

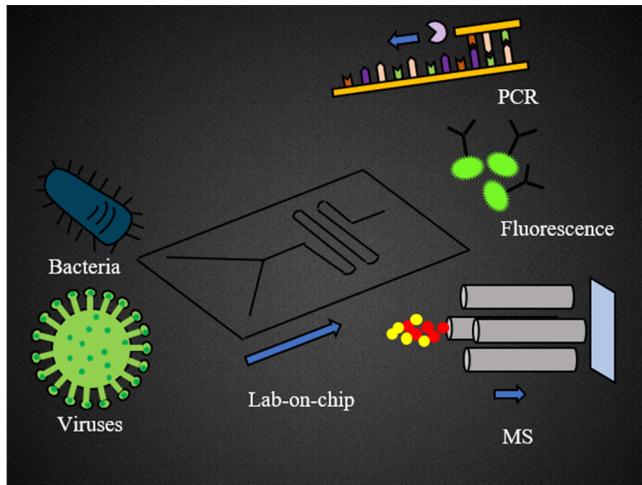
Detection of Pathogenic Microorganisms by Microfluidics Based Analytical Methods

Microfluidics based biochemical analysis shows distinctive advantages for fast detection of pathogenic microorganisms. This Feature summarizes the progress in the past decade on microfluidic methods for purification and detection of pathogenic bacteria and viruses as well as their applications in food safety control, environmental monitoring, and clinical diagnosis.

Dongxue Zhang,[†] Hongyan Bi,^{*,‡} Baohong Liu,[†] and Liang Qiao^{*,†}

[†]Department of Chemistry, Shanghai Stomatological Hospital, and Shanghai Key Laboratory of Molecular Catalysis and Innovative Materials, Fudan University, Shanghai, China 200433

[‡]College of Food Science and Engineering, Shanghai Ocean University, Shanghai, China 201306



CHALLENGES IN THE DETECTION OF PATHOGENIC MICROORGANISMS

Pathogenic microorganisms refer to any microorganisms capable of causing human or animal diseases, including viruses, bacteria, fungi, protozoa, helminthes, etc. They can be transferred from one host to another by air, body fluids, food, water, etc., causing public panic and economic losses. Rapid and accurate identification of pathogenic microorganisms is crucial in ensuring proper therapy. Nevertheless, the current methods used in hospital cannot fully address the concern. For instance, semiquantitative plate culture method is the “gold standard” for bacterial identification, which is however time-consuming, limited by low positive rate, and can hardly differentiate bacteria at the levels of strains and species.¹

During the recent years, modern immunological and molecular diagnostics have been developed for microbial identification. Immunological diagnostics relies on the usage of specific antibodies to recognize the corresponding microorganisms, followed by spectrophotometric or fluorescence spectrometric detection. Molecular diagnostics has been developed based on proteomic and genomic approaches, including mass spectrometry (MS), polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), high-throughput next generation sequencing (NGS), etc.^{2–5}

Mass spectrometry is a technique that can elucidate the molecular structure and molecular weight of analytes. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) has been widely used for bacterial identification since 1990s.⁶ Because of the significant differences in molecular compositions of different species of bacteria, MALDI-TOF mass spectra collected from intact bacterial cells have fingerprint characteristics and can be used for bacterial identification.⁷ The MS based system for bacterial identification has been commercialized by several producers, e.g., bioMérieux as Vitek MS and Bruker as Biotyper.^{8,9} However, the method can only identify highly abundant and purified bacterial samples, i.e., single colonies after plate culture, and thereby is still limited by the long procedure and low positive rate of bacterial culture. By using affinity mass spectrometry, bacteria with concentrations as low as 10^3 cells/mL in blood serum can be collected by antibodies modified magnetic beads and then directly identified by MALDI-TOF MS without bacterial culture.¹⁰

PCR is a technique to amplify segments of DNA. Based on the knowledge of specific gene sequences of a target microorganism, primers corresponding to the sequences can be designed to identify the microorganism by PCR. For instance, a method based on multiplex real-time PCR has been developed for the diagnosis of bloodstream infections (BSI) in intensive care unit (ICU).¹¹ During PCR, a series of repeated temperature changes is required. In contrast, LAMP is an isothermal nucleic acid amplification technique. Therefore, it is more suitable than PCR to be applied in resource-limited laboratories and is also widely used for microbial identification.^{12,13} The high-throughput NGS can obtain the whole genome of a microorganism for highly accurate microbial typing.¹⁴ New gene sequencing techniques, such as Oxford nanopore techniques, make it much easier and faster to obtain the whole genome, which may lead to revolutionary changes in microbial typing.^{15,16}

To date, the development of modern diagnostic methods targets at rapid identification of a wide range of microorganisms with high sensitivity and accuracy. However, time-consuming and complex sample preparation steps are always required to

Published: March 29, 2018

extract pathogenic microorganisms from specimens with highly abundant background matrixes. It is desirable to develop new methods with the capability to greatly shorten and simplify pretreatment procedures and to automatically couple the pretreatment procedures with various detection techniques, e.g., PCR, LAMP, MS, fluorescence spectrometry, electrochemistry, etc. For clinical applications, the goal is to achieve accurate microbial typing within hours or 1 day by disposable and highly automated techniques.

MICROFLUIDIC TECHNIQUES FOR MICROBIAL ANALYSIS: OVERVIEW

Microfluidics is a multidiscipline that has been rapidly developing since 1980s and early 1990s.¹⁷ Microfluidic techniques integrate sample preparation, reaction, separation and detection by controlling the movement of fluids within microfluidic chips, which are portable devices in the sizes of few centimeters that usually contain valves, microchannels, reaction chambers, pressure systems, and detection systems. The devices consume a low amount of sample (\leq few microliters) and lead to fast analysis.²⁵ In addition, microfluidic chips are safe platforms for chemical, radiological, and biological studies, e.g., for rapid genetic analysis using a 96-sample inlet device.²⁶

The first generation of microfluidic chips were developed with silica or glass using photolithography.²⁷ Soft lithography is now widely used to obtain complex structures with extensive materials.^{28,29} Polydimethylsiloxane (PDMS) is among the most popular elastomers in building microfluidic chips. Compared to glass, silicon, poly(methyl methacrylate) (PMMA), and polycarbonate (PC), PDMS is gas permeable, which makes it more suitable for on-chip cell culture.²⁷ The advantages of PDMS microfluidic chips also include ease-to-realize, nontoxicity, low cost in fabrication, and high elasticity.³⁰ Since it is elastomeric, valves made of PDMS can be easily incorporated into microfluidic systems. Active and passive microvalves have been developed employing mechanical and nonmechanical, e.g., magnetic, electric, and thermal-mechanical methods.³¹

Microfluidic chips can integrate various functions for high-throughput and automated analysis of microbes, as illustrated in Figure 1. The most charming aspect of microfluidics in microbial analysis is on-chip microbial extraction and concentration using physical, chemical, and biochemical methods. The curving microchannel has been used to separate bacteria and viruses from large particles by centrifugal force,²⁰ electrodes has been embedded in microchambers for microbial capturing.³² The physical methods are rapid, convenient, and accurate for the concentration of a broad range of microorganisms. For the enrichment and purification of specific microorganisms, immunoaffinity interaction has been performed on microchips using antibodies modified magnetic beads.³³ In order to increase magnetic force from magnets outside of microchips, micropillar arrays made of paramagnetic material could be embedded in microchambers for highly efficient concentration of microorganisms.²¹ Microfluidic chips can be coupled with various detection techniques, i.e., PCR, LAMP, mass spectrometry, fluorescence spectroscopy, electrochemistry, etc., in the form of on-chip or after-chip detection, for the analysis of microorganisms. It is promising to make microfluidic chips as portable devices for ambient sampling to test environmental pollution, food contaminants, and body fluids.

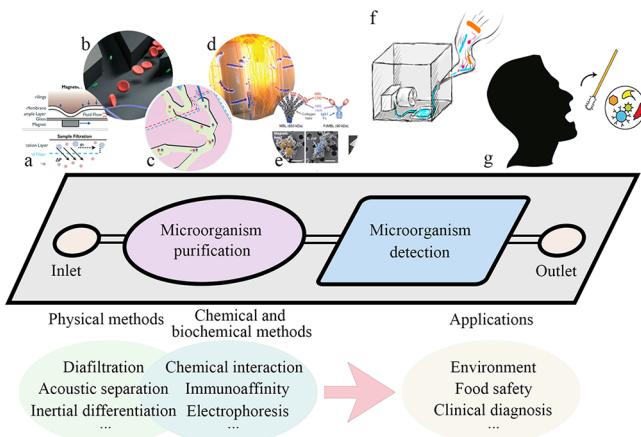


Figure 1. Schematic illustration of on-microchip microbial analysis, combining (a–e) on-chip purification of target microbes and detection methods for (f, g) applications, e.g. environmental monitoring and clinical diagnosis: (a) reproduced from Liu, J. F.; Yadavali, S.; Tsourkas, A.; Issadore, D. *Lab Chip* 2017, 17, 3796–3803 (ref 18), with permission of The Royal Society of Chemistry; (b) reproduced from Ohlsson, P.; Evander, M.; Petersson, K.; Mellhammar, L.; Lehmusvuori, A.; Karhunen, U.; Soikkeli, M.; Seppa, T.; Tuunainen, E.; Spangar, A.; von Lode, P.; Rantakokko-Jalava, K.; Otto, G.; Scheding, S.; Soukka, T.; Wittfooth, S.; Laurell, T. *Anal. Chem.* 2016, 88, 9403–9411 (ref 19). Copyright 2016 American Chemical Society; (c) reproduced from Hong, S. C.; Kang, J. S.; Lee, J. E.; Kim, S. S.; Jung, J. H. *Lab Chip* 2015, 15, 1889–1897 (ref 20), with permission of The Royal Society of Chemistry; (d) reproduced from Malic, L.; Zhang, X.; Brassard, D.; Clime, L.; Daoud, J.; Luebbert, C.; Barrere, V.; Boutin, A.; Bidawid, S.; Farber, J.; Corneau, N.; Veres, T. *Lab Chip* 2015, 15, 3994–4007 (ref 21), with permission of The Royal Society of Chemistry; (e) reprinted by permission from Macmillan Publishers Ltd.: Nat. Med., Kang, J. H.; Super, M.; Yung, C. W.; Cooper, R. M.; Do-mansky, K.; Graveline, A. R.; Mammoto, T.; Berthet, J. B.; Tobin, H.; Cartwright, M. J.; Watters, A. L.; Rottman, M.; Waterhouse, A.; Mammoto, A.; Gamini, N.; Rodas, M. J.; Kole, A.; Jiang, A.; Valentin, T. M.; Diaz, A., et al. *Nat. Med.* 2014, 20, 1211–1216 (ref 22). Copyright 2014; (f) reproduced from Bian, X. J.; Lan, Y.; Wang, B.; Zhang, Y. S.; Liu, B. H.; Yang, P. Y.; Zhang, W. J.; Qiao, L. *Anal. Chem.* 2016, 88, 11504–11512 (ref 23). Copyright 2016 American Chemical Society; (g), reproduced from Ferguson, B. S.; Buchsbaum, S. F.; Wu, T.-T.; Hsieh, K.; Xiao, Y.; Sun, R.; Soh, H. T. *J. Am. Chem. Soc.* 2011, 133, 9129–9135 (ref 24). Copyright 2011 American Chemical Society).

This Feature summarizes the progress in the past decade on microfluidics based methods for microbial analysis as well as their applications in food safety control, environmental monitoring, and clinical diagnosis, especially focusing on the most widely studied pathogenic microorganisms, i.e., bacteria and viruses.

MICROCHIP FOR SAMPLE PREPARATION

Purification and enrichment of microorganisms are key steps required for accurate microbial identification. Traditional methods for bacterial purification relies on bacterial culture, which is time-consuming and can only be applied to culturable species. Furthermore, the purified bacteria from single colonies cannot fully represent the original components of samples, especially when the original samples contain a number of microbes. To achieve fast and on-site identification and to overcome the biases during culture, different microfluidic chips have been designed to purify and enrich microbes from air, water, food samples, clinical samples, etc., by physical, chemical,

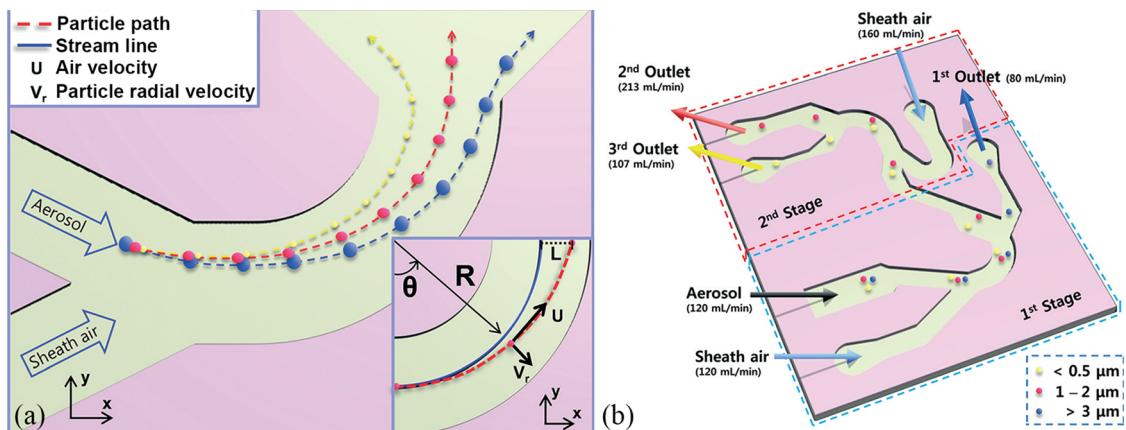


Figure 2. (a) Separation of airborne viral and bacterial bioaerosols from large particles by inertial difference in a curved microchannel and (b) schematic diagram of the microfluidic chip. Reproduced from Hong, S. C.; Kang, J. S.; Lee, J. E.; Kim, S. S.; Jung, J. H. *Lab Chip* 2015, 15, 1889–1897 (ref 20), with permission of The Royal Society of Chemistry.

and biochemical methods. On-chip lysis of bacterial cells and purification of biomolecules have also been developed for on-chip and after chip identification of microbes based on the biomolecules.

Physical Methods for Microbial Extraction. Physical methods for microbial extraction rely on inertial differentiation, size differentiation, diafiltration, acoustic separation, and special design of microchannel structure.^{18–20,34}

Based on the principle of inertial differences, Hong et al. designed a microfluidic chip with a curved microchannel to separate microbes from large particles. When passing the curved channel as shown in Figure 2, large particles had larger radial motion and flowed into outward outlet; while small particles maintained their streamline and passed the radially inner channel.²⁰ Using the methods, particles with the sizes of $>3\text{ }\mu\text{m}$ in diameter, $1\text{--}2\text{ }\mu\text{m}$ in diameter, i.e., bacteria, and $<0.5\text{ }\mu\text{m}$ in diameter, i.e., viruses, could be separated into different outlets. Bioaerosols containing *S. epidermidis* and Adenovirus were used to demonstrate the performance of the system, $>70\%$ of *S. epidermidis* bioaerosols and $\sim 70\%$ Adenovirus bioaerosols were collected in the second and third outlets, respectively. Based on inertial force, bacteria in whole blood could be separated from blood cells.^{35,36}

Membrane filtration methods are also used to separate particles with different sizes. Liu et al. designed a microfluidic device, which consisted of a magnetic pump and a size-based filter to separate small particles from large ones.¹⁸ Compared with dialysis, this method has smaller dead volume. Figure 3 shows the schematic diagram of the microfluidic chip. Under the force of iron fillings in the upper microchannel affected by a moving magnet, the flexible PDMS membrane could deform to drive fluidic flow. Pressure generated by a long thin pinch channel drove small particles permeating the size-based filter for separation.

Margination methods have also been used to separate bacteria from matrixes. A microfluidic chip consisting of a straight microchannel with two bifurcations (1:8:1 in width) was fabricated to separate bacteria from other cells in whole blood, i.e., red blood cells, platelets, and leukocytes.³⁷

Chemical and Biochemical Methods for Microbial Extraction. Chemical and biochemical methods separate microorganisms based on specific recognition, such as immunoaffinity interaction³⁸ and affinity of mannose binding lectin to mannose on the cell membranes of bacteria.²² With

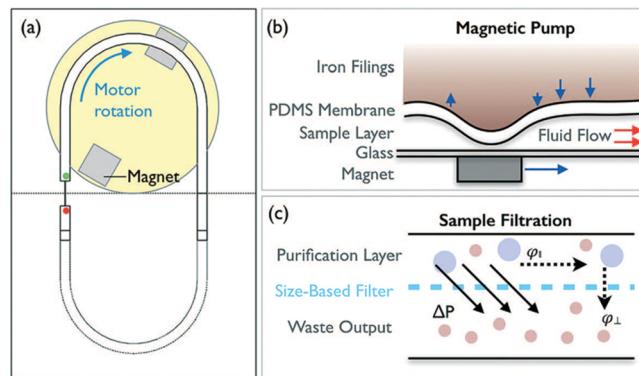


Figure 3. Schematic diagram of the microfluidic chip with a magnetic pump and a size-based filter to separate particles with different sizes. Reproduced from Liu, J. F.; Yadavali, S.; Tsourkas, A.; Issadore, D. *Lab Chip* 2017, 17, 3796–3803 (ref 18), with permission of The Royal Society of Chemistry.

the specific interactions, microorganisms are linked to probe molecules, which can be immobilized on magnetic beads or in microchannels, to be separated from other particles. Comparing to physical methods, the biochemical methods can extract target pathogenic microorganisms from particles with similar sizes and densities.

Guo et al. developed a microfluidic chip with a magnetic field to separate magnetic beads tagged *Salmonella typhimurium*.³⁹ The limit of detection (LOD) of *Salmonella typhimurium* in milk reached 5.4×10^3 CFU/mL. Pereiro et al. also utilized antibody-functionalized superparamagnetic beads to capture bacteria, as shown in Figure 4.³⁸ In the microfluidic system, the magnetic beads were retained in a chamber by the balance between magnetic and drag forces to form microscale-fluidized bed. Bacteria captured by the magnetic beads were then *in situ* cultivated by infusing nutrient rich medium, resulting in an expansion of the fluidized bed because of the growth of the captured bacteria, which could be directly observed by naked eyes, for bacterial detection. This system achieved detection of *Salmonella typhimurium* from undiluted and unskimmed milk with the capture rate of about 71% and quantitative detection of bacteria down to 4 CFU.

Combining with specific affinities between probe molecules and microbial cells, electrophoresis in microchips has also been used to purify and concentrate microorganisms. Wang et al.

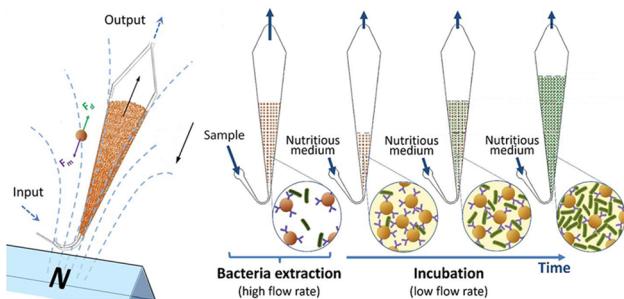


Figure 4. Scheme of the microfluidic fluidized bed and the processes of bacterial extraction and incubation in the fluidized bed. Reproduced from Pereiro, I.; Bendali, A.; Tabnaoui, S.; Alexandre, L.; Srbova, J.; Bilkova, Z.; Deegan, S.; Joshi, L.; Viovy, J. L.; Malaquin, L. *Chem. Sci.* 2017, 8, 1329–1336 (ref 38), with permission of The Royal Society of Chemistry.

utilized chitosan to integrate bacterial cells and as a sweeping carrier. A multiple on-chip concentration approach was then developed for bacteria by combining chitosan sweeping, field-amplified sample stacking, and reversed-field stacking.⁴⁰ Yang et al. developed a microfluidic chip, where the microchannel was on top of an array of oxide covered interdigitated electrodes. Positive dielectrophoresis was performed on the chip to concentrate bacterial cells, followed with selective capture of the concentrated bacteria using specific antibodies.³²

To analyze bacteria in BSI specimens, lysing all blood cells is a method of choice to collect bacteria. Zelenin et al. designed a microfluidic system that used mild detergent and osmotic shock by deionized water to lyse blood cells and get bacterial cells from blood samples.⁴¹ Almost 100% viable bacteria were readily recovered by the microchip for further analysis.

On-Chip Lysis of Bacterial Cells and Purification of Biomolecules. Following on-chip bacterial extraction, lysis of bacterial cells and purification of biomolecules could be performed to collect molecular biomarkers for subsequent analysis. Van Heirstraeten et al. integrated sample (bacterial and viral pathogens) resuspension, cell lysis, nucleic acid purification and concentration into a microfluidic chip, as shown in Figure 5.⁴² Once microbial cells were lysed, ethanol was added to the swab-containing reservoir (No. 1) to stimulate nucleic acid binding on the solid phase extraction (SPE) membrane (No. 7) embedded in the microfluidic chip. Cell lysates were then flown through the membrane (No. 7) and rinsed by an ethanol-based buffer. After 1 min of incubation, distilled water as elution buffer was used to elute nucleic acid from the SPE membrane into the recovery chamber (No. 8). The microchip provides a more efficient and secure solution for extracting bacterial DNA and viral RNA comparing to manual benchtop experiments. Microfluidic chip with SPE method was also used to extract bacterial DNA from blood.⁴³

MICROFLUIDIC CHIPS COUPLED WITH VARIOUS ANALYTICAL METHODS FOR MICROBIAL DETECTION

Microfluidic chips can couple with various analytical methods to detect and identify microorganisms. Genetic and mass spectrometric techniques are the methods of choice to identify microorganisms without the need of labeling procedures. Techniques, such as spectrophotometry, fluorescence spectrometry, and electrochemistry, are also used for microbial

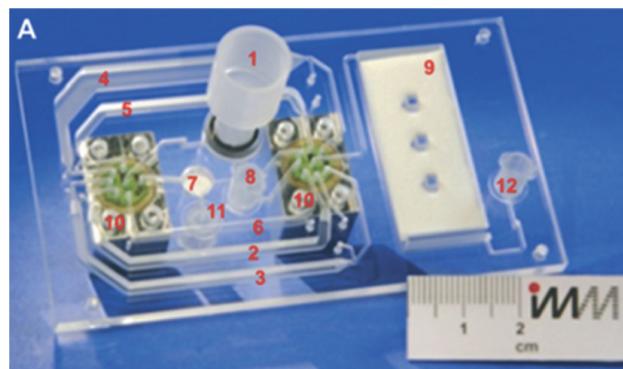


Figure 5. (A and B) Microfluidic device and its layout, respectively, for on-chip extraction and purification of nucleic acids from microorganisms: (1) swab/sample reservoir, (2) suspension/pretreatment buffer, (3) lysis buffer, (4) ethanol, (5) washing buffer, (6) elution buffer, (7) SPE membrane, (8, 11, 12) Luer connections, (9) waste chamber, and (10) turning valves. Red arrows in part B show turning-valves rotating sense. Reproduced from Van Heirstraeten, L.; Spang, P.; Schwind, C.; Dresen, K. S.; Ritzel-Lehnert, M.; Nieto, B.; Camps, M.; Landgraf, B.; Guasch, F.; Corbera, A. H.; Samitier, J.; Goossens, H.; Malhotra-Kumar, S.; Roeser, T. *Lab Chip* 2014, 14, 1519–1526 (ref 42), with permission of The Royal Society of Chemistry.

detection but usually need specific labeling by antibodies that can interact with target pathogenic microorganisms.

PCR on Microfluidic Chips. PCR can be performed on microfluidic chips by carefully controlling reaction conditions, e.g., temperature, and introducing primers as well as other PCR reagents into microchips. Microfluidic chips integrated with PCR and sample preparation can work as point-of-care devices for rapid and accurate microbial analysis. Cao et al. developed a microfluidic chip that is functionalized with SPE and reverse transcription (RT)-PCR for the detection of viruses.^{44,45} The system consumed low amount of reagents, was more portable than benchtop RT-PCR, and more sensitive than rapid immunoassay tests. It was used to detect influenza A directly from clinical specimens with concentrations as low as 10^3 copies/mL in 3 h. Microfluidic chips integrated with PCR/RT-PCR could also be applied to differentiate subtypes of viruses. Sun et al. described a microfluidic chip integrated with DNA microarray-based solid-phase PCR for Avian Influenza virus (AIV) screening.⁴⁶ Using the microchip assay, three AIV strains, H₁N₁, H₅N₁, and H₇N₅, were successfully identified.

LAMP on Microfluidic Chips. Similar to PCR, LAMP can also be performed on microfluidic chips. Comparing to PCR, LAMP based microbial analysis has the advantages of reduced analysis time and simplified procedure and equipment. Wang et al. integrated magnetic beads based rapid RNA extraction with RT-LAMP on a microfluidic chip to detect viruses from tissue

samples.⁴⁸ Sensitivity of the method was about 100-fold higher than a typical traditional RT-PCR process. Dou et al. developed a microfluidic system for sensitive diagnosis of infectious disease without laborious sample preparation processes and the use of a centrifuge.⁴⁷ A 3D illustration of the microchip is shown in Figure 6. The microchip consisted of two PDMS

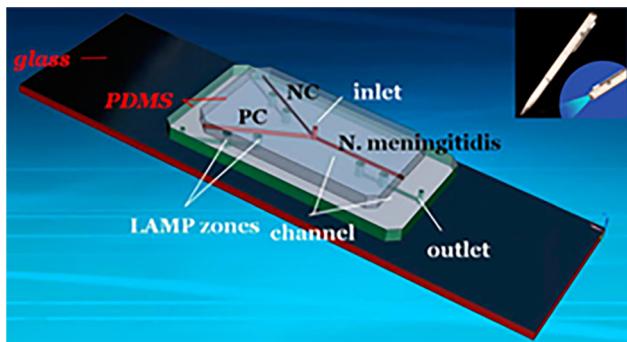


Figure 6. Layout of the PDMS/paper hybrid microfluidic device for on-chip LAMP based detection of *Neisseria meningitidis*. Reproduced from Dou, M.; Dominguez, D. C.; Li, X.; Sanchez, J.; Scott, G. *Anal. Chem.* 2014, 86, 7978–7986 (ref 47). Copyright 2014 American Chemical Society.

layers, one glass slide, and a chromatography paper disk situated inside each LAMP zone to preload LAMP primers. A UV penlight was used to illuminate LAMP products, and the fluorescence produced as a result of the LAMP reaction was recorded by a camera. The LOD of ~3 copies of *Neisseria meningitidis* was achieved using the microchip within 45 min. Chen et al. designed a microfluidic chip containing agarose and LAMP reagents to detect multiple pathogens in human serum.⁴⁹ The microchip could be kept at 4 °C for long-term storage. It was used to detect four species of foodborne bacteria in serous samples, including *Escherichia coli*, *Proteus hauseri*, *Vibrio parahemolyticus*, and *Salmonella* subsp.. Centrifugal microdevices integrated with RT-LAMP and immunochromatographic strip (ICS) based amplicon detection have also been developed for the detection of pathogenic microbes, e.g., influenza A virus.⁵⁰

Microfluidics in Combination with Mass Spectrometry. MS based bacterial detection relies on spectra pattern matching against a library of MALDI-TOF mass spectra from standard strains^{51,52} or relies on proteomic strategies.⁵³ Comparing to PCR and LAMP, MS can identify much wider types of bacteria. A distinctive advantage of MALDI-TOF MS based bacterial identification is its low cost, where expensive reagents for gene amplification and biochemical experiments can be avoided. Coupling microfluidic chips with mass spectrometry can greatly improve the overall analytical performance of MS based approaches and expand their potential applications.⁵⁴ Microfluidic chips have been coupled with mass spectrometers via various interfaces, e.g., microchip electrospray ionization⁵⁵ and droplets collection from microchips on MALDI target plates.⁵⁶ Bian et al. designed a microfluidic chip, which could catch and enrich bacteria in air with high efficiency.²³ They used liquid chromatography–mass spectrometry (LC–MS) based proteomic strategy to identify multiple bacterial species, i.e., *Vibrio parahemolyticus*, *Listeria monocytogenes*, and *Escherichia coli*, from the samples collected within the microchip. Cho et al. utilized MALDI-TOF MS in combination with a microchip integrated with magnetic beads-

based affinity chromatography to detect RNA polymerase of hepatitis C virus from patient serum.⁵⁷ Aptamer was used as affinity ligand and immobilized on the magnetic beads to capture the proteins. UV irradiation was performed to elute the captured proteins. The eluted target proteins were then digested and analyzed by MALDI-TOF MS.

Microfluidics in Combination with Fluorescence Spectrometry

Spectrometry. Comparing with genetic and mass spectrometric methods, fluorescent detection assay could provide a visual signal. With fluorescence microscopy, it is convenient to observe captured bacteria and to visualize fluidic flow in microfluidic devices.³⁴ Zhang et al. reported a microdevice integrated with immunofluorescence for point-of-care AIV detection.³³ AIV H₉N₂ was captured specifically by antibodies modified magnetic beads, and the beads were trapped in a magnetic zone for detection by fluorescence spectroscopy, as shown in Figure 7. The LOD for AIV H₉N₂ was down to 3.7 ×

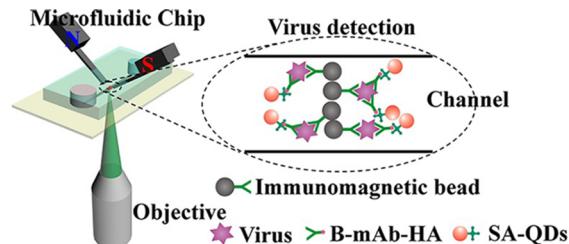


Figure 7. Working principle of the on-chip magnetic immunofluorescence assay for the detection of AIV. Reproduced from Zhang, R.-Q.; Liu, S.-L.; Zhao, W.; Zhang, W.-P.; Yu, X.; Li, Y.; Li, A.-J.; Pang, D.-W.; Zhang, Z.-L. *Anal. Chem.* 2013, 85, 2645–2651 (ref 33). Copyright 2013 American Chemical Society.

10⁴ copies/μL with only 2 μL of sample consumption. Similarly, on-chip magnetic immunofluorescence assay was also used for bacterial detection.³⁹

Microfluidics in Combination with Electrochemistry

By integrating electrodes in microfluidic chips, electrochemical measurements can be on-chip performed to assist sensitive detection of microbes. Altintas et al. designed a fully automated microfluidic-based electrochemical biosensor for *Escherichia coli* detection in water sample.⁵⁸ The microfluidic biosensor contained eight Au electrodes. Bacterial cells could be concentrated on the surface of the Au electrodes by immunoaffinity. Horse radish peroxidase (HRP) labeled antibodies were then introduced to form a sandwich structure with the captured bacterial cells for electrochemical measurement of HRP-TMB (3,3',5,5'-tetramethylbenzidine) interaction. The system achieved waterborne pathogenic *Escherichia coli* detection, with a LOD as low as 50 CFU/mL. Ölcer et al. developed a sensing microsystem that consisted of Au electrode arrays and Au nanoparticles for real-time amperometric measurements.⁵⁹ The platform was applied to detect cyanobacterial gene fragments.

To summarize this section, more works of on-chip PCR and LAMP have been reported for bacterial and viral detection than microchip electrochemistry, fluorescence, and mass spectrometry. The techniques of PCR and LAMP gain the advantages that the techniques do not rely on the usage of antibodies and can detect a wide range of microbes based on their specific gene sequences. Furthermore, because of gene amplification, the techniques are very sensitive. Electrochemistry and fluorescence cannot directly identify microbes. Specific detection of

pathogenic microbes normally relied on the usage of specific antibodies. Nevertheless, the techniques are very fast and sensitive, suitable for the detection of specific pathogenic microbes of interest. Mass spectrometry has the advantage of identifying a wide range of microbes based on proteomic approach or spectra matching. Nevertheless, the current technique requires large amount of cell copies for proteomic analysis ($\sim 10^7$ cell copies) or MALDI-TOF MS identification ($\sim 10^5$ cell copies). Therefore, MS based techniques still need to couple with bacterial culture for bacterial amplification. For specific applications, suitable techniques can be selected or several techniques can be combined, based on analysis purpose and the nature of sample.

■ APPLICATION

Microfluidics based microbial detection has been applied in the fields of environmental monitoring, food safety control, clinical diagnosis, etc.

Application to Environmental Monitoring. Airborne bioaerosols consist of microorganisms, such as fungi, bacteria, and viruses as well as their excretions.⁶⁰ Exposure to bioaerosols could cause immunologic, infectious, and toxic lung diseases.⁶¹ There is an urgent demand of portable analytical devices to monitor airborne pathogenic bioaerosols. Sui's lab in Fudan University has done a lot of work on the collection and detection of airborne pathogenic microorganisms. They developed microfluidic chips with staggered herringbone mixer (SHM) structured channels.³⁴ The devices were capable of fast capturing and enriching airborne bacterial aerosols. Capture efficiency could reach almost 100% in 9 min. They further integrated the capturing microchips with detection microchips based on immunofluorescence assay, as shown on Figure 8.⁶² The SHM structured microfluidic chip was also

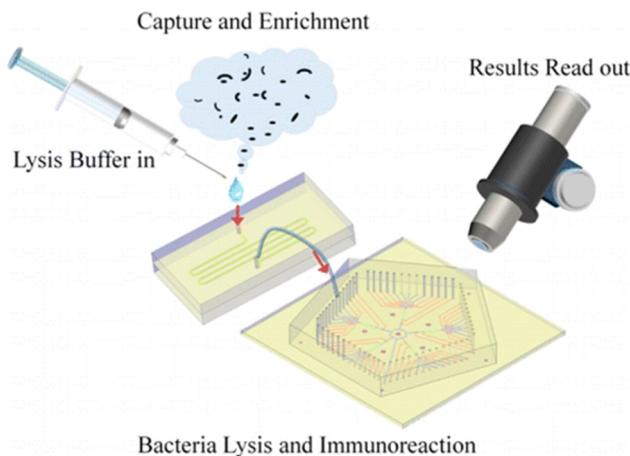


Figure 8. Schematic illustration of the microfluidic system for on-chip airborne bacterial collection and immunofluorescence based detection. Reproduced from Jing, W. W.; Jiang, X. R.; Zhao, W.; Liu, S. X.; Cheng, X. J.; Sui, G. D. *Anal. Chem.* 2014, 86, 5815–5821 (ref 62). Copyright 2014 American Chemical Society.

integrated with a high-throughput continuous-flow PCR chip.⁶³ Detection limit down to approximately 118 cells was achieved for *Escherichia coli* without any additional DNA purification processes. The SHM structured microfluidic chip was also integrated with LAMP analysis to detect airborne *Staphylococcus aureus*,⁶⁴ where bacteria were collected from polluted

air and then directly subjected to LAMP analysis without any procedure for DNA purification.

Application to Food Safety Control. Food safety is a global issue and always a focus of attention. Foodborne pathogenic microbes, e.g., *Campylobacter jejuni*, Shiga toxin-producing *Escherichia coli* (STEC), *Listeria monocytogenes*, and *Salmonella*, can be easily transmitted via food and severely threaten public health.⁶⁵ It is strongly essential to have a device for rapid and accurate identification of foodborne pathogenic microorganisms.

Chen et al. designed a microfluidic biosensor including a separation chip and a detection chip for *Listeria* identification from food samples.⁶⁶ In the separation chip, the immuno-magnetic assay was applied. A complex of anti-*Listeria* monoclonal antibodies modified magnetic nanoparticles, *Listeria* cells, anti-*Listeria* polyclonal antibodies, and urease modified gold nanoparticles (MNP-*Listeria*-AuNP-urease) was formed. In the detection part, electrochemical signal of ammonium ions and carbonate ions produced by urea hydrolysis was detected to quantify *Listeria* cells. The LOD was as low as 160 CFU/mL. The recovery rates of *Listeria* cells from spiked lettuce samples were ranging from 82.1% to 89.6%. Pathogens in drinking water can also be detected using similar microfluidic devices.⁵⁹

Clinical Applications. According to the Review on Antimicrobial Resistance, worldwide deaths attributable to antimicrobial resistant bacteria could reach 10 million by 2050.⁶⁷ Accurate diagnosis plays an important role in the whole therapy process of infectious diseases and is a key step for precision medicine. Ferguson et al. presented a microfluidic platform integrating electrochemical DNA (E-DNA) sensors based on PCR or LAMP and sample preparation by immunomagnetic separation to make genetic detection at clinically relevant concentrations possible.^{24,68} The magnetic integrated microfluidic electrochemical detector (MIMED) device could accomplish all the processes of viral detection directly from throat swabs, i.e., immunomagnetic extraction of target viruses, RT-PCR, single stranded DNA generation, and sequence-specific E-DNA detection, as shown in Figure 9. LOD of the method was 4 orders of magnitude lower than traditional clinical titers.

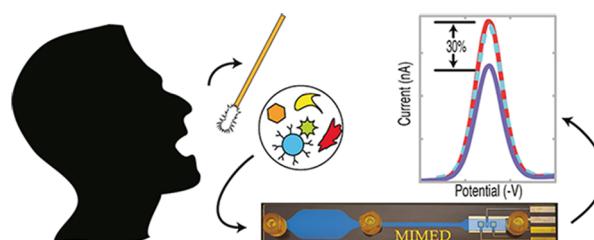


Figure 9. Overview of the MIMED device assay. Reproduced from Ferguson, B. S.; Buchsbaum, S. F.; Wu, T.-T.; Hsieh, K.; Xiao, Y.; Sun, R.; Soh, H. T. *J. Am. Chem. Soc.* 2011, 133, 9129–9135 (ref 24). Copyright 2011 American Chemical Society.

Oblath et al. developed a microfluidic chip that integrated with DNA extraction and real-time PCR to detect *Streptococcus mutans* from saliva.⁶⁹ They used monolithic aluminum oxide membrane to extract DNA. One advantage of this assay is that different primers can be used in different reaction wells to detect multiple microorganisms without the complex processes of multiplex PCR reaction. The LOD of 100–125 cell copies

was achieved for both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) spiked into saliva samples.

For the analysis of bacteria in blood samples, Ohlsson et al.¹⁹ designed an acoustophoresis microchip. Using the microchip, red blood cells were focused to the center of the channel by an acoustic standing wave and sequentially removed. Then bacteria in plasma moved into a glass capillary with a localized acoustic standing wave field, where bacteria were trapped and separated from plasma. Finally, bacteria were released into a polymer microchip for PCR reaction. The LOD of *Pseudomonas putida* spiked into whole blood was about 10^3 cells/mL by using the analysis system.

Kang et al. developed a blood-cleaning device for sepsis therapy (biospleen), which can continuously remove harmful particles, e.g., pathogens and toxins (Figure 10).²² They used

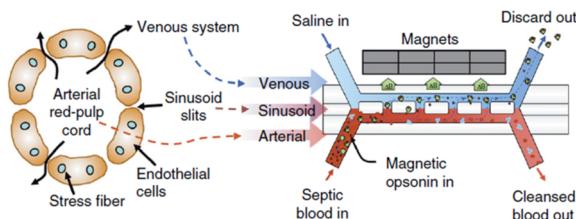


Figure 10. Depletion of pathogens in blood by continuous *in vitro* cleaning using the biospleen device. Reprinted by permission from Macmillan Publishers Ltd.: *Nat. Med.*, Kang, J. H.; Super, M.; Yung, C. W.; Cooper, R. M.; Domansky, K.; Graveline, A. R.; Mammoto, T.; Berthet, J. B.; Tobin, H.; Cartwright, M. J.; Watters, A. L.; Rottman, M.; Waterhouse, A.; Mammoto, A.; Gamini, N.; Rodas, M. J.; Kole, A.; Jiang, A.; Valentin, T. M.; Diaz, A., et al. *Nat. Med.* 2014, 20, 1211–1216 (ref 22). Copyright 2014.

magnetic nanobeads coated with engineered human opsonin-mannose-binding lectin to capture a broad range of pathogens and toxins, thereby to clean the blood of BSI patients for therapy. According to experiments on model rats infected with *Staphylococcus aureus* and *Escherichia coli*, the biospleen could clean >90% of bacteria from blood.

Antibiotic resistance of bacteria is a significant reason for excess morbidity, mortality, and economic costs.⁷⁰ Microfluidic chips are also used to accomplish antimicrobial susceptibility testing (AST). Xu et al. designed a microfluidic chip containing culture chamber array with paper substrates for cell culture.⁷¹ By using the multiplexed chromogenic assay, multiple bacteria were identified successfully. Besides, AST was accomplished by using the lowest concentration of antibiotics to inhibit the chromogenic reaction. A microfluidic chip that integrated with multiple functions, i.e., bacterial dispensation, broth transportation, and antibiotic dilution, for AST was developed by Lee et al.⁷²

■ CONCLUSION AND PERSPECTIVES

The early warning capability of the presence of biological threats is an urgent civilian and military safety demand. Efficient and rapid extraction of pathogenic microbes from relevant specimens is a key step for subsequent microbial analysis. A variety of microfluidic chips have been designed for microbial extraction and have been coupled with various analytical methods to detect pathogenic microorganisms, e.g., bacteria and viruses, existing in environment, food, and human body fluids. Designing and fabricating functionally structured micro-

fluidic chips integrated with external force field, e.g., magnetic field, and developing new interfaces to couple various analytical techniques with microchips would contribute to the progressing of microfluidics in pathogenic microbial detection.

Microfluidic chips for clinical diagnosis of pathogens are developing toward point-of-care, rapid analysis, automation, high throughput, high specificity, high accuracy, uninjurious and portable type, low cost, and convenience. Key requirements for clinical usages of microchips are stability, reproducibility, and mass production, which call for the development of new fabrication techniques and new materials for microfluidic chips. Among various clinical applications, one important topic is to separate bacteria from blood samples for clinical diagnosis of BSI and for BSI treatment. Comparing to other infections, BSI is more dangerous, where fast identification of pathogens is urgently required. In the future, various microfluidics based approaches for bacterial extraction from blood and identification methods could be combined for the purpose of fast, untargeted, and accurate identification of a broad range of bacteria from patients' blood samples. A series of microfluidic platforms with preloaded reagents could be designed and manufactured for the purpose.

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: liang_qiao@fudan.edu.cn.

*E-mail: hybi@shou.edu.cn.

ORCID

Baohong Liu: 0000-0002-0660-8610

Liang Qiao: 0000-0002-6233-8459

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Dongxue Zhang is currently pursuing her Ph.D. studies in the Chemistry Department of Fudan University. She earned her B.S. degree in Applied Chemistry from East China Normal University in 2016. Her research topic is focused on microfluidic chip based bacterial analysis.

Hongyan Bi is an associate professor at Shanghai Ocean University with the expertise in analytical chemistry. She received her Ph.D. degree in Chemistry from Ecole Polytechnique Fédérale de Lausanne in 2010. Her current interests include developing microfluidic devices, biosensors, and microsensors for food quality control.

Baohong Liu is a professor in the Chemistry Department of Fudan University. She obtained her B.S., Masters, and Ph.D. degrees in Chemistry from Fudan University in 1991, 1994, and 1997, respectively. She was visiting professor in Ecole Polytechnique Fédérale de Lausanne (2002, 2006, 2008), Ecole Normale Supérieure (2005), and Perpignan University (2006). She was vice chair of Bioelectrochemistry Division of International Society of Electrochemistry. Her research interests include electroanalytical chemistry, microfluidics, and mass spectrometry.

Liang Qiao is a professor in the Chemistry Department of Fudan University. He obtained his B.S. degree in Chemistry and Master degree in Chemical Biology from the Chemistry Department of Fudan University in 2006 and 2009, respectively, and his Ph.D. degree in Chemistry from Ecole Polytechnique Fédérale de Lausanne in 2013.

His research focuses on fundamental and applied aspects of analytical science, especially in mass spectrometry and microfluidics. His recent research interests include mass spectrometry-based bacterial typing, microfluidics for bacterial extraction from blood samples, bacterial metabolomics, and computational mass spectrometry.

ACKNOWLEDGMENTS

This work is supported by National Natural Science Foundation of China (NSFC, Grant 81671849), and Science and Technology Commission of Shanghai Municipality (Grant 17JC1400900). H.B. acknowledges the Eastern Scholar Professorship program (Grant No. QD2016054). L.Q. acknowledges the Thousand Talents Program of China. The authors acknowledge the American Chemical Society, the Royal Society of Chemistry, and the Springer Nature publication groups for the reprints of the figures used in this Feature.

REFERENCES

- (1) Yu, F.; Li, Y.; Li, M. Y.; Tang, L. H.; He, J. J. *Biosens. Bioelectron.* **2017**, *89*, 880–885.
- (2) Pietrowska, M.; Marczak, L.; Polanska, J.; Behrendt, K.; Nowicka, E.; Walaszczyk, A.; Chmura, A.; Deja, R.; Stobiecki, M.; Polanski, A.; Tarnawski, R.; Widlak, P. *J. Transl. Med.* **2009**, *7*, 60.
- (3) Han, E. T. *Expert Rev. Mol. Diagn.* **2013**, *13*, 205–218.
- (4) Barr, N. B.; Copeland, R. S.; De Meyer, M.; Masiga, D.; Kibogo, H. G.; Billah, M. K.; Osir, E.; Wharton, R. A.; McPheron, B. A. *Bull. Entomol. Res.* **2006**, *96*, 505–521.
- (5) Mirsaidov, U. M.; Wang, D.; Timp, W.; Timp, G. *Wires Nanomed. Nanobi.* **2010**, *2*, 367–381.
- (6) Claydon, M. A.; Davey, S. N.; EdwardsJones, V.; Gordon, D. B. *Nat. Biotechnol.* **1996**, *14*, 1584–1586.
- (7) Ziegler, D.; Pothier, J. F.; Ardley, J.; Fossou, R. K.; Pflueger, V.; de Meyer, S.; Vogel, G.; Tonolla, M.; Howieson, J.; Reeve, W.; Perret, X. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 5547–5562.
- (8) Buchan, B. W.; Riebe, K. M.; Ledebroer, N. A. *J. Clin. Microbiol.* **2012**, *50*, 346–352.
- (9) Huang, A. M.; Newton, D.; Kunapuli, A.; Gandhi, T. N.; Washer, L. L.; Isip, J.; Collins, C. D.; Nagel, J. L. *Clin. Infect. Dis.* **2013**, *57*, 1237–1245.
- (10) Zhu, Y. D.; Qiao, L.; Prudent, M.; Bondarenko, A.; Gasilova, N.; Möller, S. B.; Lion, N.; Pick, H.; Gong, T. Q.; Chen, Z. X.; Yang, P. Y.; Lovey, L. T.; Girault, H. H. *Chem. Sci.* **2016**, *7*, 2987–2995.
- (11) Lehmann, L. E.; Hunfeld, K.-P.; Steinbrucker, M.; Brade, V.; Book, M.; Seifert, H.; Bingold, T.; Hoeft, A.; Wissing, H.; Stueber, F. *Intensive Care Med.* **2010**, *36*, 49–56.
- (12) Tomita, N.; Mori, Y.; Kanda, H.; Notomi, T. *Nat. Protoc.* **2008**, *3*, 877–882.
- (13) Mori, Y.; Notomi, T. *J. Infect. Chemother.* **2009**, *15*, 62–69.
- (14) Schadt, E. E.; Turner, S.; Kasarskis, A. *Hum. Mol. Genet.* **2010**, *19*, R227–R240.
- (15) Branton, D.; Deamer, D. W.; Marziali, A.; Bayley, H.; Benner, S. A.; Butler, T.; Di Ventra, M.; Garaj, S.; Hibbs, A.; Huang, X.; Jovanovich, S. B.; Krstic, P. S.; Lindsay, S.; Ling, X. S.; Mastrangelo, C. H.; Meller, A.; Oliver, J. S.; Pershin, Y. V.; Ramsey, J. M.; Riehn, R.; et al. *Nat. Biotechnol.* **2008**, *26*, 1146–1153.
- (16) Laver, T.; Harrison, J.; O'Neill, P. A.; Moore, K.; Farbos, A.; Paszkiewicz, K.; Studholme, D. *J. Biomol. Detect. Quantif.* **2015**, *3*, 1–8.
- (17) Ducre, J. *Diagnostics* **2012**, *2*, 1.
- (18) Liu, J. F.; Yadavali, S.; Tsourkas, A.; Issadore, D. *Lab Chip* **2017**, *17*, 3796–3803.
- (19) Ohlsson, P.; Evander, M.; Petersson, K.; Mellhammar, L.; Lehmusvuori, A.; Karhunen, U.; Soikkeli, M.; Seppa, T.; Tuunainen, E.; Spangar, A.; von Lode, P.; Rantakokko-Jalava, K.; Otto, G.; Scheding, S.; Soukka, T.; Wittfooth, S.; Laurell, T. *Anal. Chem.* **2016**, *88*, 9403–9411.
- (20) Hong, S. C.; Kang, J. S.; Lee, J. E.; Kim, S. S.; Jung, J. H. *Lab Chip* **2015**, *15*, 1889–1897.
- (21) Malic, L.; Zhang, X.; Brassard, D.; Clime, L.; Daoud, J.; Luebbert, C.; Barrere, V.; Boutin, A.; Bidawid, S.; Farber, J.; Corneau, N.; Veres, T. *Lab Chip* **2015**, *15*, 3994–4007.
- (22) Kang, J. H.; Super, M.; Yung, C. W.; Cooper, R. M.; Domansky, K.; Graveline, A. R.; Mammoto, T.; Berthet, J. B.; Tobin, H.; Cartwright, M. J.; Watters, A. L.; Rottman, M.; Waterhouse, A.; Mammoto, A.; Gamini, N.; Rodas, M. J.; Kole, A.; Jiang, A.; Valentin, T. M.; Diaz, A.; et al. *Nat. Med.* **2014**, *20*, 1211–1216.
- (23) Bian, X. J.; Lan, Y.; Wang, B.; Zhang, Y. S.; Liu, B. H.; Yang, P. Y.; Zhang, W. J.; Qiao, L. *Anal. Chem.* **2016**, *88*, 11504–11512.
- (24) Ferguson, B. S.; Buchsbaum, S. F.; Wu, T.-T.; Hsieh, K.; Xiao, Y.; Sun, R.; Soh, H. T. *J. Am. Chem. Soc.* **2011**, *133*, 9129–9135.
- (25) Feng, X.; Du, W.; Luo, Q.; Liu, B. F. *Anal. Chim. Acta* **2009**, *650*, 83–97.
- (26) Simpson, P. C.; Roach, D.; Woolley, A. T.; Thorsen, T.; Johnston, R.; Sensabaugh, G. F.; Mathies, R. A. *Proc. Natl. Acad. Sci. U. S. A.* **1998**, *95*, 2256–2261.
- (27) Ren, K. N.; Zhou, J. H.; Wu, H. K. *Acc. Chem. Res.* **2013**, *46*, 2396–2406.
- (28) Qin, D.; Xia, Y. N.; Whitesides, G. M. *Nat. Protoc.* **2010**, *5*, 491–502.
- (29) Xia, Y. N.; Whitesides, G. M. *Annu. Rev. Mater. Sci.* **1998**, *28*, 153–184.
- (30) McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H. K.; Schueller, O. J. A.; Whitesides, G. M. *Electrophoresis* **2000**, *21*, 27–40.
- (31) Oh, K. W.; Ahn, C. H. *J. Micromech. Microeng.* **2006**, *16*, R13–R39.
- (32) Yang, L.; Banada, P. P.; Chatni, M. R.; Lim, K. S.; Bhunia, A. K.; Ladisch, M.; Bashir, R. *Lab Chip* **2006**, *6*, 896–905.
- (33) Zhang, R.-Q.; Liu, S.-L.; Zhao, W.; Zhang, W.-P.; Yu, X.; Li, Y.; Li, A.-J.; Pang, D.-W.; Zhang, Z.-L. *Anal. Chem.* **2013**, *85*, 2645–2651.
- (34) Jing, W.; Zhao, W.; Liu, S.; Li, L.; Tsai, C.-T.; Fan, X.; Wu, W.; Li, J.; Yang, X.; Sui, G. *Anal. Chem.* **2013**, *85*, 5255–5262.
- (35) Wu, Z.; Willing, B.; Bjerketorp, J.; Jansson, J. K.; Hjort, K. *Lab Chip* **2009**, *9*, 1193–1199.
- (36) Mach, A. J.; Di Carlo, D. *Biotechnol. Bioeng.* **2010**, *107*, 302–311.
- (37) Hou, H. W.; Gan, H. Y.; Bhagat, A. A. S.; Li, L. D.; Lim, C. T.; Han, J. *Biomicrofluidics* **2012**, *6*, 024115–024115–13.
- (38) Pereiro, I.; Bendali, A.; Tabnaoui, S.; Alexandre, L.; Srbova, J.; Bilkova, Z.; Deegan, S.; Joshi, L.; Viovy, J. L.; Malaquin, L. *Chem. Sci.* **2017**, *8*, 1329–1336.
- (39) Guo, P.-L.; Tang, M.; Hong, S.-L.; Yu, X.; Pang, D.-W.; Zhang, Z.-L. *Biosens. Bioelectron.* **2015**, *74*, 628–636.
- (40) Wang, Z. F.; Cheng, S.; Ge, S. L.; Wang, H.; Wang, Q. J.; He, P. G.; Fang, Y. Z. *Anal. Chem.* **2012**, *84*, 1687–1694.
- (41) Zelenin, S.; Hansson, J.; Arbabili, S.; Ramachandraiah, H.; Brismar, H.; Russom, A. *Biotechnol. Lett.* **2015**, *37*, 825–830.
- (42) Van Heirstraeten, L.; Spang, P.; Schwind, C.; Drese, K. S.; Ritzel-Lehnert, M.; Nieto, B.; Camps, M.; Landgraf, B.; Guasch, F.; Corbera, A. H.; Samitier, J.; Goossens, H.; Malhotra-Kumar, S.; Roeser, T. *Lab Chip* **2014**, *14*, 1519–1526.
- (43) Breadmore, M. C.; Wolfe, K. A.; Arcibal, I. G.; Leung, W. K.; Dickson, D.; Giordano, B. C.; Power, M. E.; Ferrance, J. P.; Feldman, S. H.; Norris, P. M.; Landers, J. P. *Anal. Chem.* **2003**, *75*, 1880–1886.
- (44) Cao, Q.; Mahalanabis, M.; Chang, J.; Carey, B.; Hsieh, C.; Stanley, A.; Odell, C. A.; Mitchell, P.; Feldman, J.; Pollock, N. R.; Klapperich, C. M. *PLoS One* **2012**, *7*, e33176.
- (45) Cao, Q. Q.; Fan, A.; Klapperich, C. J. *Visualized Exp.* **2013**, DOI: 10.3791/50325.
- (46) Sun, Y.; Dhumpa, R.; Bang, D. D.; Hogberg, J.; Handberg, K.; Wolff, A. *Lab Chip* **2011**, *11*, 1457–1463.
- (47) Dou, M.; Dominguez, D. C.; Li, X.; Sanchez, J.; Scott, G. *Anal. Chem.* **2014**, *86*, 7978–7986.
- (48) Wang, C. H.; Lien, K. Y.; Wang, T. Y.; Chen, T. Y.; Lee, G. B. *Biosens. Bioelectron.* **2011**, *26*, 2045–2052.
- (49) Chen, C.; Liu, P.; Zhao, X.; Du, W.; Feng, X. J.; Liu, B. F. *Sens. Actuators, B* **2017**, *239*, 1–8.

- (50) Jung, J. H.; Park, B. H.; Oh, S. J.; Choi, G.; Seo, T. S. *Lab Chip* **2015**, *15*, 718–725.
- (51) Bohme, K.; Fernandez-No, I. C.; Barros-Velazquez, J.; Gallardo, J. M.; Canas, B.; Calo-Mata, P. *Electrophoresis* **2011**, *32*, 2951–2965.
- (52) De Bruyne, K.; Slabbinck, B.; Waegeman, W.; Vauterin, P.; De Baets, B.; Vandamme, P. *Syst. Appl. Microbiol.* **2011**, *34*, 20–29.
- (53) Nomura, F. *Biochim. Biophys. Acta, Proteins Proteomics* **2015**, *1854*, 528–537.
- (54) Feng, X. J.; Liu, B. F.; Li, J. J.; Liu, X. *Mass Spectrom. Rev.* **2015**, *34*, 535–557.
- (55) Jiang, Y.; Wang, P. C.; Locascio, L. E.; Lee, C. S. *Anal. Chem.* **2001**, *73*, 2048–2053.
- (56) Lee, J.; Musyimi, H. K.; Soper, S. A.; Murray, K. K. *J. Am. Soc. Mass Spectrom.* **2008**, *19*, 964–972.
- (57) Cho, S.; Lee, S. H.; Chung, W. J.; Kim, Y. K.; Lee, Y. S.; Kim, B. G. *Electrophoresis* **2004**, *25*, 3730–3739.
- (58) Altintas, Z.; Akgun, M.; Kokturk, G.; Uludag, Y. *Biosens. Bioelectron.* **2018**, *100*, 541–548.
- (59) Olcer, Z.; Esen, E.; Ersoy, A.; Budak, S.; Kaya, D. S.; Gok, M. Y.; Barut, S.; Ustek, D.; Uludag, Y. *Biosens. Bioelectron.* **2015**, *70*, 426–432.
- (60) Frohlich-Nowoisky, J.; Kampf, C. J.; Weber, B.; Huffman, J. A.; Pohlker, C.; Andreae, M. O.; Lang-Yona, N.; Burrows, S. M.; Gunthe, S. S.; Elbert, W.; Su, H.; Hoor, P.; Thines, E.; Hoffmann, T.; Despres, V. R.; Poschl, U. *Atmos. Res.* **2016**, *182*, 346–376.
- (61) Rose, C. S. *Semin. Respir. Crit. Care Med.* **1999**, *20*, 511–520.
- (62) Jing, W. W.; Jiang, X. R.; Zhao, W.; Liu, S. X.; Cheng, X. J.; Sui, G. D. *Anal. Chem.* **2014**, *86*, 5815–5821.
- (63) Jiang, X.; Jing, W.; Zheng, L.; Liu, S.; Wu, W.; Sui, G. *Lab Chip* **2014**, *14*, 671–676.
- (64) Jiang, X.; Liu, Y.; Liu, Q.; Jing, W.; Qin, K.; Sui, G. *Micromachines* **2016**, *7*, 169.
- (65) Oliver, S. P.; Jayarao, B. M.; Almeida, R. A. *Foodborne Pathog. Dis.* **2005**, *2*, 115.
- (66) Chen, Q.; Wang, D.; Cai, G.; Xiong, Y.; Li, Y.; Wang, M.; Huo, H.; Lin, J. *Biosens. Bioelectron.* **2016**, *86*, 770–776.
- (67) O'Neill, J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations, Review on Antimicrobial Resistance*, 2014.
- (68) Hsieh, K.; Ferguson, B. S.; Eisenstein, M.; Plaxco, K. W.; Soh, H. T. *Acc. Chem. Res.* **2015**, *48*, 911–920.
- (69) Oblath, E. A.; Henley, W. H.; Alarie, J. P.; Ramsey, J. M. *Lab Chip* **2013**, *13*, 1325–1332.
- (70) Reed, S. D.; Laxminarayan, R.; Black, D. J.; Sullivan, S. D. *Ann. Pharmacother.* **2002**, *36*, 148–154.
- (71) Xu, B.; Du, Y.; Lin, J.; Qi, M.; Shu, B.; Wen, X.; Liang, G.; Chen, B.; Liu, D. *Anal. Chem.* **2016**, *88*, 11593–11600.
- (72) Lee, W. B.; Fu, C. Y.; Chang, W. H.; You, H. L.; Wang, C. H.; Lee, M. S.; Lee, G. B. *Biosens. Bioelectron.* **2017**, *87*, 669–678.