced contamination risks from the user, and enhanced efficiency in the clinical diagnostics workflow[44]. Yao et al.proposed a microfluidic system for automated insulin detection that utilized a chemiluminescence immunoassay. The system demonstrated high sensitivity and sample throughput (results were generated in less than 10 minutes). The principles of chemiluminescence and overall automation of the proposed system demonstrate the feasibility of detecting pathogens, even though the system was initially designed to detect insulin (Figure 3C)[45].

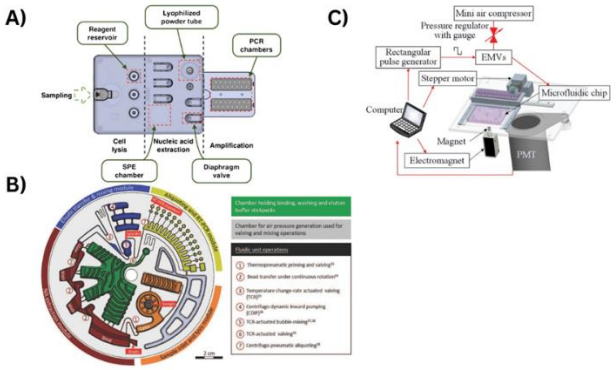

Figure 3. Automated Microfluidic Platforms for Integrated Nucleic Acid Testing and Pathogen Detection

The Figure 3. shows that Automated Microfluidic Platforms for Integrated Nucleic Acid Testing and Pathogen Detection A) Schematic

of the Onestart system, illustrating automated sample preparation, nucleic acid extraction, and PCR-based amplification for pathogen detection[3]. B) The schematic representation of the RespiDisk system demonstrates centrifugal microfluidic operations and RT-PCR-based respiratory pathogen detection[12]. C) A schematic diagram illustrating the working principle of a fully automated microfluidic device integrated with chemiluminescence detection[45].

The emerging field of automation in microfluidic platforms enhances the throughput, reproducibility, and accuracy of testing for pathogens, ultimately reducing human errors in testing for pathogen detection. An example of this is the FeverDisk designed by Hin et al., which is a fully automated centrifugal microfluidic platform that can rapidly detect the pathogens responsible for acute febrile illness-causing diseases, such as dengue virus, Plasmodium falciparum, and Salmonella enterica. In about 2 hours, the FeverDisk completes sample-to-result bacteriophage testing and generates highly actionable results designed for clinical diagnostics[46]. Similarly, Oh and Seo constructed their autonomous centrifugal microfluidic device that used a solution-loading cartridge to test liquid for foodborne pathogens. This eliminated the need for the user to pipette manually, and microfluidics techniques were used for sample elution, amplification, and detection in only 65 minutes for 18 unique pathogens[47]. To progress automation, Guo et al. developed a centrifugal microfluidic system capable of nucleic acid sequencing foodborne pathogens while automating key processes of enzymatic DNA fragmentation, adding the adapter, and PCR amplification. Automating those key procedures resulted in similar performance as a manual method with substantial time and cost savings, which proved viable in resource quality settings[48]. Similarly, when extending the application of microfluidic automation from pathogens detection, Sunkara et al. examined Lab-on-a-Disc (LOAD) technology that used centrifugal force to improve some or all steps of diagnostics on one disc; the LOAD also showed promise for infectious disease diagnosis, liquid biopsy, and environmental monitoring[49]. Intelligent chemiluminescence-based microfluidic automation has the most explicit implications for point-of-care testing (POCT). The designs' speed, accuracy, and efficiency incorporated with clinical decision support may improve pathogen detection in clinical care settings or remote locations. The applicability of a fully automated microfluidics pathogen detection system represents positive epidemiological advances in testing for pathogens that will surely enhance diagnostic capabilities, the patient journey, and public health response.

Table 2. Key features of fully automated chemiluminescence-based microfluidic systems.

System Name	Pathogen Detected	Throughput	Automation Level	POCT Feasibility	Ref.
Onestart	21 pathogens (various)	High	Fully	Yes	[3]
Centrifugal Microfluidic Device	Foodborne pathogens (various)	Moderate	Fully	Yes	[47]
Portable Microfluidic Device	Ebola virus	Low	Fully	Yes	[50]
CL-Biosensor	E. coli O157:H7	High	Fully	Yes	[51]
Centrifugal LabDisk	Bacterial pathogens	High	Fully	Yes	[52]
Automated RT-LAMP System	SARS-CoV-2	High	Fully	Yes	[53]
Automated Detection of HBV	Hepatitis B virus	Moderate	Fully	Yes	[54]
Automated Detection of Influenza A	Influenza A	High	Fully	Yes	[55]
Automated Detection of Zika Virus	Zika virus	Moderate	Fully	Yes	[56]
Smartphone-Controlled Microfluidic Device	MRSA	Moderate	Fully	Yes	[57]

The Table. 2 provides an overview of fully automated detection systems based on microfluidics, which use chemiluminescence and biosensing for pathogen detection. Automated detection systems such as Onestart, CL-Biosensor, and the Centrifugal LabDisk are high-throughput systems that can detect multiple pathogens to improve clinical diagnostics and disease surveillance. The Centrifugal Microfluidic Device and CL-Biosensor also offer automation strategies that rapidly detect foodborne pathogens, further addressing potential contamination. Many systems are also dedicated to viral detection, with recommended systems including the Portable Microfluidic Device for Ebola Virus, RT-LAMP system for SARS-

CoV-2, as well as automated systems for Hepatitis B Virus, Influenza A, and Zika virus. These devices serve helpful purposes in response to pandemic conditions and remote diagnostics for various differential diagnoses. The Smartphone-Controlled Microfluidic Device, for example, specifically targets the detection of MRSA and is representative of trends in digital biosensing and point-of-care testing (POCT) applications. They offer complete automation of microfluidic detection, and based on information provided by the manufacturers, they can significantly improve sensitivity, specificity, and reproducibility while reducing human error and improving diagnostic accuracy. Nonetheless, barriers to acceptance and widespread use remain relating to moderate throughput tempo across bioassays or microfluidic devices, scalability, and design costs. Future device recommendations should include improving multiplex detection pan pathogens, AI-supported data analysis processes, and incorporating stable reagent formulations. In summary, fully automated microfluidic systems based on chemiluminescence are transforming the field of pathogen detection. They can significantly increase diagnostics' speed, sensitivity, and reliability across clinical, food safety, and environmental domains and ultimately lead to a change on a global scale for disease surveillance and outbreak identification and management.

v. Commercial Microfluidic Chemiluminescence Systems for Pathogen Detection

The convergence of chemiluminescence with microfluidic systems has resulted in numerous exciting diagnostic systems that leverage sensitivity, time response, and size. Commercial systems have demonstrated the benefits of chemiluminescence paired with microfluidics in several applications. The LumiraDx SARS-CoV-2 Ab Test was presented to detect SARS-CoV-2 antibodies in fingerstick blood samples utilizing a microfluidic immunofluorescent assay inline microfluidic chemistry. It agreed with laboratory-based methods and efficacy as a point-of-care rapid clinical diagnostic test[58]. A 3D-printed microfluidic device utilizing in-line amperometric and chemiluminescent detection demonstrates the simultaneous detection of norepinephrine and adenosine triphosphate (ATP) and is compact, efficient biochemical detection (Figure 4A)[59]. Another system is an integrated microfluidic chip designed for multiplexed detection of respiratory pathogens, allowing nucleic acid extraction, amplification, and chemiluminescent detection with high sensitivity and specificity[60]. In food safety, a portable automated microfluidic platform integrates chemiluminescence imaging that detects multiple mycotoxins simultaneously in actual samples while minimizing manual steps and providing sensitivity (Figure 4B)[61]. An integrated design for portable diagnostics and easy use via a smartphone application is based on colourimetric detection of foodborne pathogens by detecting colour changes during amplification[62].

The landscape of chemiluminescent microfluidic device commercial tools is rapidly evolving and driven by advances in miniaturisation, automation, and molecular detection technologies. Notable manufacturers include LumiraDx, which markets various point-of-care diagnostic systems; Fluidigm Corporation, which commercialises genomic microfluidic manufacturing systems for high throughput; and Bio-Rad Laboratories, proficient in chemiluminescence for nucleic acid detection systems. Recent developments in chemiluminescence-based microfluidic diagnostics illustrate an increasingly directed focus on improving sensitivity, portability, and multiplex detection capabilities. For example, CRISPR-based detection processes with greater sensitivity and specificity for pathogen detection have recently been established[21]. Furthermore, there is a strong trend towards developing portable devices that can be used in field settings, allowing for rapid diagnostics without complex laboratory infrastructure[62]. Finally, novel multiplexing technologies are being developed to support the detection of multiple pathogens in one assay, a critical process in disease management[60], [63]. These improvements support many application areas, such as infectious disease diagnostics for cases of SARS-CoV-2, influenza, bacterial infections such as E. coli and Salmonella from food sources, and food safety applications in detecting mycotoxins and even microbial contaminants[19], [61], [64]. The applications of chemiluminescence-based microfluidic diagnostics can also be expanded into environmental tracking with pathogen detection capabilities in water and other biological sampling as public health measures[65]. Integrating chemiluminescence and microfluidic technology creates unique diagnostic methods that are fast, sensitive, and point-of-care applicable. The market continues to progress creatively, and technological advancements and increased demands for efficient diagnostic devices will lead to further developments within the field.

Table 3. Performance Comparison of Self-Prepared vs. Commercial Chemiluminescence Systems

Features	Self-Prepared Systems	Commercial Systems	Ref.
System Type	Experimental	Ready-to-use	[66], [67]
Detection Time	Slow	Fast	[21], [61]
Sensitivity	Variable	High	[68], [69]
Specificity	Dependent	Optimised	[70], [71]
Cost	Low (initial), High (development)	High (initial), Cost-effective (long run)	[72], [73]
Ease of Use	Complex	User-friendly	[74], [75]
Integration with Other Systems	Limited	Seamless	[61], [76]
Regulatory Approval	Unregulated	Approved	[19], [77]

The Table.3 compares self-prepared and commercial chemiluminescence-based microfluidics, especially looking at detection time, sensitivity, specificity, cost, ease of use, integration, and regulatory approval. Self-prepared systems are experimental and require manual optimising, longer analysis time, and a skilled hand. They have varying sensitivity and specificity based on biomarker selection and assay optimisation. While making self-prepared systems is less expensive in a time frame, complete development pushes costs

higher. There is no regulatory approval for self-prepared systems, meaning no real-world applications. Commercial systems are completely ready-to-use with high sensitivity to pathogen detection, optimised to detect pathogens in a limited timeframe (~30 minutes-2 hours). Commercial systems are also highly specific, easy to automate, and easily integrated into a diagnostic platform, making them ideal for point-of-care testing (POCT) of food and clinical applications. Although the upfront cost of commercial systems is high, they remain cost-efficient over time due to the efficiency and reproducibility levels expected with commercial systems. Additionally, commercial systems undergo extensive regulatory validation and other variables to ensure reliability in the clinical setting and food safety applications. Overall, this comparison highlights the need to balance the customisation that self-prepared systems provide for research purposes and the scalability and real-world applicability of commercial microfluidics for pathogen detection systems.

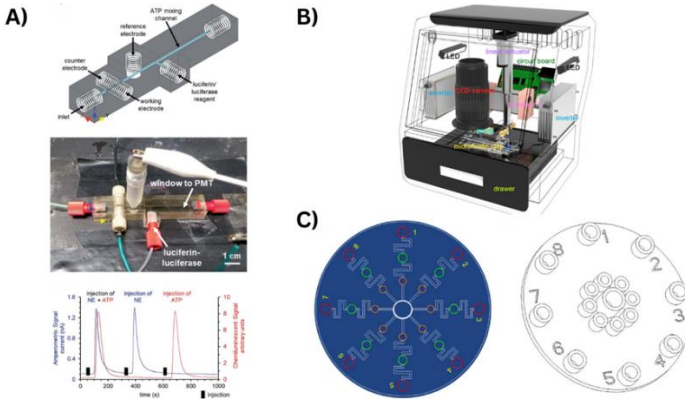

Figure 4. Integrated Chemiluminescence-Based Microfluidic Platforms for Pathogen Detection A), B) and C)

The Figure 4. shows that Integrated Chemiluminescence-Based Microfluidic Platforms for Pathogen Detection A) Design and Operation of a 3D-printed Microfluidic Chemiluminescence Device Integrating Luciferin-Luciferase Reactions for ATP and Norepinephrine Detection[59]. B) Schematic of a portable automated chemiluminescence microfluidic detection system with integrated optics and control components[61]. C) Schematic and structural design of the multiplexed microfluidic immunoassay chip for simultaneous respiratory virus detection.

The commercialisation of chemiluminescence-based microfluidic devices has significantly impacted pathogen detection in public health. Several studies have identified and reported successful cases using these technologies to demonstrate real-world performance in detecting infectious diseases. For example, an automated chemiluminescence enzyme immunoassay (CLEIA) for the detection of IgG against Porcine Circovirus 3 (PCV3) in pig serum was developed by Wang et al. The platform exhibited high analytical sensitivity with a diagnostic sensitivity of 96.8% and specificity of 97.3%, making the CLEIA a reliable tool for exemption epidemiological surveillance of PCV3. Notably, the CLEIA platform's automated system minimised operating time while providing diagnostic capability, demonstrating the promise of using advanced, automated microfluidic diagnostic platforms in veterinary medicine[78]. Another example of chemiluminescence use in microfluidics was the multiplexed microfluidic immunoassay chip developed by Wu et al., which allowed for detecting eight respiratory viruses, including several SARS-CoV-2 and influenza viruses. This multiplexed hybrid immunoassay detection device incorporated enzyme technology to increase detection sensitivity, achieving a resulting detection limit of 0.1 pg/mL. The high specificity and accuracy for detecting multiple respiratory viruses using this platform further demonstrate the potential of these devices for future rapid diagnostics in clinical or point-of-care settings (Figure 4C)[79]. A notable case study is a digital microfluidic platform for syndromic pathogen detection created by Bai et al., which combines nucleic acid extraction, quantitative polymerase chain reaction (qPCR) amplification, and real-time fluorescence analysis to enable the simultaneous detection of various pathogen targets (e.g., multiple respiratory pathogens) from a single unprocessed sample within 80 minutes. The device's high specificity and sensitivity indicate suitability for point-of-care testing, especially in low-resource settings[80]. These case studies illustrate chemiluminescence-based microfluidic devices' impact on pathogen detection. Each study case highlights the promise of integrating new advanced chemiluminescent technology and allowing for the testing of multiple pathogen targets using multiplexing capabilities and advanced technologies to improve accessibility and detection accuracy and therapeutically improve public health outcomes in various areas.

3. Summary of Key Advancements

Microfluidic assays utilising chemiluminescence have emerged as transformative tools for detecting pathogenic microorganisms. The main advantage of these platforms is the combination of many essential diagnostic steps, such as sample preparation, amplification, and signal detection, into a fully automated platform. Numerous recent examples reflect this evolution, including the Onestart PCR-array system[3], a RespiDisk centrifugal RT-PCR chip[12], and integrated systems that combine CRISPR-Cas13a detection with rotary-valve-based microfluidics. These systems offer significantly reduced run durations, frequently yielding results under 30 minutes, and exhibit exceptional sensitivity, detecting as few as eight copies of the target organism per response in certain instances, rendering them appropriate for clinical, environmental, and food safety applications[21].

Advances in chemiluminescence and microfluidics have paved the way for many new detection strategies and device designs. Hybrid systems have combined loop-mediated isothermal amplification (LAMP) with CRISPR-based sequence recognition to detect pathogens, and some utilise artificial intelligence to control fluid flow and mitigate error. There are also concurrent multiplexing developments, including systems that utilise enzyme-enhanced immunoassays and microvalve-controlled reaction chambers, allowing simultaneous detection of multiple pathogens in real time. Commercial products are beginning to demonstrate many of these features, including LumiraDx, and academic and commercial collaborations such as the smartphone-based pathogen analyser, portable chemiluminescence enzyme immunoassay (CLEIA) systems, and field-deployable mycotoxin detection systems, indicating that these systems and assays are advancing from research.

Despite the progress, many barriers to application remain. While commercial systems often report satisfactory sensitivity and specification, entirely experimental or self-prepared systems can generate inconsistent results based on biomarker selection, the extent to which the involved assays can be standardised, and the inherent variability of collecting samples to run diagnostic assays. Cost is also a consideration, as self-fabricated systems can be more cost-effective but require significant trial and error expertise and time to customise the assays. The commercial systems, while being more expensive up front, will save you money on labor in the long run by reducing time spent customizing the system and increasing assay throughput. These cost comparisons can be compared to facilitate decision-making (Table 4). A critical barrier is scalability, especially in a low-resource setting where the scale of mass production, regulatory approval, and systems procurement are limited. As we move forward, considering

strategies to overcome each of the listed barriers through device design, cost-effective materials, and regulatory compliance frameworks will be pivotal in realising chemiluminescence-based microfluidic diagnostics in global health.

Table 2 Comprehensive performance achievement matrix comparing designed functional requirements to experimentally achieved prototype performance.

Functional Requirement	**Target Specification**	**Achieved (Mean ± SD)**	**Sample Size (n)**	**Compliance Status**
(Mean ± SD)	Sample Size (n)	Compliance Status		
Detection Limit	<5 μM	1.8±0.3 μM	10 devices	✓ PASS (2.8× margin)
Sensitivity	>50 μA/mM	85±12 μA/mM	10 devices	✓ PASS (1.7× margin)
Response Time (95%)	<5 min	3.2±0.4 min	8 replicates	✓ PASS (1.6× margin)
Linearity (R^2)	>0.95	0.987±0.008	10 devices	✓PASS(1.0 4× margin)
Accuracy (MARE)	<15%	11.2±2.1%	47 measurements	✓PASS(1.3 4× margin)
Selectivity (vs AA)	>10:1	20:1±3:1	5 devices	✓ PASS (2.0× margin)
Operating Lifetime	>8 hours	12–16 hours*	5 devices	✓PASS(1.5 -2.0× margin)
Drift Rate	<2%/hour	1.8±0.4%/hour	5 devices	✓ PASS (1.1× margin)
Biocompatibility	Grade 0–1 ISO 10993-5	Grade 1 (minor irritation)	MTT/LDH assay	✓ PASS
Power Consumption	<5 mW	2.1±0.3 mW (biosensor core)	1 prototype	✓ PASS (2.4× margin)

CONCLUSION

Integrating chemiluminescence with microfluidic technologies represents a powerful new technology for pathogen biosensing systems. The chemiluminescence's strengths of a high signal-to-noise ratio, low backgrounds, and compatibility with microfluidic platforms highlight the attractive technologies for incorporation into fully automated microfluidic devices for the rapid, multiplexed detection of pathogenic microorganisms. As discussed in this review, these systems present unique advantages in laboratory-scale diagnostics and whether clinical diagnostics can move to decentralized, point-of-care applications. Nevertheless, despite the advances in research, significant challenges remain in achieving standardized assays, scaling systems, and providing cost-effective tests. Future directions should seek to increase sensitivity and reproducibility while preserving ease of use by investigating the integration of CRISPR-based detection, AI-assisted automation, and stratified microfluidics. Furthermore, moving away from existing fabrication methods and regulatory approval is necessary to develop products with clinical and market viability. Fully automated chemiluminescent microfluidics are up-and-coming methods to increase global health surveillance that has implications in infectious disease diagnostics, environmental testing, food safety testing, and personalized medicine. These rapid outcomes can also improve epidemic preparedness and public health targeting, particularly in resource-poor and field-based applications. In conclusion, chemiluminescent microfluidics refers to an attractive and rapidly evolving biosensing technology that will alter diagnostic workflows for the betterment of human health by improving current models for efficacy, portability, and access. Realizing these outcomes will require updates from engineering and biotechnology, collaborations with clinical science, and sophisticated biosensing technologies to have a meaningful, real-world impact on global health outcomes.

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How to cite this article:

Shah Muhammad Adnan, Md Mudassir Chowdhury, Daloar Hossan, Mohammes Imran Hossain, Muhammad Tahir Zaman "Recent Advantages of Chemiluminescence-Based Fully Automated Microfluidic Devices in the Detection of Pathogenic Microorganisms" International Journal of Engineering Works, Vol. 13, Issue 07, PP. 49-60, July 2026. https://doi.org/10.5281/zenodo.21551530.