УДК 577.29
doi: https://dx.doi.org/10.22328/2413-5747-2025-11-4-28-38

ПРИМЕНЕНИЕ ТЕХНОЛОГИИ МУЛЬТИПЛЕКСНОЙ ДЕТЕКЦИИ ПАТОГЕНОВ: ДОСТИЖЕНИЯ И ПЕРСПЕКТИВЫ

*Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь**
Военно-морской медицинский университет, Шанхай, Китай
Шанхайская ключевая лаборатория медицинской защиты, Шанхай, Китай

ВВЕДЕНИЕ. Патогены инфекционных заболеваний обладают такими характеристиками как видовое разнообразие и геномная сложность, что делает их быструю и точную совместную детекцию серьёзной задачей современности. Микрофлюидные технологии, благодаря своим преимуществам, таким как миниатюризация, функциональная интеграция, высокая пропускная способность и воспроизводимость, демонстрируют огромный потенциал в этой области.
ЦЕЛЬ. Провести систематическое обобщение последних достижений в области многофункциональных микрофлюидных диагностических устройств, изучить возможность их применения при наличии нескольких патогенов, а также технические ограничения и перспективные направления их клинического применения.
МАТЕРИАЛЫ И МЕТОДЫ. В данной статье рассматриваются многофункциональные устройства для детекции, основанные на таких распространённых технологических подходах, как электрохимические сенсоры, оптические сенсоры, иммуносенсоры и бумажные микрофлюидные платформы; особое внимание уделяется инновационным методам использования микрофлюидной технологии амплификации нуклеиновых кислот для повышения чувствительности детекции.
РЕЗУЛЬТАТЫ. Различные типы микрофлюидных устройств значительно повысили эффективность и интеграцию детектирования патогенов. При этом микрофлюидная технология амплификации нуклеиновых кислот благодаря инновационным решениям продемонстрировала значительный потенциал в оптимизации чувствительности. Однако, перекрёстное взаимодействие между анализируемыми веществами, по-прежнему остается ключевым фактором, влияющим на эффективность совместного тестирования.
ОБСУЖДЕНИЕ. Несмотря на значительный прогресс в области технологий, совместная детекция патогенов по-прежнему сталкивается с вызовами, связанными с разнообразием, сложностью и надежностью практического применения. В будущем развитие должно быть направлено на повышение универсальности, стабильности и экономической эффективности платформы.
ЗАКЛЮЧЕНИЕ. Мультиплексная микрофлюидная технология детектирования уже достигла значительных успехов в диагностике инфекционных заболеваний и обладает широкими перспективами клинического применения. Дальнейшее технологическое развитие является ключом к внедрению данных технологий в клиническую практику.

КЛЮЧЕВЫЕ СЛОВА: морская медицина, микрофлюидика, мультиплексная детекция, патогены, чувствительность

***For correspondence:** *Cao Guangwen, e-mail: gcao@smmu.edu.cn*
***Для корреспонденции:** *Цао Гуанвэнь, e-mail: gcao@smmu.edu.cn*

For citation: Shi Yiwei, Fang Letian, Cao Guangwen. Application of multiplex pathogen detection technology: achievements and prospects // *Marine Medicine*. 2025. Vol. 11, № 4. P. 28–38, doi: https://dx.doi.org/10.22328/2413-5747-2025-11-4-28-38; EDN: https://elibrary.ru/VOTXRO
Для цитирования: Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь. Применение технологии мультиплексной детекции патогенов: достижения и перспективы // *Морская медицина*. 2025. Т. 11, № 4. С. 28–38,
doi: https://dx.doi.org/10.22328/2413-5747-2025-11-4-28-38; EDN: https://elibrary.ru/VOTXRO

APPLICATION OF MULTIPLEX PATHOGEN DETECTION TECHNOLOGY: ACHIEVEMENTS AND PROSPECTS

*Shi Yiwei, Fang Letian, Cao Guangwen**
Naval Medical University, Shanghai, China
Shanghai Key Laboratory of Medical Defense, Shanghai, China

INTRODUCTION. Infectious disease pathogens exhibit species diversity and genomic complexity, making their rapid and accurate combined detection a major challenge today. Microfluidic technologies, with their advantages such as miniaturization, functional integration, high throughput, and reproducibility, demonstrate enormous potential in this field.
OBJECTIVE. To systematically summarize the latest advances in multifunctional microfluidic diagnostic devices, explore the feasibility of their use in the presence of multiple pathogens, as well as technical limitations and promising areas for their clinical application.
MATERIALS AND METHODS. This article reviews multifunctional detection devices based on common technological approaches such as electrochemical sensors, optical sensors, immunosensors, and paper-based microfluidic platforms, with a particular focus on innovative methods for using microfluidic nucleic acid amplification technology to improve detection sensitivity.
RESULTS. Various types of microfluidic devices have significantly improved the efficiency and integration of pathogen detection. Thanks to innovative solutions, microfluidic nucleic acid amplification technology has demonstrated significant potential for optimizing sensitivity. However, cross-reactivity between analytes remains a key factor affecting the effectiveness of combined testing.
DISCUSSION. Despite significant technological advances, collaborative pathogen detection still faces challenges related to the diversity, complexity, and reliability of practical applications. Future developments should focus on improving the platform's versatility, stability, and cost-effectiveness.
CONCLUSION. Multiplexed microfluidic detection technology has already achieved significant success in infectious disease diagnostics and holds broad clinical application potential. Further technological development is key to implementing these technologies into clinical practice.

KEYWORDS: marine medicine, maritime medicine, microfluidics, multiplex detection, pathogens, sensitivity

Introduction. Infectious diseases are a major challenge to global public health and seriously threaten human health. According to the World Health Organization (WHO) Global Health Statistics 2019 report, infectious diseases rank fourth among the causes of death worldwide, and are particularly serious in developing countries due to poor sanitation and lack of medical resources. It is worth noting that developed countries also face multiple challenges such as food safety, outbreaks of infectious diseases and sexually transmitted diseases [1]. With the continuous emergence of new pathogens, the development of rapid and economical multiplex pathogen detection technology is of great significance to curb the spread of infection. Early diagnosis and precise treatment are key links in the prevention and control of infectious diseases. Therefore, the development of efficient pathogen detection technology has important scientific value and practical significance. Microfluidics has become a research hotspot in this field due to its unique advantages in pathogen detection. This technology platform provides strong support for the development of efficient detection methods through its excellent flexibility and functionality (Fig. 1). The core of microfluidics technology lies in the design of its micron-level channels, which can handle very small amounts of liquid (10-9~10-18 L), thereby achieving high sensitivity and high throughput detection [2]. Over the past decade, microfluidics has made significant progress, particularly in integrating complex functional units into miniaturized platforms. These platforms are typically only centimeters in size, or even smaller, yet possess extremely powerful functions, enabling multiple processes such as specimen pretreatment, reaction, detection, and cell culture [3, 4]. Furthermore, combining various biosensors with microfluidics has further expanded its applications in pathogen detection. This combination not only enables rapid diagnosis and highly repeatable testing but also significantly improves the cost-effectiveness of detection. These characteristics make microfluidics a promising field for pathogen detection, providing strong support for the early diagnosis and control of infectious diseases.

Based on their detection principles, multi-mode microfluidic devices can be categorized into elec-

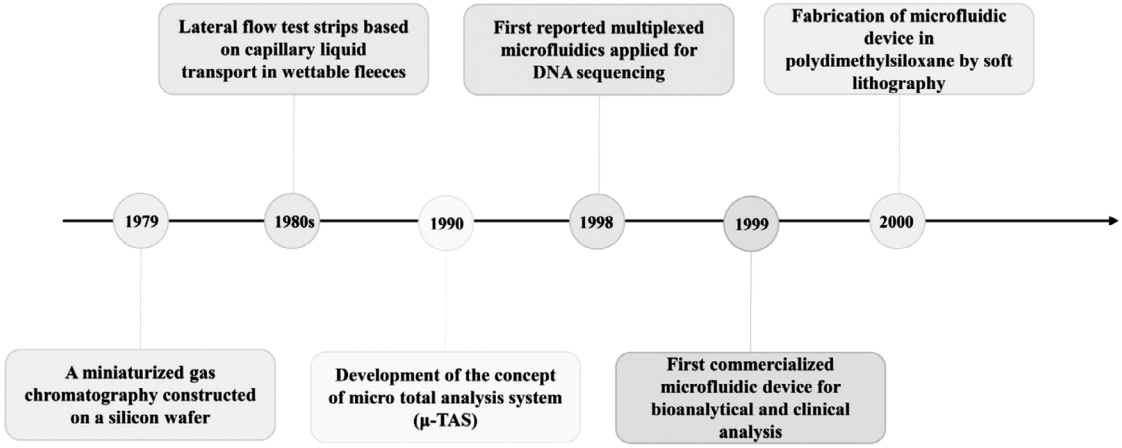

Fig. 1. Timeline for the development of microfluidic technology and multiplexed microfluidics
Рис. 1. Хронология развития микрофлюидной технологии и мультиплексной микрофлюидики

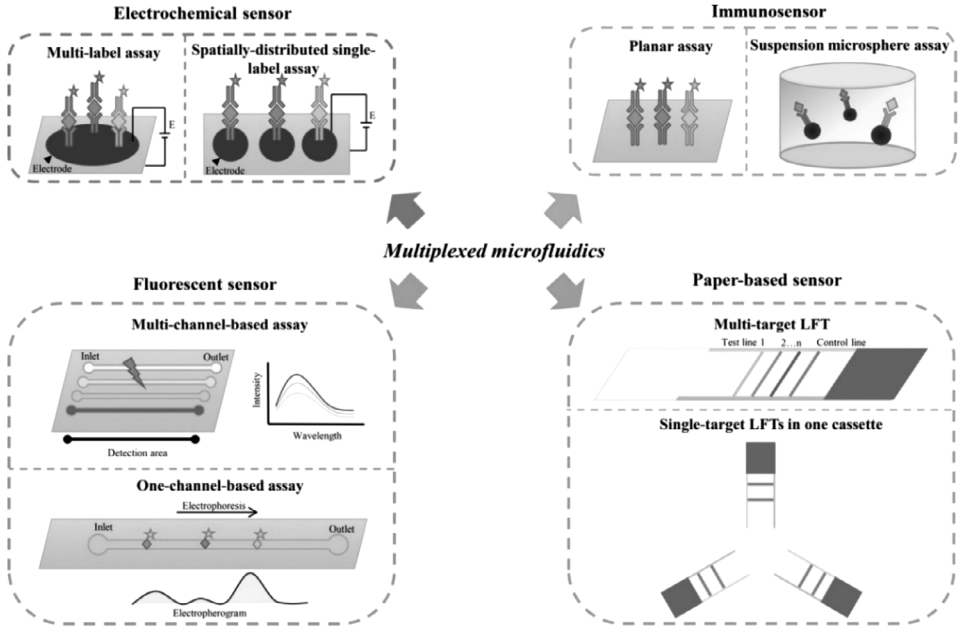

Fig. 2. Schematic representation for multiplexed microfluidics
Рис. 2. Схематическое изображение мультиплексной микрофлюидики

trochemical sensor-based, optical sensor-based, immunosensor-based, and paper-based multi-mode microfluidic technologies (Fig. 2). Each of these technologies has its advantages and is suitable for different detection scenarios and practical needs. Electrochemical sensors have attracted widespread attention due to their high sensitivity and rapid response; optical sensors, with their multi-channel detection capabilities and high sensitivity, are widely used in the detection of complex samples; immunosensors use specific antibody-antigen binding to achieve highly selective and sensitive detection; and paper-based multi-mode microfluidic technology, with its low cost and portability, has become an ideal choice for rapid on-site detection. This review systematically summarizes the design of multi-mode microfluidic chips to achieve simultaneous detection, reduce cross-reactivity, and improve sensitivity.

Multi-microfluidic technology

Multi-microfluidic technology based on electrochemical sensors

Electrochemical methods, including amperometric, impedance, and voltammetric methods, have been widely used in the detection of infectious diseases. The working principle of an elec-

trochemical sensor is that the biochemical reaction between the analyte and the recognition element fixed on the electrode causes a change in the electronic signal, which is then converted by the sensor [5]. In microfluidic technology, the combination of electrochemical sensors and micro-channels significantly shortens the diffusion distance between reactants, thereby accelerating the interaction between the recognition element and the target analyte. This design not only improves detection efficiency but also enhances the sensitivity and specificity of detection [6].

In the multi-target analysis of infectious diseases, the key is to accurately distinguish two or more signals while minimizing mutual interference. To improve the selectivity of electrochemical sensors, there are two methods, which are divided according to the number of tags in a device.

The first method is to integrate multiple tags into the microfluidic device. This method is achieved by immobilizing multiple antibodies on one electrode. For example, researchers have successfully constructed a multi-label electrochemical sensor by co-immobilizing two antibodies on the surface of Fe3O4 nanoparticles. This sensor distinguishes signals by observing different voltammetric peaks generated by different probes, thus achieving simultaneous detection of multiple pathogens [7].

The second method is spatially distributed single-label detection using an electrode array. This method usees a patterned microelectrode array based on microfluidic technology, capable of simultaneously and independently sensing multiple ions in an electrolyte solution [8]. A major problem with this method is the mutual interference between adjacent electrodes. To address this, researchers have proposed various electrode spatial separation methods, such as independent channel or chamber designs, which can effectively reduce signal interference between electrodes [9]. Electrochemical sensors integrated into microfluidic technology provide a promising platform for infection detection because they do not require external instruments and are compatible with integrated circuits, making them relatively suitable for point-of-care applications. However, the design and application of electrochemical sensors must be customized according to specific needs, including the type of target pathogen, the characteristics of the biological sample, and the sample preparation method [10]. Currently, the level of system integration and automation still needs improvement to further optimize the detection process and increase detection efficiency. The integration of microfluidic technology with electrochemical sensors provides a promising platform for infection detection. This platform requires no external instrumentation and is compatible with integrated circuits, making it suitable for point-of-care applications.

Multiplex Microfluidics Based on Optical Sensors

Optical sensors have attracted much attention due to their significant advantages in rapid multiplex detection of infectious diseases. These sensors can complete detection in a short time (within 15 minutes) [14]. Optical sensors emit signals of different wavelengths, enabling them to detect multiple pathogens simultaneously, thereby significantly improving detection efficiency and accuracy [15].

Fluorescent sensors are classified into the following categories based on their detection principles and design characteristics: First, multichannel sensors, which can detect multiple biomarkers simultaneously and in parallel. For example, researchers have developed a multichannel droplet array chip pre-loaded with detection reagents in a “water-in-oil” form for bacterial lysis, RNA extraction, and pathogen detection [16]. Although this design has been widely used in multiplex detection, its application is limited by the need for external fluid control and the large size of the equipment. Second, single-channel multiplex sensors based on various separation techniques, in which a single tag is used for detection. Microfluidic electrophoresis technology has been developed for the separation and enrichment of targets [17]. The core of this technology lies in achieving efficient separation and enrichment of targets through electrophoresis. Specifically, it includes two basic steps: first, the specific binding of the probe to the target; second, the separation and differentiation of the target by electrophoresis. Third, the use of multicolor probes to distinguish the signal. This method can simultaneously detect multiple targets by using fluorescent probes of different colors, thus achieving multiple detection. However, this method is limited by the number of available probes and the mutual interference between different probes. All three methods are homogeneous sensors, in

which the optical sensor floats in the solution and is detected by the fluorescence signal in the solution. In multiplex microfluidics, different probes must be used to distinguish various fluorescence signals to achieve multiple detection [18]. In optical sensor-type microfluidics that do not require external instruments, colorimetric sensors have received widespread attention due to their ease of operation, low cost, and ability to determine positive results by observing color changes with the naked eye. Calcein is a commonly used colorimetric indicator that allows to determine positive results by observing color changes with the naked eye, thereby detecting pathogens [19]. In addition, hydroxynaphthol blue, as an auxiliary indicator, does not inhibit the sensitivity of calcein, so it can be used in combination with calcein to improve the accuracy and reliability of detection [20]. In addition to calcein and hydroxynaphthol blue, naked-eye monitoring by observing precipitates or DNA-binding dyes has also been incorporated into microfluidic technology. These methods indicate the presence of target substances through color changes or the formation of precipitates, thereby enabling rapid detection of pathogens. However, these colorimetric sensors still face some challenges in practical applications, such as limited detection sensitivity and the possibility of color changes being affected by environmental factors [21-23].

Multiplex Microfluidics Based on Immunosensors Immunosensor technology is a detection technique based on the binding of target antigens to specific antibodies or antigens, enabling the detection of various pathogens, biomolecules, and small molecules with high sensitivity and selectivity. Multiplex microfluidics based on immunosensors can be divided into planar detection and suspended microsphere detection. In two-dimensional (2D) planar multiplex detection, the surface of the microfluidic chip forms a heterogeneous pattern by fixing multiple capture ligands to spatially discrete micro-dots. For example, a thermoplastic microfluidic device fabricated using laser-assisted protein micropatterning technology can detect four cancer-related biomarkers [25]. Planar microfluidic detection can be further divided into spatial segmentation, temporal segmentation, frequency segmentation, and barcode multiplexing [26]. In three-dimensional (3D) suspended arrays, microspheres serve as substrates to capture antibodies or target antigens in solution, forming a homogeneous pattern. Unlike planar detection, which relies on planar position for identification, microspheres use classifiers such as size or internal fluorophores for detection allocation [27]. Immunomagnetic assays serve as a highly efficient pathogen capture platform. Pathogens can be efficiently separated and enriched by using magnetic beads of varying sizes and magnetite content carrying pathogen-specific biotinylated recombinant phages [28]. Planar heterogeneous immunoassays benefit from high surface-to-volume ratios, while homogeneous suspension assays use multiplexing and electrophoretic separation techniques in microfluidics. The combination of microfluidics and immunoassays solves many problems in traditional immunoassays; they are characterized by low reagent and sample consumption, rapid antibody-antigen interaction due to high surface-to-volume ratios and small length scales, and high reproducibility and throughput due to automated fluid handling [29]. Despite the significant advantages of microfluidic immunosensors in pathogen detection, current devices still face many limitations in terms of system integration, sensitivity, and multiplexing capabilities. To improve the practicality and convenience of these devices, the integration of sample pretreatment and data analysis must be strengthened. Furthermore, the sensitivity of immunosensors can be further enhanced by using different tags and introducing signal transduction pathways. Currently commonly used tags include metal tags, redox tags, optical tags, and enzyme tags. Enzyme tags catalyze the conversion of substrates into detectable products, and their numbers increase exponentially, thus significantly amplifying the signal and improving sensitivity. Alkaline phosphatase and horseradish peroxidase are representative enzyme markers [30, 31]. Optical markers, on the other hand, identify and locate antigens in specimens by linking to antibodies, reacting with antigens, and generating light signals emitted by antigen-antibody complexes. These signals can be observed under a microscope or a charge-coupled device camera.

Due to its hydrophilicity and porosity, paper-based multi-channel microfluidics exhibits many significant advantages over traditional capillary microfluidics, such as small size, low cost, the ability to operate liquids without complex equipment, and ease of manufacturing, thus providing an innovative and efficient method for

pathogen detection. Microfluidic paper-based analytical devices (μPADs) and lateral flow tests (LFTs), as typical representatives of paper-based multiplexing microfluidics in infectious disease detection, have been widely used. The conversion between two-dimensional and three-dimensional structures can be achieved by folding, bending, and twisting paper, thereby establishing multi-layer microfluidic channel connections between layers [33]. This design not only improves the flexibility and multifunctionality of detection but also provides a more effective platform for the multiplex quantitative detection of various analytes. In particular, the 3D-μPAD with a multi-layer fluid network has a particularly prominent advantage in multiplex quantitative analysis, capable of simultaneously detecting multiple pathogens and significantly improving detection efficiency and accuracy [34]. LFT has long been widely used in the detection of human chorionic gonadotropin (hCG) for self-monitoring of pregnancy. A typical LFT device consists of a sample pad, a conjugate pad, a nitrocellulose membrane, and an absorbent pad. The sample pad ensures sufficient contact between the liquid sample and the test strip; the conjugate pad is pre-loaded with biomarkers conjugated to specific molecules, which can bind to the target analyte and generate a detectable signal; the nitrocellulose membrane is the core region that immobilizes the trapped molecules for specific binding to the target analyte; the absorbent pad collects excess liquid by driving capillary forces, thereby maintaining the flow balance of the test strip [35-36].

Based on the design of LFT and methods to improve its reusability, LFT-based reusable detection can be divided into the following types. The first type integrates multiple single-target LFTs into the same detection kit. This method is relatively simple for multi-target LFTs. In recent years, researchers have developed a variety of innovative LFT designs, such as multi-directional designs (including origami-style, peace sign-style, orthogonal sign-style, and disc designs), bidirectional detection, and multi-line detection (such as tree-like and forked signs). The second type integrates multi-target detection into a single LFT, applying strategies such as: using spatial isolation within the same detection area (point or line) to observe different targets; using different signal converters for multiple tags on a single detection line; and using barcode-assisted design [38-41].

Nucleic Acid Amplification Methods in Multiplex Microfluidic Technology

In recent years, the combination of microfluidic technology and nucleic acid amplification technology has made significant progress in the field of infectious disease detection. Polymerase chain reaction (PCR), as a classic nucleic acid amplification technology, is widely used for the amplification of specific DNA fragments. For PCR technology, researchers have designed two types of microfluidic devices. The first is fixed-chamber PCR microfluidic technology, which working principle is similar to traditional PCR equipment, where PCR reagents are fixed in the reaction chamber, and nucleic acid amplification is achieved through temperature cycling. This design retains the advantages of precise temperature control in traditional PCR while improving reaction efficiency and portability by leveraging the miniaturization of microfluidic chips. The second is continuous-flow PCR microfluidic technology, which accelerates the analysis process by providing fluid flow between two or three fixed heaters. Compared with fixed-chamber PCR microfluidic technology, continuous-flow PCR microfluidic technology does not require complex temperature cycling equipment, enabling faster nucleic acid amplification, and is particularly suitable for scenarios requiring rapid detection, such as on-site point-of-care testing. The development of microfluidic PCR technology has not only improved the efficiency of nucleic acid amplification, but also provided new technical means for the rapid and accurate detection of infectious diseases. Multiplex polymerase chain reaction (mPCR), as a highly efficient multi-target pathogen detection technology, achieves simultaneous amplification of multiple targets by designing multiple primers complementary to the target gene and mixing them in the same reaction unit or physically isolating them into different units. Currently, mPCR technology has developed various methods, including standard endpoint methods based on gel or capillary electrophoresis, probes labeled with different fluorophores, and intercalation dyes based on melt curve analysis. In probe-based multiplex detection, the detection results are identified by detecting dyes with different fluorescent colors, among which mul-

tiplex probes include TaqMan hydrolysis probes and molecular beacons. However, the limitation of fluorescence channels means that probe-based multiplex detection can usually only detect a maximum of six sequences [43]. To overcome this limitation, combinatorial probe encoding technology has emerged, which combines fluorescent groups into as many indicator probes as possible, and can simultaneously amplify up to ten target fragments even with limited detection channels, significantly improving detection efficiency. Microfluidic mPCR technology, based on microfluidics, pre-fills microchambers with different primer pairs and uses precise fluid control to split samples into different microwells for spatially independent and specific PCR, effectively avoiding non-specific amplification caused by the formation of dimers or polymers between nucleotide chains. Furthermore, the combination of microfluidics and PCR can divide samples into thousands or even millions of tiny units, enabling digital detection, also known as digital PCR. This technology has significant advantages in improving detection sensitivity and accuracy. However, when the template concentration is low, the probability of splitting the template into microwells containing specific primers decreases, potentially causing false negative results. Table 1 shows commercially available microfluidic devices for infectious disease detection.

Summary and Outlook

In the field of pathogen detection, there is no absolutely optimal detection method, but rather only the most suitable technical solutions for detecting pathogens of different properties (Table 2). Microfluidic technologies based on electrochemical sensors, fluorescence sensors, and immunosensors, due to their high sensitivity and rapid response, can achieve multiplex detection of pathogens. Due to their ease of operation and lack of external analytical equipment, microfluidic technologies, based on colorimetric sensors, have become an easy-to-implement pathogen detection method. Surface-enhanced Raman scattering (SERS) technology, with its non-invasive detection advantage, has become an ideal choice for non-destructive testing. However, while these types of multi-pathogen microfluidic technologies perform well in laboratory environments, they have certain limitations in field testing scenarios. In contrast, due to their portability and ability to manipulate liquids without external equipment, microfluidic paper-based analytical devices and transverse flow assays can meet the needs for field pathogen detection. Material sustainability is also an important consideration in the early stages of device design. Currently, most microfluidic devices are made from unsustainable fossil polymers, the disposal of which often requires incineration, resulting in additional carbon dioxide emissions and toxic chemicals. Therefore, sustainable materials, such as recyclable and biodegradable plastics and natural fibers, should be prioritized in development and production to reduce environmental impact.

Microfluidic technology still holds great promise for the simultaneous detection of multiple pathogens. First, the isolation and purification of pathogens from complex clinical samples remains a pressing problem. Direct pathogen detection is difficult to achieve without appropriate sample preparation steps. Microfluidic technology has multiple functions, such as cell capture, enrichment, and nucleic acid extraction and purification [47, 48]. Microfluidics based on size exclusion filtration has been applied to the capture and enrichment of pathogens. The principle is to use the physical properties of nanoparticles or microbeads with high specific surface areas, combined with rapid diffusion and dielectrophoresis techniques in microfluidics [49-52].

Primer design constitutes the second major challenge in multiplex nucleic acid detection. Given the limited number of target nucleic acids, separating samples for parallel single-nucleic acid amplification is impractical. Currently, researchers have designed specific single primer sets and multiple primer sets for multiplex nucleic acid amplification, aiming to reduce sample and reagent consumption. However, the difference in amplification efficiency between different primers often causes reduced detection sensitivity. Using single primer sets targeting phylogenetically conserved sequences, nucleic acid amplification can achieve the detection of a wide range of pathogens without sacrificing sensitivity [53]. Furthermore, broad-spectrum PCR can identify unknown, variant, or emerging causes of disease infection [54]. Microfluidic chip sequencing and high-resolution melting curve analysis have been used to detect pathogens without amplicon, providing a better option for large-scale pathogen identification [55-57]. Finally, research should not be limited to laboratories and specialized operators; improving the scalability of multiplex

detection devices is crucial. For example, microfluidic materials commonly used in academic laboratories (such as polydimethylsiloxane) are not suitable for large-scale production, while traditional soft lithography techniques are not only time-consuming but also have low throughput. In contrast, microfluidic paper-based analytical devices are more efficient and less expensive to pro-

Table 1

Summary of the commercial microfluidic devices used for infectious disease testing

Таблица 1

Краткая информация о коммерческих микрофлюидных устройствах, используемых для тестирования на инфекционные заболевания

Company	Website	Equipment Name	Technology	Detection Target
Cephid	https://www.cepheid.com/	GeneXpert®	PCR	NA
BioFire	https://www.biofiredx.com/	FilmArray®	Nested multiplex PCR	NA
BD	https://www.bd.com/	BD MAX™	Real-time PCR	NA
Mobidiag	https://www.mobidiag.com	Amplidiag® Novodiag®	Multiplex real-time PCR	NA
Baicare	https://www.bai-care.com	iChip-400 Onestart-1000	Isothermal amplification PCR	NA
Curetis	https://www.curetis.com	Unyvero A50	Multiplex PCR	NA
GenePOC	https://www.genepoc-diagnostics.com	Revogene®	PCR	NA
GeneMark	https://www.genmarkdx.com/	ePlex®	Real-time PCR	NA
Lumex	https://www.lumex.com.cn/	AriaDNA	Real-time qPCR	NA

NA: Nucleic acid; PCR, polymerase chain reaction
NA: Нуклеиновая кислота; ПЦР, полимеразная цепная реакция

Table 2

Comparison of multiplex microfluidic techniques for simultaneous pathogen detection

Таблица 2

Сравнение мультиплексных микрофлюидных технологий для одновременного обнаружения патогенов

Types	Advantages	Disadvantages:
Electrochemical sensors	Easy to use; rapid analysis; high sensitivity; high repeatability; accuracy	Poor electrode stability;
Fluorescence sensors	High sensitivity; high selectivity; fast response	Sensitive to interference;
Colorimetric sensors	Fast and simple analysis; no external equipment required; low cost	Low sensitivity; limited quantitative capability; limited multiplex detection capability;
Surface-enhanced Raman scattering (SERS)	Fast analysis; small sample size; high sensitivity; high signal strength	Low repeatability; high cost; short lifespan; complex manufacturing;
Immunosensors	Fast analysis; high selectivity; high sensitivity; low cost; high repeatability	Non-specific binding;
Paper-based microfluidics	Small sample size; low cost; liquid manipulation without external equipment; easy to manufacture; miniaturization and portability; field detection	Limited sensitivity; limited quantitative capability; limited multiplex detection capability;
Multiplex PCR	Small sample size; high specificity; high sensitivity	False negative results may occur at low template concentrations; high-precision detection instruments are required.

duce. Furthermore, future microfluidic technologies for infectious disease detection need to meet the needs of end-users and the requirements of big data analytics. This can be achieved by integrating smartphone technology or AI-driven big data solutions.

Information about the authors:

Shi Yiwei – PhD, Lecturer, Naval Medical University, Shanghai Key Laboratory of Medical Defense, China, 200433, Shanghai, Yangpu, Xiangyin Road, 800; e-mail: shiyiwei9773@163.com

Fang Letian – MSc, Naval Medical University, Shanghai Key Laboratory of Medical Defense, China, 200433, Shanghai, Yangpu, Xiangyin Road, 800; e-mail: letianfang@163.com

Cao Guangwen – PhD, Professor, Doctoral Supervisor, Naval Medical University, Shanghai Key Laboratory of Medical Defense, China, 200433, Shanghai, Yangpu, Xiangyin Road, 800; e-mail: gcao@smmu.edu.cn

Сведения об авторах:

Ши Ивэй – доктор наук, преподаватель, Военно-морской медицинский университет, Шанхайская ключевая лаборатория медицинской защиты, Китай, 200433, Шанхай, Янпу, дорога Сянъинь, 800; e-mail: shiyiwei9773@163.com

Фан Лэтянь – магистр наук. Военно-морской медицинский университет, Шанхайская ключевая лаборатория медицинской защиты, Китай, 200433, Шанхай, Янпу, дорога Сянъинь, 800; e-mail: letianfang@163.com

Цао Гуанвэнь – доктор наук, профессор, научный руководитель докторантов, Военно-морской медицинский университет, Шанхайская ключевая лаборатория медицинской защиты, Китай, 200433, Шанхай, Янпу, дорога Сянъинь, 800; e-mail: gcao@smmu.edu.cn

Вклад авторов. Все авторы подтверждают соответствие своего авторства, согласно международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией).

Наибольший вклад распределен следующим образом. Концепция и план исследования – Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь; сбор данных – Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь; анализ данных и выводы – Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь; подготовка рукописи – Ши Ивэй, Фан Лэтянь, Цао Гуанвэнь.

Author contribution. All authors according to the ICMJE criteria participated in the development of the concept of the article, obtaining and analyzing factual data, writing and editing the text of the article, checking and approving the text of the article.

Special contribution: ShI, FL, TcG contribution to the concept and plan of the study. ShI, FL, TcG contribution to data collection. ShI, FL, TcG contribution to data analysis and conclusions. ShI, FL, TcG contribution to the preparation of the manuscript.

Disclosure. The authors declare that they have no competing interests.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Funding. The study was carried out without additional funding.

Финансирование. Исследование проведено без дополнительного финансирования.

Поступила/Received: *10.10.2025*
Принята к печати/Accepted: *15.12.2025*
Опубликована/Published: *30.12.2025*

ЛИТЕРАТУРА/REFERENCES

1. Batt C. A. Materials science. Food pathogen detection [J]. *Science*, 2007, 316 (5831), 1579-80.
2. Whitesides G. M. The origins and the future of microfluidics [J]. *Nature*, 2006, 442 (7101), 368-73.
3. Shi Y., Cai Y., Cao Y., et al. Recent advances in microfluidic technology and applications for anti-cancer drug screening [J]. *TrAC Trends in Analytical Chemistry*, 2021, 134, 116118.
4. Park J., Han D. H., Park J. K. Towards practical sample preparation in point-of-care testing, user-friendly microfluidic devices [J]. *Lab Chip*, 2020, 20 (7), 1191-203.
5. Ayub K., Mohammad H., Nasrin S., et al. Electrochemical biosensing using N-GQDs, Recent advances in analytical approach [J]. *TrAC Trends in Analytical Chemistry*, 2018, 105, S0165993618301663.
6. Labib M., Sargent E. H., Kelley S. O. Electrochemical Methods for the Analysis of Clinically Relevant Biomolecules [J]. *Chem Rev*, 2016, 116 (16), 9001-90.
7. Rong Q., Feng F., Ma Z. Metal ions doped chitosan–poly(acrylic acid) nanospheres, Synthesis and their application in simultaneously electrochemical detection of four markers of pancreatic cancer [J]. *Biosens Bioelectron*, 2016, 75, 148-54.
8. Wu C., Selberg J., Nguyen B., et al. A Microfluidic Ion Sensor Array [J]. *Small (Weinheim an Der Bergstrasse, Germany)*, 2020, 16(6), e1906436.
9. Li C., Fu Z., Li Z., et al. Cross-talk-free multiplexed immunoassay using a disposable electrochemiluminescent immunosensor array coupled with a non-array detector [J]. *Biosens Bioelectron*, 2011, 27 (1), 141-7.
10. Hsieh K., Ferguson B. S., Eisenstein M., et al. Integrated electrochemical microsystems for genetic detection of pathogens at the point of care [J]. *Acc Chem Res*, 2015, 48 (4), 911-20.
11. Zhang J., Yang Z., Liu Q., et al. Electrochemical biotoxicity detection on a microfluidic paper-based analytical device via cellular respiratory inhibition [J]. *Talanta*, 2019, 202, 384-91.
12. Nwankire C. E., Venkatanarayanan A., Glennon T., et al. Label-free impedance detection of cancer cells from whole blood on an integrated centrifugal microfluidic platform [J]. *Biosens Bioelectron*, 2015, 68, 382-9.

13. Salahandish R., Hassani M., Zare A., et al. Autonomous electrochemical biosensing of glial fibrillary acidic protein for point-of-care detection of central nervous system injuries [J]. *Lab Chip*, 2022, 22 (8), 1542-55.
14. Cooper M. A. Optical biosensors, where next and how soon? [J]. *Drug Discov Today*, 2006, 11 (23-24), 1061-7.
15. Liao Z., Zhang Y., Li Y., et al. Microfluidic chip coupled with optical biosensors for simultaneous detection of multiple analytes, A review [J]. *Biosens Bioelectron*, 2019, 126, 697-706.
16. Shu B., Lin L., Wu B., et al. A pocket-sized device automates multiplexed point-of-care RNA testing for rapid screening of infectious pathogens [J]. *Biosens Bioelectron*, 2021, 181, 113145.
17. Lu Y., Yu S., Lin F., et al. Simultaneous label-free screening of G-quadruplex active ligands from natural medicine via a microfluidic chip electrophoresis-based energy transfer multi-biosensor strategy [J]. *Analyst*, 2017, 142 (22), 4257-64.
18. Stanisavljevic M., Krizkova S., Vaculovicova M., et al. Quantum dots-fluorescence resonance energy transfer-based nanosensors and their application [J]. *Biosens Bioelectron*, 2015, 74, 562-74.
19. Zhang M., Liu J., Shen Z., et al. A newly developed paper embedded microchip based on LAMP for rapid multiple detections of foodborne pathogens [J]. *BMC Microbiol*, 2021, 21 (1), 197.
20. Wastling S. L., Picozzi K., Kakembo A. S., et al. LAMP for human African trypanosomiasis, a comparative study of detection formats [J]. *PLoS Negl Trop Dis*, 2010, 4 (11), e865.
21. Banerjee I., Aralaguppe S. G., Lapins N., et al. Microfluidic centrifugation assisted precipitation based DNA quantification [J]. *Lab Chip*, 2019, 19 (9), 1657-64.
22. Sharma S., Thomas E., Caputi M., et al. RT-LAMP-Based Molecular Diagnostic Set-Up for Rapid Hepatitis C Virus Testing [J]. *Biosensors (Basel)*, 2022, 12 (5).
23. Chen H., Das A., Bi L., et al. Recent advances in surface-enhanced Raman scattering-based microdevices for point-of-care diagnosis of viruses and bacteria [J]. *Nanoscale*, 2020, 12 (42), 21560-70.
24. Yang K., Zong S., Zhang Y., et al. Array-Assisted SERS Microfluidic Chips for Highly Sensitive and Multiplex Gas Sensing [J]. *ACS applied materials & interfaces*, 2020, 12 (1), 1395-403.
25. Kling A., Dirscherl L., Dittrich P. S. Laser-assisted protein micropatterning in a thermoplastic device for multiplexed prostate cancer biomarker detection [J]. *Lab Chip*, 2023, 23 (3), 534-41.
26. Gil Rosa B., Akingbade O. E., Guo X., et al. Multiplexed immunosensors for point-of-care diagnostic applications [J]. *Biosens Bioelectron*, 2022, 203, 114050.
27. Ellington A. A., Kullo I. J., Bailey K. R., et al. Antibody-based protein multiplex platforms, technical and operational challenges [J]. *Clin Chem*, 2010, 56 (2), 186-93.
28. Moeller M. E., Fock J., Pah P., et al. Evaluation of commercially available immuno-magnetic agglutination in comparison to enzyme-linked immunosorbent assays for rapid point-of-care diagnostics of COVID-19 [J]. *J Med Virol*, 2021, 93 (5), 3084-91.
29. Ngamsom B., Esfahani M. M., Phurimsak C., et al. Multiplex sorting of foodborne pathogens by on-chip free-flow magnetophoresis [J]. *Anal Chim Acta*, 2016, 918, 69-76.
30. Lin J. H., Yang Y. C., Shih Y. C, et al. Photoinduced electron transfer between Fe(III) and adenosine triphosphate-BODIPY conjugates, Application to alkaline-phosphatase-linked immunoassay [J]. *Biosens Bioelectron*, 2016, 77, 242-8.
31. Pinto V., Sousa P., Catarino S. O., et al. Microfluidic immunosensor for rapid and highly-sensitive salivary cortisol quantification [J]. *Biosens Bioelectron*, 2017, 90, 308-13.
32. Nilghaz A., Wicaksono D. H. B., Gustiono D., et al. Flexible microfluidic cloth-based analytical devices using a low-cost wax patterning technique [J]. *Lab Chip*, 2012, 12 (1), 209-18.
33. Noviana E., Ozer T., Carrell C. S., et al. Microfluidic Paper-Based Analytical Devices, From Design to Applications [J]. *Chem Rev*, 2021, 121 (19), 11835-85.
34. Clarke O. J. R., Goodall B. L., Hui H. P., et al. Development of a SERS-Based Rapid Vertical Flow Assay for Point-of-Care Diagnostics [J]. *Anal Chem*, 2017, 89 (3), 1405-10.
35. Soh J. H., Chan H-M., Ying J. Y. Strategies for developing sensitive and specific nanoparticle-based lateral flow assays as point-of-care diagnostic device [J]. *Nano Today*, 2020, 30, 100831.
36. Wang X., Li F., Guo Y. Recent Trends in Nanomaterial-Based Biosensors for Point-of-Care Testing [J]. *Frontiers in chemistry*, 2020, 8, 586702.
37. Wu Y., Zhou Y., Leng Y., et al. Emerging design strategies for constructing multiplex lateral flow test strip sensors [J]. *Biosens Bioelectron*, 2020, 157, 112168.
38. Han M., Gong L., Wang J., et al. An octuplex lateral flow immunoassay for rapid detection of antibiotic residues, aflatoxin M1 and melamine in milk [J]. *Sensors and Actuators B, Chemical*, 2019, 292, 94-104.
39. Zhang D., Huang L., Liu B., et al. Rapid and Ultrasensitive Quantification of Multiplex Respiratory Tract Infection Pathogen via Lateral Flow Microarray based on SERS Nanotags [J]. *Theranostics*, 2019, 9 (17), 4849-59.
40. Di Nardo F., Alladio E., Baggiani C., et al. Colour-encoded lateral flow immunoassay for the simultaneous detection of aflatoxin B1 and type-B fumonisins in a single Test line [J]. *Talanta*, 2019, 192, 288-94.
41. Yang M., Zhang W., Zheng W., et al. Inkjet-printed barcodes for a rapid and multiplexed paper-based assay compatible with mobile devices [J]. *Lab Chip*, 2017, 17 (22), 3874-82.
42. Mullis K., Faloona F., Scharf S., et al. Specific enzymatic amplification of DNA in vitro, the polymerase chain reaction [J]. *Cold Spring Harb Symp Quant Biol*, 1986, 51, 263-73.
43. Madic J., Jovelet C., Lopez J., et al. EGFR C797S, EGFR T790M and EGFR sensitizing mutations in non-small cell lung cancer revealed by six-color crystal digital PCR [J]. *Oncotarget*, 2018, 9 (100), 37393-406.
44. Miotke L., Lau B. T., Rumma R. T., et al. High Sensitivity Detection and Quantitation of DNA Copy Number and Single Nucleotide Variants with Single Color Droplet Digital PCR [J]. *Anal Chem*, 2014, 86 (5), 2618-24.

45. Tanaka J., Nakagawa T., Shiratori A., et al. KRAS genotyping by digital PCR combined with melting curve analysis [J]. *Sci Rep*, 2019, 9 (1), 2626.
46. Gaňová M., Zhang H., Zhu H., et al. Multiplexed digital polymerase chain reaction as a powerful diagnostic tool [J]. *Biosens Bioelectron*, 2021, 181, 113155.
47. Chen X., Liang Z., Li D., et al. Microfluidic dielectrophoresis device for trapping, counting and detecting Shewanella oneidensis at the cell level [J]. *Biosens Bioelectron*, 2018, 99, 416-23.
48. Su Y., Tian Q., Pan D., et al. Antibody-Functional Microsphere-Integrated Filter Chip with Inertial Microflow for Size-Immune-Capturing and Digital Detection of Circulating Tumor Cells [J]. *ACS applied materials & interfaces*, 2019, 11 (33), 29569-78.
49. Kastania A. S., Petrou P. S., Loukas C. M., et al. Poly-L-histidine coated microfluidic devices for bacterial DNA purification without chaotropic solutions [J]. *Biomed Microdevices*, 2020, 22 (3), 44.
50. Ramachandran A., Huyke D. A., Sharma E., et al. Electric field-driven microfluidics for rapid CRISPR-based diagnostics and its application to detection of SARS-CoV-2 [J]. *Proc Natl Acad Sci U S A*, 2020, 117 (47), 29518-25.
51. Hu F., Li J., Zhang Z., et al. Smartphone-Based Droplet Digital LAMP Device with Rapid Nucleic Acid Isolation for Highly Sensitive Point-of-Care Detection [J]. *Anal Chem*, 2020, 92 (2), 2258-65.
52. Coudron L., Mcdonnell M. B., Munro I., et al. Fully integrated digital microfluidics platform for automated immunoassay, A versatile tool for rapid, specific detection of a wide range of pathogens [J]. *Biosens Bioelectron*, 2019, 128, 52-60.
53. Bouchiat C., Ginevra C., Benito Y., et al. Improving the Diagnosis of Bacterial Infections, Evaluation of 16S rRNA Nanopore Metagenomics in Culture-Negative Samples [J]. *Frontiers in microbiology*, 2022, 13, 943441.
54. Renvoisé A., Brossier F., Sougakoff W., et al. Broad-range PCR, past, present, or future of bacteriology? [J]. *Med Mal Infect*, 2013, 43 (8), 322-30.
55. Zhang H., Xu T., Li C. W., et al. A microfluidic device with microbead array for sensitive virus detection and genotyping using quantum dots as fluorescence labels [J]. *Biosens Bioelectron*, 2010, 25 (11), 2402-7.
56. Rezaei M., Radfar P., Winter M., et al. Simple-to-Operate Approach for Single Cell Analysis Using a Hydrophobic Surface and Nanosized Droplets [J]. *Anal Chem*, 2021, 93 (10), 4584-92.
57. Rolando J. C., Jue E., Barlow J. T., et al. Real-time kinetics and high-resolution melt curves in single-molecule digital LAMP to differentiate and study specific and non-specific amplification [J]. *Nucleic Acids Res*, 2020, 48 (7), e42.