

# A rapid multiplex nucleic acid detection system of airborne fungi by an integrated DNA release device and microfluidic chip

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## ARTICLE INFO

### Keywords:

The LMC method  
Integrated DNA release device  
Portable microfluidic chip  
Airborne fungi  
*Aspergillus fumigatus*  
Multiplex nucleic acid detection

## ABSTRACT

Occupational health problems, such as asthma, in specific work environments arise from the presence of airborne fungi. Rapid detection of pathogenic airborne fungi is therefore important to reduce or avoid any adverse effects on staff health. Herein, we established a new integrated rapid Lyticase-Motor-Chemical reagent nucleic acid releasing (LMC) method for the release of fungal DNA. *Aspergillus fumigatus*, *Aspergillus flavus*, and *Cryptococcus neoformans* were chosen to evaluate the LMC method. The results of Loop-Mediated Isothermal Amplification (LAMP) analyses showed that this method could release the nucleic acid of  $4 \times 10^4$  fungal spores, equaling to 400 copies per microliter. This rapid multiplex nucleic acid detection system of airborne fungi included an integrated DNA release device and a portable microfluidic chip. The integrated DNA release device combined mechanical lysing and biochemical reagent treatment to automate DNA release. The microfluidic chip was capable of multiplex nucleic acid detection. The detection limit of this system was  $4 \times 10^4$  spores per test, meeting the requirement of early warnings. The whole analysis from the sample input to readout could be completed within 90 min, including 30 min for fungal DNA release and 45 min for LAMP analysis. The integrated DNA release device and microfluidic chip were portable, showing tremendous potential in point-of-care tests of airborne fungi.

## 1. Introduction

Worldwide, over 300 million people suffer from fungal-related severe diseases, causing the death of more than 1.6 million people annually, more than malaria and similar to the tuberculosis death toll [1]. Moreover, fungal infection rates are rising continuously all over the world, threatening public health [2]. Inhalation is the main route of fungal infection [3]. Depending on their size, fine fungal particles can enter the upper respiratory tract ( $>10 \mu\text{m}$ ) [4], reach the lower respiratory tract ( $5\text{--}10 \mu\text{m}$ ) [4–6], or penetrate deep into the lungs ( $<5 \mu\text{m}$ ) [7]. Moreover, certain types of indoor mold may release microbial

volatile organic compounds that are adverse to human health [8]. More importantly, high concentrations of airborne fungi are enriched in specific work environments, such as farms, sawmills, and waste management, causing adverse effects on workers. Farmer's lung disease is a well-known case of long-term exposure to high loads of fungi that leads to hypersensitivity pneumonitis. Among pathogenic species, *Aspergillus fumigatus* is one of the most common which can cause bronchial hyper-response, cough, allergic sinusitis, and other pulmonary symptoms [9]. It is nearly impossible to completely avoid fungal aerosols due to their wide distribution and ability to easily spread. Methods of rapid detection of specific fungal aerosols, portability, and ease of use

**Abbreviations:** LMC, Lyticase-Motor-Chemical reagent nucleic acid releasing (LMC); LAMP, Loop-Mediated Isothermal Amplification; PCR, polymerase chain reaction; PMMA, Polymethyl Methacrylate; HNB, hydroxyl naphthol blue; dsDNA, double-stranded DNA; CT, cycle threshold.

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<https://doi.org/10.1016/j.talanta.2022.123467>

Received 17 February 2022; Received in revised form 4 April 2022; Accepted 5 April 2022

Available online 9 April 2022

0039-9140/© 2022 Published by Elsevier B.V.

are of utmost importance.

Culture-based methods are considered the standard approaches for the detection of airborne fungi [10]. However, these methods are time-consuming and highly dependent on the culturability and species of the airborne fungi. Thus, some molecular methods, such as polymerase chain reaction (PCR) and isothermal DNA amplification, have been developed and widely used in microorganism measurement with the development of molecular biology [11–13]. LAMP, one of the most popular isothermal DNA amplification methods, is first invented by Notomi [14] and possesses high sensitivity, specificity, and rapidity [15]. Compared with PCR, LAMP is economic with no need for expensive thermal cycling equipment, suitable for on-site analysis.

DNA extraction is the speed-limiting step of fungal nucleic acid detection. DNA extraction of fungal spores or cells is more difficult than bacteria and viruses because fungal spores or cells have tough cell walls and abundant chitin. Tedious and expensive fungal DNA extraction methods such as commercial extraction kits [16–22], liquid nitrogen grinding, ultrasonic crushing, and high-pressure crushing [17,23], have to be used in the laboratory. Though these methods can yield high-quality DNA, they are time-consuming, laborious, expensive, dependent on equipment, and not suitable for rapid or point-of-care tests. Meanwhile, although there are some commercial rapid DNA extraction products on the market, such as miniaturized equipment and nucleic acid releaser. But they can only be used for DNA extraction of bacteria and viruses. More research should be done if miniaturized equipment such as a handheld sonicator was used for fungi DNA extraction. Therefore, it is necessary to develop a simple, user-friendly, and low equipment-dependent method of fungal DNA extraction for rapid on-site detection.

Three-dimensional (3D) printing has been widely applied in portable device manufacturing [24–26], accelerating the application of miniaturized devices in point-of-care tests. Microfluidic chips have also been widely used in point-of-care tests as they can achieve simultaneous multiplex detection. The capillary microfluidic chip is one of the most important aspects for achieving a self-driven liquid flow. Herein, we established sensitive fungus-specific LAMP assays. A simple and stable Lyticase-Motor-Chemical reagents nucleic acid releasing method (LMC) was then developed based on the fungus-specific LAMP assays. For convenience, an integrated DNA release device was 3D printed. A multiplex nucleic acid testing microfluidic chip was designed for airborne fungi detection. Thus, we established an entire nucleic acid detection system, and the process took only 90 min from sample input to readout. Furthermore, the system was economical and user-friendly without resource restrictions. The proposed system could be widely used in rapid nucleic acid detection, particularly in resource-limited areas.

## 2. Materials and methods

### 2.1. Materials and reagents

The fungal strains *A. flavus* (CICC No.2436) and *A. fumigatus* (CICC No.41022) were obtained from the China Center of Industrial Culture Collection (CICC). *C. neoformans* was gifted from Shanghai Huashan Hospital. Petrifilm and mold count MoldTM was purchased from 3 M (Saint Paul, USA). DNeasy Blood & Tissue kit was purchased from QIAGEN (Hilden, Germany). Magnetic Universal Genomic DNA kit was purchased from Hiquialbio Biotechnology Co., Ltd (Luoyang, China). Bst DNA polymerase, large fragment, and dNTPs were purchased from Novoprotein (Suzhou, China). SYBR Green I and UltraPure Low Melting Point Agarose were purchased from ThermoFisher (Waltham, USA). Polymethyl Methacrylate (PMMA) boards were purchased from Jianchuan Technology Co., Ltd (Wenzhou, China). Platemax UltraClear Sealing Film was purchased from Axygen (Union, USA). EC UV resin was purchased from Anycubic (Shenzhen, China). Zirconia beads and other reagents were purchased from Aladdin (Shanghai, China).

### 2.2. Pathogenic fungi culture and collection

*A. flavus*, and *A. fumigatus* were grown at 25 °C for 7–10 days in Salt Czapek-Dox Agar to induce sporulation. The fungal spores were collected using sterile cotton swabs and washed several times with 0.5% Tween-20. The collected spores were suspended in 0.5% tween-20. And *C. neoformans* was grown at 37 °C for 3–4 days in Sabouraud's Dextrose Agar. Some independent colons of *C. neoformans* were picked up by sterile pipettor tips. Then these independent colons were harvested in saline solution. The suspensions were stored at 4 °C and used within 2 weeks.

### 2.3. Fungus-specific LAMP assays establishment and evaluation

Standard fungi genomic DNA extraction was conducted by using DNeasy Blood & Tissue kit according to instructions. LAMP primers were designed with specific nucleotide sequences of fungi (*A. fumigatus*: GenBank accession no. AB259964, *A. flavus*: GenBank accession no. KF562227) using the Primer Explorer V5 software (Eiken Chemical Co., Ltd., Tokyo, Japan; <http://primerexplorer.jp/LAMPv5e/index.html>). The primer sequences of *C. neoformans* were obtained from previous research in the lab [27]. The primers were synthesized by Invitrogen (Carlsbad, CA, USA). The primer details were listed in Table S1. To determine the specificity of the LAMP assays, three sets of primers cross-reacted respectively with the total genomic DNA of six common fungi and sterile water. Three parallel experiments were carried out. To estimate the sensitivity of these assays, a recombinant plasmid containing three target gene sequences including calmodulin gene of *A. fumigatus* (GenBank accession no. AB259964), calmodulin gene of *A. flavus* (GenBank accession no. KF562227) and capsular-associated protein 10 gene of *C. neoformans* (CAP10) (GeneBank accession no. MK413182.1) was constructed by General Biol (Chuzhou, Anhui, China). Recombinant plasmid with  $10^7$ – $10^8$  copies and sterile water were respectively used as templates of LAMP reactions to provide precise sensitivities of these assays. LAMP experiments were conducted in a QuantStudio® 3 Real-Time PCR System (Thermo Fisher, Waltham, American).

### 2.4. Establishment and evaluation of a new fungal DNA extraction method (LMC)

DNA releasing ability of the LMC method was evaluated using  $4 \times 10^2$ – $4 \times 10^6$  fungal spores or cells. These were incubated with 10 U of lyticase (Yeasen, Shanghai, China) and 100 µL of 1 M sorbitol buffer at 30 °C for 10 min. After incubation, 0.01–0.03 g of 1 µm and 10 µm (1:1) zirconia beads were added to the suspension. Gears powered by a coreless motor (3.7 V, 48,000 rpm) (Jiechuangsen, Shenzhen, China) were placed into the tube for 10 min to lyse the fungal spores and then removed. Finally, 100 µL of homemade nucleic acid releaser was added to the tube at 63 °C for 10 min. The supernatant of the mix was used as DNA templates for the following rapid detections. The development of the homemade nucleic acid releaser was listed in supplementary methods. The reasons for the selection of chemical lysis reagents were detailed in Table S2. Evaluation of these chemical lysis reagents and compatibility of the final homemade nucleic acid releaser for the following LAMP assays were shown in Figs. S1–S2. Two commercial DNA extraction kits were used to compare to the LMC method. DNA extraction with two commercial kits was conducted strictly according to use instructions. The LMC method was conducted as described above. After extraction, the DNA was verified by LAMP.

### 2.5. Development of an integrated DNA release device for the LMC method

To automate DNA extraction, an integrated DNA release device for the LMC method was developed. Schematic diagrams of simulation and

physical maps of the whole integrated DNA release device and some components were shown in Fig. 1. The integrated DNA release device mainly consisted of four key modules: a fluid control module, a reagent storage and DNA release module, a heating module, and a power supply module. The first module was applied for automatic fluid control. The movement of the upper reagent storage structure (Fig. 1E) was driven by a micro servo (Telesky, Shenzhen, China) controlled by an Arduino board (YwRobot, Tianjin, China). A Bluetooth module (YwRobot, Tianjin, China) was connected to the Arduino board for control by an application on a smartphone. The second module which was the key component of the integrated DNA release device was used for reagent storage and the reaction vessel. All components of this module, except the microcentrifuge tubes, were designed by Solidworks 2019 Software (Dassault Systèmes, Paris, France) and manufactured by a 3D printer (Anycubic, Shenzhen, China). A coreless motor (Fig. 1E) provided power for the gears (Senmeichuang Technology Co., Ltd., Shenzhen, China). The third module, including a heating film (50 mm × 100 mm) (Huashi, Guangzhou, China) and a temperature probe (Huashi), was used for heating the reagents in the microcentrifuge tubes. Lastly, a power supply unit was included in the device, along with additional modules. The power supply included two parts: one to power the heating module and a second to power the micro servo and coreless motor. The power supply could be rechargeable and the full-charged power supply could be used at least 30 times. Because the power of the heating module, micro servo, and the coreless motor was fairly low, respectively 10 W, 3.6 W, 0.148 W. A pressure-reducing plate (Sinilink, Taobao.com, China) was used to provide stable voltage for the Arduino plate. An optocoupler isolation relay (Flourishing, Ningbo, China) was used to control the power of the micro servo and the Arduino plate.

## 2.6. Microfluidic chip fabrication and operation

The 25 mm × 25 mm × 5 mm microfluidic chip included three layers: I) a PCR plate sealing film top; II) a 5 mm PMMA chip; III) and a PCR plate sealing film bottom. The chip body was composed of three LAMP reaction chambers, three microchannels, one inlet, and three air vents. It was designed by Solidworks 2019 Software and fabricated by a computer-controlled milling machine (Snapmaker, Shenzhen, China). The specific primers mixed with 0.5% low melt agarose (1:1) were

preloaded into individual LAMP reaction chambers before the bottom of the chip body was bonded by a PCR plate sealing film. After that, the chip can be sealed tightly and stored at 4 °C. In the experiment, pre-treated samples mixed with LAMP reaction solution (without primers) were injected into the chip through the inlet. Then, dimethyl silicone oil with highly viscous (200 Mpa) (Rhawn, Shanghai, China) was added to the inlet to form separate chambers. Finally, the chip's inlet and three air vents were sealed by a PCR plate sealing film. And the chip was placed at 63 °C for LAMP amplification.

## 2.7. System verification with air samples

The procedure of the whole detection system was shown in the graphical abstract. To validate the performance of the system, simulated spore-aerosol of *A. fumigatus* were generated and collected. The schematic diagram of the fungi aerosol generation system was shown in Fig. S4. The aerosol was collected into 4 mL 1 M sorbitol containing 0.5% Tween 20 by a wet cyclone sampler (RH Genetech, Shenzhen, China). More details about the wet cyclone sampler were illustrated in supplementary materials. After collecting air samples, 100 µl of the sample was used to count using petrifilm and mold count Mold™ to provide a clear *A. fumigatus* spores amount of the simulated air samples while the rest (3900 µl) were used to validate the feasibility and sensitivity of the whole system. The rest sample was centrifuged with a handheld mini centrifuge and then concentrated to 100 µl. After DNA release with the new LMC method, the LAMP amplification reactions were conducted both in centrifuge tubes and microfluidic chips.

## 2.8. Other methods were listed in the supplementary materials

See [Supplementary materials](#)

## 3. Results and discussion

### 3.1. Specificity and sensitivity of airborne fungi LAMP assays with SYBR green I and HNB

LAMP is a high-efficiency isothermal DNA amplification method, comparable to PCR [28]. Two readout methods, including real-time

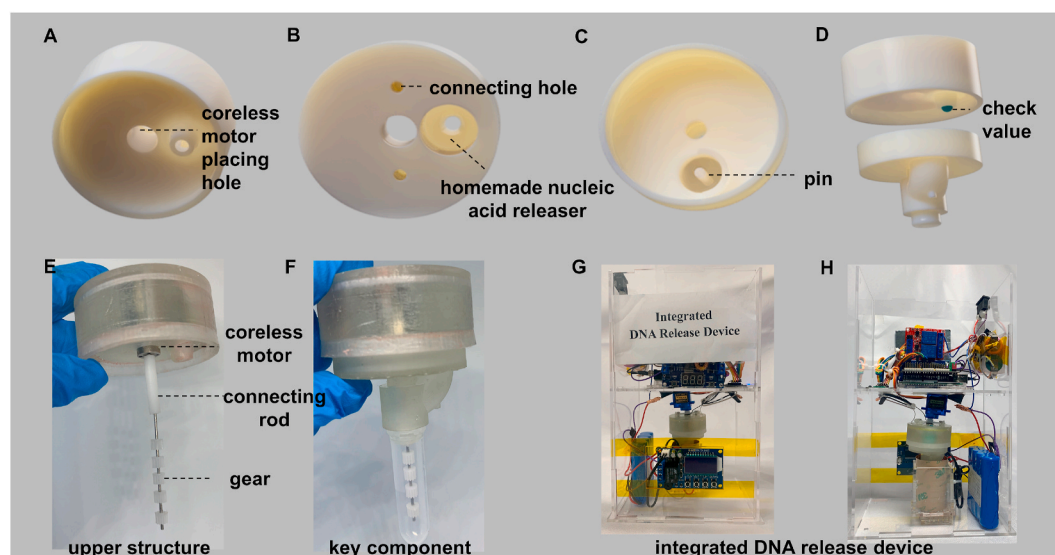


**Fig. 1.** Schematic diagrams of simulation (A–D). (A) Upper structure. A coreless motor placing hole was left in the upper structure. (B) Upper structure. There were two connecting holes on the upper structure, which were used to connect the upper structure and micro servo. And there was a hole that was used for the storage of homemade nucleic acid releaser. (C) Lower part. A pin was on the lower part which was used to trigger the release of liquid. (D) Upper structure and lower part. There was a check valve that was key for liquid control. Physical maps of the integrated DNA release device (E–H). (E) Upper structure. (F) The key component of the integrated DNA release device. (G) The front of the integrated DNA release device. (H) The back of the integrated DNA release device.

fluorescence LAMP with SYBR Green I and colorimetric LAMP with hydroxyl naphthol blue (HNB), were used to determine the specificity and sensitivity of the airborne fungi LAMP assays. Real-time fluorescence LAMP was primarily used in the development process, while colorimetric LAMP was primarily used in sample verification and on-site detection due to its lesser dependence on equipment.

SYBR Green I was commonly used in real-time fluorescence LAMP. Because it fluoresced when it inserted or bound to double-stranded DNA (dsDNA) [29]. High specificity LAMP primers were used for the accurate detection of fungal DNA. Fig. 2A demonstrated that there were no false positives or cross-reactions with other non-target genes from related fungi, proving the high specificity of the LAMP assays used in this study. Fluorescence signals could be detected within 10 min, and showed exponential growth from 10 to 20 min, demonstrating the rapidity of the LAMP assays.

Sensitivity was an important aspect of pathogenic microorganism detection. Fig. 2B showed that the detection limits of *A. fumigatus*, *A. flavus*, and *C. neoformans* were 100 copies per reaction, meeting the general airborne fungi detection requirements. The results indicated that the time in which the signal could be detected was related to the template concentration. The more concentrated the template, the earlier the signal could be detected. Moreover, the result was defined as negative if no fluorescence signal could be detected within 35 min.

The real-time fluorescence LAMP confirmed the feasibility of the three sets of primers. To better apply this method to resource-limited areas and on-site detection, colorimetric LAMP assays with HNB, a metal indicator for calcium and a colorimetric reagent for alkaline earth metal ions, were developed. The addition of HNB prior to LAMP reactions did not inhibit amplification efficiency in previous studies [30]. Fig. 2C demonstrated that there were no false positives or cross-reactions with non-target genes, showing the same specificity as real-time fluorescence LAMP. The sensitivity of the three sets of primers in the colorimetric LAMP assays was also tested. Fig. 2D showed the detection limits of *A. fumigatus*, *A. flavus*, and *C. neoformans* were 100 copies per reaction, which were the same as real-time fluorescence LAMP assays.

Real-time fluorescence LAMP and colorimetric LAMP assays were successfully established for the detection of fungal DNA. Both real-time fluorescence LAMP and colorimetric LAMP showed high specificity and sensitivity, meeting the general requirement of pathogenic microorganism detection. LAMP could easily produce aerosol pollution due to its high efficiency and high loads of amplification products. Therefore, it was necessary to try to avoid open-cap in the process of result readout. These two readout methods could completely avoid the risk of open-cap contamination, ensuring the accuracy of the results.

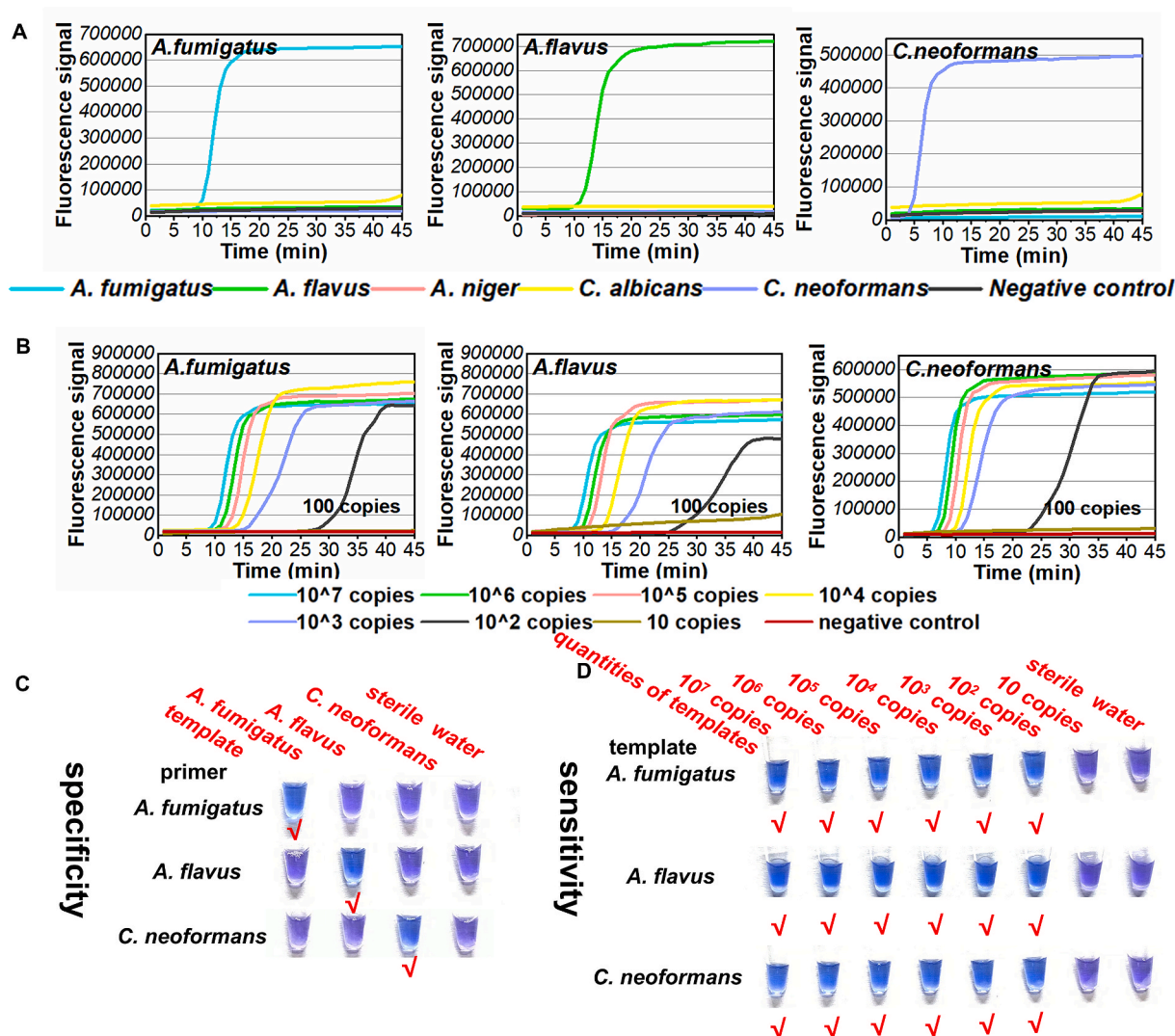


Fig. 2. Specificity and sensitivity of LAMP assays. (A) Specificity of real-time fluorescence LAMP assays. (B) Sensitivity of real-time fluorescence LAMP assays. (C) Specificity of colorimetric LAMP assays. (D) Sensitivity of colorimetric LAMP assays.

### 3.2. Evaluation of the LMC method

To compare the three fungal DNA extraction methods (two commercial DNA extraction kits and LMC) for rapid detection, DNA extracts were used as templates for LAMP assays. Results were illustrated in Fig. 3. After a lot of verifying with fungus-specific LAMP assays used in this study, the results were considered effective when the cycle threshold (CT) value was less than 35. The LMC method could be certain to successfully release the DNA of  $4 \times 10^4$  spores or cells, equating to 400 copies/ $\mu\text{L}$  (Fig. 3). When the number of spores or cells was equal to or more than  $4 \times 10^4$ , there were few differences when compared to the other two commercial DNA extraction methods (Fig. 3). However, the LMC method was more accessible and cost-effective, demonstrating the greater potential for rapid, on-site detection. The details of the comparison among the three methods were illustrated in Table S3. The extracted DNA with the LMC method met the requirement of the following nucleic acid tests. Because our purpose was to establish a rapid and on-site detection system of fungal aerosol, applied in some specific workplaces where the amount of mold ranged from  $10^4$  to  $10^9$  CFU/ $\text{m}^3$  for early warnings [31–33].

### 3.3. Workflow of the integrated DNA release device

An integrated DNA release device was designed for automated DNA release. The basic workflow and control program of the device were shown in Fig. 4A and Video S1. The critical component of the device was the check valve. The check valve (Fig. 1D) was a ball with a diameter of 3 mm that could be used to trigger the homemade nucleic acid releaser release. The mechanism of it was illustrated in Fig. 4B. This valve concept was inspired by the mechanism of ballpoint pens where ink was dispensed onto paper by pressing the metal ball at the tip of the pen. The check valve could block the liquid when the pin staggered it. While the stored reagent could flow into the lower microcentrifuge tube when the check valve was pushed up by the pin. Inspired by the PureLyse® bacteria gDNA extraction kit (PureLyse® technology, Claremont Bio-Solutions LLC), a coreless motor, combined with a microcentrifuge tube formed a miniature bead blender for lysing fungal spores. The integrated DNA release device had potential advantages. First, the integrated DNA release device could be controlled by a smartphone application to achieve automated DNA template preparation. Furthermore, the device could reduce the risk of contamination between different samples. The commonly used microcentrifuge tubes could be changed for each sample to prevent cross-contamination. Finally, the device was small and economic that could be used in rapid point-of-care tests.

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.talanta.2022.123467>.

### 3.4. Preparation and feasibility of the microfluidic chip

A microfluidic chip was developed for multiplex nucleic acid detection. The details of the chip were shown in Fig. 5A and B. Overview of the microfluidic chip and the adapter for the chip were shown in

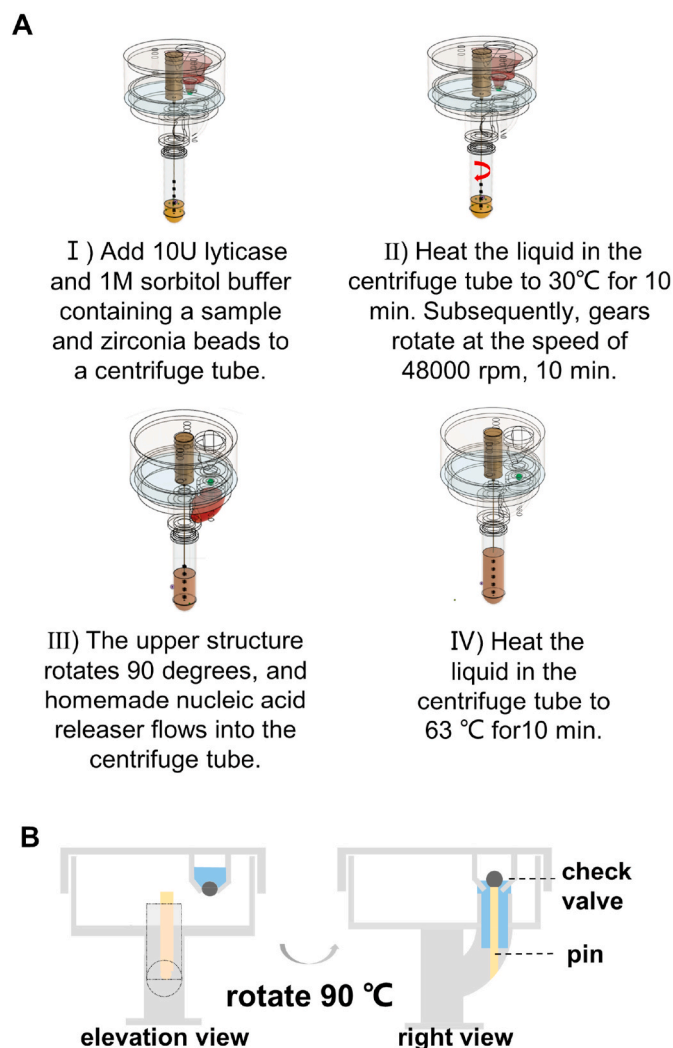


Fig. 4. (A) Workflow of the integrated DNA release device. (B) Two-dimensional, cross-sectional view of the check valve mechanism.

Fig. 5C and D. The solution could fill three reaction chambers within 30 s and each reaction chamber of the chip could work successfully (Fig. 5E). There was no cross-contamination between adjacent chambers (Fig. 5F). Thus, the microfluidic chip could be successfully used in LAMP amplification. The chip seemed very simple but was stable for LAMP amplification. The design was inspired by the principle of communicating vessels and capillary action. The distributary channels were just 1 mm in width and 6 mm in length. We found that these channels could serve as three capillary valves which could block the solution in the reaction chambers. The 3D-printed adapter was 10 mm in length, providing

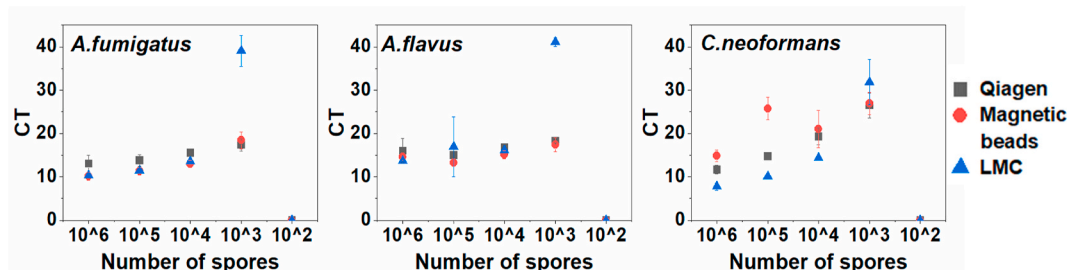


Fig. 3. The results of LAMP with three DNA extraction methods.  $4 \times 10^2$ – $4 \times 10^6$  spores or cells were used as samples. The CT value of negative results was defined as 0. CT: cycle threshold.

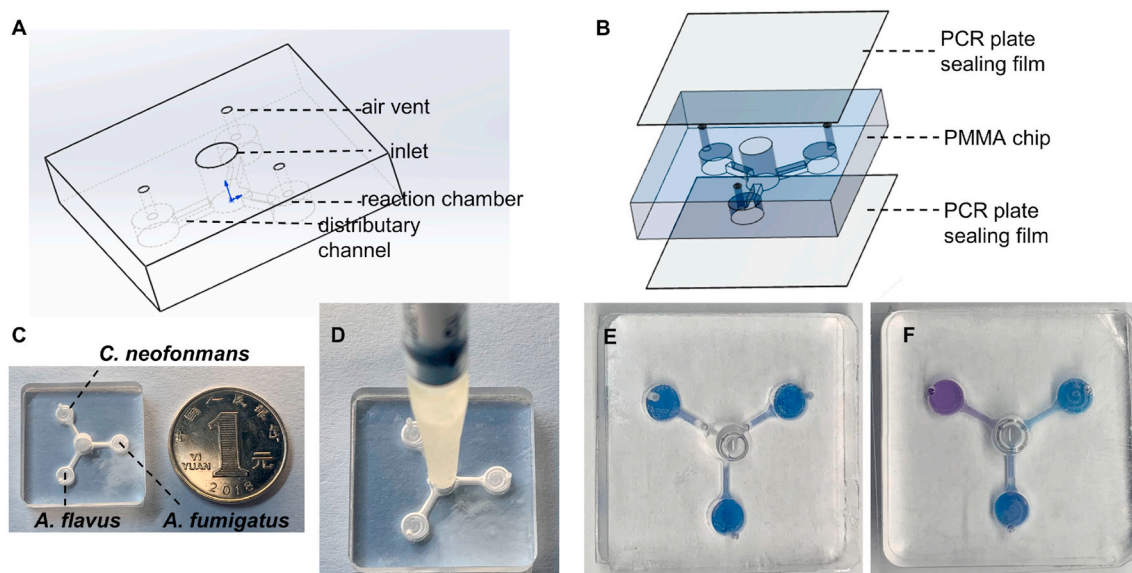


**Fig. 5.** (A) The details of the chip. (B) Chip assembly. (C) The appearance of the chip. And reaction chambers were pre-loaded with three sets of primers with 0.5% low melt agarose. (D) An adapter of the chip. (E) Feasibility verification of LAMP assays in the chip. (F) Cross-contamination verification of LAMP assays in the chip.

higher pressure of a liquid column to overcome the capillary force of the distributary channels, achieving the successful flow of liquid into the reaction chambers. The location of the air vents was the key for whether reaction chambers could be filled with liquid (Fig. S3). The air vents were located farthest from the distributary channels to keep the chambers consistent with the atmosphere, making sure the liquid flowed into the chambers without the air pressure being sealed in the chamber.

### 3.5. System assembly and validation

The system was made for rapid, economic, and user-friendly detection of airborne fungi. The amount of collected simulated spore-aerosols of *A. fumigatus* contained  $10^5$ ,  $10^4$ ,  $10^3$ , and  $10^2$  spores, as analyzed with Petrifilm and the mold counter, Mold™. Therefore, the amount of *A. fumigatus* spores in the simulated samples used for system validation

were about  $4 \times 10^6$ ,  $4 \times 10^5$ ,  $4 \times 10^4$ ,  $4 \times 10^3$  spores. Both the results from microcentrifuge tubes and the microfluidic chips demonstrated that the detection limit of the system was  $4 \times 10^4$  spores/sample (Fig. 6A and B). The whole detection from sample input to readout was completed within 90 min.

The rapid detection system that we developed had many advantages. First, the detection occurred rapidly within 90 min due to the rapid LMC DNA release method. 90 min was not rapid for nucleic acid tests of bacteria and viruses, but it was enough rapid for fungal spores due to the difficulty of fungal DNA extraction. After testing, the existing commercial DNA extraction methods such as silica gel column or paramagnetic particle method needed at least 3 h to complete DNA extraction of fungal spores. Together with PCR, LAMP and any other in vitro diagnostic methods, the whole process needed at least 4 h. However, our detection system just needed 90 min. Therefore, our system was efficient.

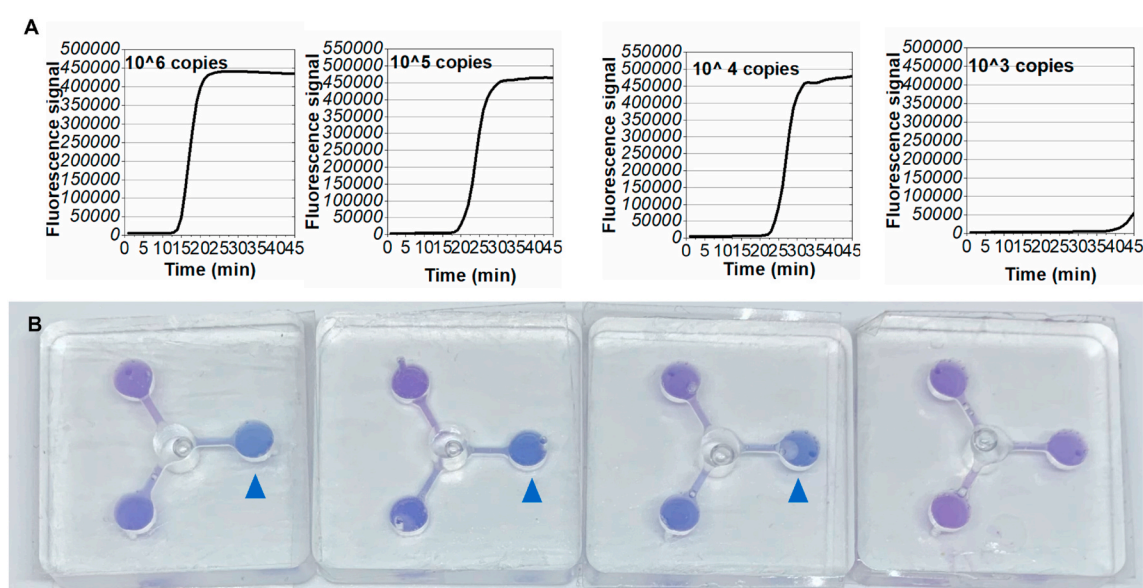


**Fig. 6.** The results of simulated air samples of *A. fumigatus* spores. (A) Results of the real-time fluorescence LAMP assays on the centrifuge tubes.  $4 \times 10^3$ – $4 \times 10^6$  spores were separately used as the samples. (B) Results of the colorimetric LAMP assays on the microfluidic chips. From left to right:  $4 \times 10^6$ ,  $4 \times 10^5$ ,  $4 \times 10^4$ ,  $4 \times 10^3$  spores/sample.

Moreover, the equipment of the system (the integrated DNA release device and microfluidic chip) could be controlled by a smartphone for ease of use. It was also small, economic, and portable. This ensured it could be applied for airborne fungi rapid detection, especially for on-site tests.

The detection limit is a concern for researchers. Our proposed system aims to be applied in a highly polluted workplace for early warning purposes. It can be used in the workplace of farm buildings (barns with moldy hay), pig farms, sawmills, medicine packing companies, buffets and cafes, libraries, mold-contaminated buildings, and waste management plants where the amount of mold ranges from  $10^4$  to  $10^9$  CFU/m<sup>3</sup> [31,32,34–38]. The maximum concentration of total fungi in the proposed guidelines is no more than  $10^3$  CFU/m<sup>3</sup> [36]. However, a less demanding threshold value of  $10^5$  CFU/m<sup>3</sup> for bacteria and fungi was proposed, specifically for waste treatment plants [39,40]. The proposed value of the limit of occupational exposure limit for industrial settings is  $5 \times 10^4$  CFU/m<sup>3</sup> [38]. The Dutch group reported that bacterial or fungal concentrations above  $10^5$  CFU/m<sup>3</sup> should be considered a threat to workers' health [35]. Therefore, our system is well suited for the rapid detection of fungi for early warning.

#### 4. Conclusions

Herein, a new rapid system was established for the on-site detection of airborne fungi. The whole process, including DNA release, multiplex LAMP, and readout, could be accomplished within 90 min. The system in this study was composed of a homemade integrated DNA release device and a multiplex fungus-specific isothermal amplification microfluidic chip. The detection sensitivity and specificity of the system were successfully verified. The limit of the detection system was  $4 \times 10^4$  spores/sample, which could meet the requirements of airborne fungi rapid detection for early warnings.

Moreover, the proposed platform could also be used for the detection of any other airborne microorganisms by simply changing the primers. This could be of great significance for some microorganisms, which could be difficult to release their DNA, such as fungal spores and pollens. Thus, our device provided for the rapid detection of airborne fungi, filling the gap in this field.

#### Credit author statement

Huijun Lu performed all the experiments and drafted the manuscript. Jinhui Zhu participated in the integrated DNA release device construction. Tong Zhang participated in the new fungal DNA releasing method establishment. Xinlian Zhang participated in the design and manufacture of microfluidic chips. Xi Chen, Wei Zhao and Yuhao Yao assisted in the experiments. Wang Zhao effectively guided experimental investigation. Guodong Sui participated in designing this study and revising the manuscript.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2022.123467>.

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