A fully automated microfluidic PCR-array system for rapid detection of multiple respiratory tract infection pathogens

Enqi Huang · Yu Wang · Na Yang · Bowen Shu · Guohao Zhang · Dayu Liu

Abstract

Rapid and accurate identification of respiratory tract infection pathogens is of utmost importance for clinical diagnosis and treatment, as well as prevention of pathogen transmission. To meet this demand, a microfluidic chip-based PCR-array system, Onestart, was developed. The Onestart system uses a microfluidic chip packaged with all the reagents required, and the waste liquid is also collected and stored on the chip. This ready-to-use system can complete the detection of 21 pathogens in a fully integrated manner, with sample lysis, nucleic acid extraction/purification, and real-time PCR sequentially implemented on the same chip. The entire analysis process is completed within 1.5 h, and the system automatically generates a test report. The lower limit-of-detection (LOD) of the Onestart assay was determined to be $1.0 \times 10^3$ copies·mL$^{-1}$. The inter-batch variation of cycle threshold (Ct) values ranged from 0.08% to 0.69%, and the intra-batch variation ranged from 0.9% to 2.66%. Analytical results of the reference sample mix showed a 100% specificity of the Onestart assay. The analysis of batched clinical samples showed consistency of the Onestart assay with real-time PCR. With its ability to provide rapid, sensitive, and specific detection of respiratory tract infection pathogens, application of the Onestart system will facilitate timely clinical management of respiratory tract infections and effective prevention of pathogen transmission.

Keywords

Microfluidic · Automation · Multiplex detection · Point-of-care testing · Rapid diagnosis · Respiratory tract infection

Introduction

Acute respiratory tract infections (RTIs) are still major public health threats worldwide [1]. RTIs causing pathogens include viruses, bacteria, mycoplasma, chlamydia, and other microorganisms [2], among which viruses account for approximately 80% of these diseases [3]. RTIs caused by different types of pathogens may have similar symptoms, but the clinical management are quite different. For example, most virus-caused RTIs are self-limited in healthy hosts, so no special treatment is needed [4]. However, symptoms caused by viruses are nonspecific, thus are indistinguishable from those caused by bacterial pathogens. Due to the limitations of conventional diagnostics in providing timely and accurate microbial test results, empirical broad-spectrum antibiotic therapy is often administered [5]. Such empirical treatments not only increase medical costs and even raise concerns about the development of multidrug-resistant bacteria [6]. In immunocompromised individuals, such as young children, elderly patients with wasting diseases, and recipients of transplant, RTIs may cause serious complications or even mortality [7, 8]. Specifically, RTIs caused by certain types of viruses may even result in a global public health emergency. Such infections make impressions with the emergence of the novel H1N1 influenza virus in 2009 [9], and the pandemic of novel coronavirus pneumonia since 2019 [10]. Besides, the highly transmissible nature of the respiratory pathogens necessitates specific quarantine and protection measures. Therefore, to enable comprehensive diagnostic applications and routine surveillance, there is a need for rapid and accurate detection of respiratory tract pathogens.
for detection technologies that can immediately identify the RTI pathogens.

Rapid determination of RTI pathogens poses challenges for laboratory diagnostics in several aspects: (1) Throughput, a certain number of pathogens are known to have the potential to cause RTIs. Accordingly, high-throughput pathogen detection assays are required to identify the causative agent from a large number of candidates; (2) turnaround time, outcome of the pathogen detection assay determines the choice of clinical intervention, and therefore, it is critical to shorten the turn-around time of the test; (3) field applicability, the pathogen detection assays are often required to be performed under field conditions and accordingly require portable equipment and simple operation. Therefore, a diagnostic system that can deliver rapid, accurate, and comprehensive detection of respiratory pathogens is highly desirable in both diagnostic laboratory and clinical settings.

While molecular diagnostics can give a rapid and sensitive determination of infection-causing pathogens at an early stage of the disease, multiplexed nucleic acid detection assays using conventional PCR instruments can be both costly and time-consuming. Alternatively, microfluidic chip system enables the implementation of high-throughput detection assays under resource-limited conditions [11–15]. Microfluidic chip consisting of a group of functional units connected by microfluidic network offers a miniaturized alternative to traditional repetitive laboratory methods, as it offers a significant reduction in sample and reagent consumption and ease of operation [16]. Benefited from the high surface-to-volume ratio of microreactors, temperature response and reaction efficiency on microfluidic chips are significantly improved, thus effectively reducing the required time for analysis [17]. Fully integrated microfluidic chip systems [18–23] can implement various types of analytical operations without the need for specialized laboratories and skilled personnel [24–27]. Because of these features, microfluidic chip is an ideal tool implementing pathogen detection assays in resource-limited settings.

Recent studies have reported microfluidic systems [28–33] that are able to identify the causative pathogen from a range of candidates or even generate subtype- or strain-specific information. Depending on the structure and function of the microfluidic device used, these systems can be classified into the following three types: (1) Simple microfluidic PCR arrays, represented by the TaqMan Array system [34]. This type of microfluidic chip systems effectively extends the throughput, but their application is still limited to specialized laboratories due to the absence of sample preparation; (2) integrated microfluidic device with a single reactor, represented by the GeneXpert system [35]. This type of microfluidic systems can be used under field conditions, but the number of targets that can be detected in a microfluidic device is limited; thus, the multiplexing capability depends on the use of multiple microfluidic devices; (3) integrated microfluidic device with parallelized reactors, represented by the FilmArray system [28]. This type of microfluidic systems detects a large number of targets in a run, thereby is well suited to rapid identification of the infection-causing pathogen under field conditions.

Here we describe a novel microfluidic diagnostic platform, the “Onestart” PCR-array system, which is capable of screening multiple respiratory pathogens in a fully integrated manner. Unlike some point-of-care (POC) devices that require users to transfer liquids or use reagents during testing [36, 37], all the reagents needed for the pathogen detection assay are preloaded on the Onestart microfluidic chip, thus minimizing user manual handling and therefore, avoiding misoperation. The operation of the Onestart system consists of only three easy steps: (1) Loading the original sample onto the microchip; (2) insert the chip into the instrument; (3) start the system operation. The Onestart system is compact and easy to operate and thus can be performed by non-skilled personnel in field conditions. Compared with POC devices with a limited number of targets [38, 39], the Onestart respiratory panel can detect and distinguish up to 21 pathogens in a single run. Because the entire analytical process is performed in an enclosed microchip, there is a low risk of laboratory contamination. We validated this system and demonstrated its feasibility in clinical sample analysis.

**Experimental**

**The Onestart system**

**The Onestart microchip**

As is shown in Fig. 1, the Onestart microchip contains three regions corresponding to sample lysis, nucleic acid extraction, and nucleic acid amplification. The sample lysis and nucleic acid extraction region are located in the main body of the microfluidic chip, while the amplification region is on a separate component connected to the main body by connectors. The main body of the microfluidic chip has a three-layer composite structure, with a middle layer made of black polycarbonate, and a top and a bottom layer made of transparent polycarbonate. The raw polycarbonate material (PC-110) for microchip fabrication was from Chi Mei Corporation. All the three layers of the microfluidic chip are injection-molded and are ultrasonically bonded to form an entire device. Afterward, the liquid storage cartridge is installed onto the microchip with mechanical clamps.

In the sample lysis region, a liquid storage cassette is installed on the main body of the microfluidic chip. The liquid storage cassette has four reservoirs, for the loading of sample (4 mL), MB-lysis buffer (2 mL), washing buffer (2 mL), and elution buffer (2 mL), respectively. Besides, the liquid storage
The cassette has a sponge-filled waste liquid collection tank. Each of the reagent reservoirs is equipped with a movable cover. Once pressure is applied onto the movable cover, the liquid stored inside is released.

In the nucleic acid extraction region, there is a 0.8-mL solid-phase extraction (SPE) chamber containing a magnetic stirring bar and a storage tube with lyophilized powder of RT-PCR reagents. The lyophilized powder storage tube collects the nucleic acid elution from the SPE chamber to form the reaction mixture. There are six diaphragm valves in the extraction region. The silicone membrane of the diaphragm valve fixed to the top layer of the microchip covers two via holes in the middle layer. Opening the diaphragm valve connects the channel on the bottom layer, while closing the diaphragm valve blocks the pathway. The synergistic control of a series of diaphragm valves allows fluid to be transferred to a designated region.

The amplification region has a primary channel divided into four parallel secondary channels, with each one extends eight branches connected to the reaction chambers where the primers and probes are pre-deposited (the sequences of primers and probes were shown in Supplementary Information (ESM) Table S1).

The analytical process of the Onestart microchip is as follows (as shown in ESM Fig. S1):

Cell lysis: After adding the sample into the sample reservoir, the MB-lysis buffer is released. Pathogens are lysed, and the released nucleic acids bind to the MBs.

Nucleic acid extraction: As the lysate is driven downstream by the micro-pump, the MBs are magnetically captured in the SPE chamber while the liquid flows to the waste liquid collection tank. The washing buffer is then released to the SPE chamber, where the MBs are washed to remove non-specifically adsorbed molecules. During rinsing, the stirring bar is rotated by an external magnetic force to increase the contact between the MBs and the washing buffer. After the rinsing, the remaining liquid is pushed into the waste liquid tank.

Sample-reagent mixing: The elution buffer released flows into the SPE chamber. The stirring bar rotates to enhance the contact between the MBs and the buffer. The SPE chamber is heated to 50 °C to accelerate the release of nucleic acids from the MBs. The elution is then pushed to the lyophilized powder storage tube to mix with the RT-PCR reagent.

Amplification: The inlet of the amplification module is open, and thus, the reaction mixture is introduced under negative pressure and distributed into the 32 reaction chambers. We control the total amount of elution buffer to determine the final amount of reaction mixture entering the amplification region, so that each chamber is allocated with 3.5 μL of the reaction mixture. The heating module below the amplification area controls the thermal cycling temperature. The fluorescence imaging module above the amplification area collects the amplification signals of the reaction chambers in real-time.

The Onestart microchip analyzer

The Onestart microchip analyzer is 39.1 cm long × 25.4 cm wide × 16.3 cm high and weighs 11 kg (Fig. 2a). It communicates with PC side software through the Ethernet port. A single PC can control 1 to 8 Onestart analyzers. The composite structure diagram schematic of the Onestart analyzer is shown in Fig. 2b.
The Onestart microchip analyzer has six pressure devices at the sites that correspond to the diaphragm valves that can move up and down under the control of the system software. The diaphragm valve is open under normal conditions, and it closes when pressure is applied onto the diaphragm. The transfer of liquid is achieved through the synergistic control of a series of diaphragm valves.

Underneath the SPE chamber, a micro motor fixed with magnets is installed. The micro motor drives the magnet to generate a rotating magnetic field to stir the stirring bar in the reservoir, to accelerate nucleic acid binding or elution by enhancing the interactions between the MBs and the solution.

At the site corresponding to the amplification region, there is a Peltier heating device responsible for thermal control of reverse transcription and PCR, a blue LED excitation light source and a CMOS camera to monitor the amplification fluorescence in the PCR array.

Materials

Reagents

The lysis buffer, washing buffer, elution buffer, magnetic beads, PCR buffer, DNA polymerase, reverse transcriptase, dNTPs, magnesium chloride solution, fluorescence probes, primers, and quality controls used in this study were all supplied by Beijing Baicare Biotechnology Co., Ltd., China.
Quality controls (QCs)

The positive control is pseudovirus contained in the lyophilized powder, and the negative control is the sample preservation buffer. Primers for the internal control, human GAPDH mRNA, are pre-deposited in a designated reaction chamber in the amplification region. The positive outcome of GAPDH amplification suggests a valid detection assay.

Clinical specimens

Ninety-three pharyngeal swab specimens were provided by Guangzhou First People’s Hospital. These samples have been confirmed to contain at least one of the pathogens within the Onestart respiratory panel by conventional PCR assays.

Healthy controls

Pharyngeal swab specimens were collected from 20 volunteers.

Reagent packaging

Lyophilized powder packaging

The RT-PCR reagent is added to a vial and then lyophilized. The resultant lyophilized powder ball was transferred to the lyophilized powder storage tube. The composition of the buffers and the RT-PCR mixture was provided in ESM (Tables S2, S3).

Deposition of primers in the reaction chambers

Before bonding the amplification module, various primers were pipetted into corresponding reaction chambers using a sample applicator and then lyophilized.

Operation of the Onestart analyzer

The Onestart analysis requires only three steps of user operations (Fig. 3): (1) Sample loading—shake the tube loaded with a pharyngeal swab 10–20 times and then add 0.8 mL of sample to the sample loading reservoir; (2) insert the microchip—close the sample loading reservoir and insert the microchip into the device; (3) start the instrument—start the software on the computer to run the apparatus.

The entire analytical process, from sample-in to answer-out, is performed automatically by the apparatus, including (1) lysis and nucleic acid binding—the instrument controls the release of the lysis-binding buffer along with MBs to the sample reservoir. The released nucleic acid fragments are therefore bound to MBs; (2) MB capturing—the MB suspension is transferred to the downstream SPE chamber, where MBs are captured by the magnetic bar, and the waste fluid is discharged into the waste liquid tank; (3) MB washing—the instrument controls the release of the washing buffer into the SPE chamber, and rotates the magnetic bar at 200 rpm. The waste fluid is collected into the waste liquid tank; (4) elution—the instrument controls the release of the elution buffer into the SPE chamber and rotates the magnetic bar at 500 rpm; (5) mixing—the nucleic acid elution was transferred to the lyophilized powder tube to form a PCR mixture; (6) amplification/detection—dispense the PCR premix to the PCR chambers, and start the temperature control and the detection modules.

The Onestart analyzer communicates with the PC. The raw data continuously collected by the CMOS camera was automatically assigned to each PCR chamber, depicting a real-time amplification curve. When the analytical procedure is complete, the instrument starts automatic data analysis and determines the Ct value using the second-order derivative algorithm. The Onestart analyzer automatically generates the report, or the user can browse previous records.

The Onestart respiratory panel

The Onestart respiratory panel is a multiplex PCR assay, targeting 21 pathogens responsible for RTIs, including (1) SARS-CoV-2 (ORF1ab gene); (2) coronavirus 229E; (3) coronavirus OC43; (4) coronavirus NL63; (5) coronavirus HKU1; (6) influenza A/−; (7) influenza A/H1N1 pdm 2009; (8) influenza A/seasonal H1N1; (9) influenza A/H3N2; (10) influenza A/H5N1; (11) influenza A/H7N9; (12) influenza B; (13) parainfluenza; (14) adenovirus 3; (15) adenovirus 7; (16) adenovirus 55; (17) respiratory syncytial virus; (18) human metapneumovirus; (19) human rhinovirus/enterovirus; (20) Mycoplasma pneumoniae; (21) Chlamydia pneumonia. The above targets and the internal quality control (RNase P gene) are simultaneously detected in a run.

Determination of results

RT-PCR assay with a Ct value < 37 is determined as a positive outcome.
Results and discussion

The Onestart system can detect multiple RTI pathogens in a “sample-in-answer-out” manner within 1.5 h. Limit-of-detection (LOD), specificity, and reproducibility of the Onestart assay were determined by analyzing the positive controls. The performance of the Onestart system was also validated by analyzing clinical samples.

Limit-of-detection

The 95% limit-of-detection (LOD$_{95}$, the concentration of target analyte that can be consistently detected at least 95% of the time) was determined by the detection of serial dilutions of each positive QCs at concentrations of $1.0 \times 10^4$, $5.0 \times 10^3$, $1.0 \times 10^2$, and $5.0 \times 10^2$ copies·mL$^{-1}$. These dilutions were tested using microfluidic chips of three batches, with each
batch repeated three times. As shown in Table 1, LOD_{95} of each pathogen was determined to be around $1.0 \times 10^3$ copies·mL$^{-1}$ based on the Probit regression analysis. Pearson’s squared test suggests that the difference between batches was not statistically significant ($p > 0.05$). The LOD_{95} was further verified by analyzing the positive QCs at a concentration of $1.0 \times 10^3$ copies·mL$^{-1}$, with microfluidic chips of three batches. The results showed that the detection rate of each positive QC was 100%. As has been demonstrated that $10^3$ copies·mL$^{-1}$ is a reliable threshold for determining a respiratory infection [40–43], the Onestart assay can meet clinical diagnosis requirements.

**Specificity**

The specificity of the Onestart assay was evaluated with reference positive samples and healthy controls. The reference sample mixes P1–P6 were prepared by mixing positive QCs following the ratios listed in Table S4 (see ESM). Microchips of three batches were used to detect each of the reference sample mixes, and the test was repeated three times for each batch of microfluidic chips. All the healthy controls were tested negative. The detection showed that the accordance rate of each reference mix was 100% (Table 2). These results demonstrate the high specificity of Onestart assay.

We also used the Onestart system to detect 15 respiratory microorganisms beyond the Onestart respiratory panel, including Corynebacterium, *Haemophilus influenzae*, *Lactobacillus acidophilus*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus salivarius*, *Candida albicans*, *Klebsiella pneumoniae*, Cytomegalovirus, Herpes simplex virus type 1, and rubella virus. Microfluidic chips of three batches were used, with the test repeated three times for each batch. The results showed that all the microorganisms beyond the Onestart respiratory panel were tested negative.

**Reproducibility**

Reproducibility of the Onestart assay was evaluated by repetitive analysis of a positive reference R1 and a negative reference R2.

### Table 1 Determination of the LOD_{95} of each pathogen

| Pathogen          | Number of samples detected | LOD_{95} (copies·mL$^{-1}$) |
|-------------------|---------------------------|-----------------------------|
|                   | 1.0 × 10^4 | 5.0 × 10^3 | 1.0 × 10^3 | 5.0 × 10^2 |
| SARS-CoV-2        | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| CoV 229E          | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| CoV OC43          | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| CoV NL63          | 9/9        | 9/9        | 9/9        | 4/9        | 898       |
| CoV HKU1          | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| Flu A/−           | 9/9        | 9/9        | 9/9        | 4/9        | 898       |
| Flu A/H1N1 pdm 2009 | 9/9     | 9/9        | 9/9        | 4/9        | 898       |
| Flu A/H1N1        | 9/9        | 9/9        | 9/9        | 4/9        | 898       |
| Flu A/H3N2        | 9/9        | 9/9        | 9/9        | 4/9        | 898       |
| Flu A/H5N1        | 9/9        | 9/9        | 9/9        | 2/9        | 998       |
| Flu A/H7N9        | 9/9        | 9/9        | 9/9        | 1/9        | 1050      |
| Flu B             | 9/9        | 9/9        | 9/9        | 1/9        | 1050      |
| ADV 3             | 9/9        | 9/9        | 9/9        | 1/9        | 1050      |
| ADV 7             | 9/9        | 9/9        | 9/9        | 1/9        | 1050      |
| ADV 55            | 9/9        | 9/9        | 9/9        | 0/9        | 1108      |
| PIV               | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| RSV               | 9/9        | 9/9        | 9/9        | 3/9        | 948       |
| MPV               | 9/9        | 9/9        | 9/9        | 1/9        | 1050      |
| EV/RV             | 9/9        | 9/9        | 9/9        | 2/9        | 998       |
| M.pne             | 9/9        | 9/9        | 9/9        | 2/9        | 998       |
| C.pne             | 9/9        | 9/9        | 9/9        | 2/9        | 998       |

*ADV*, adenovirus; *CoV*, coronavirus; *C.pne*, Chlamydia pneumoniae; *EV*, enterovirus; *Flu A*, influenza A; *Flu B*, influenza B; *M.pne*, Mycoplasma pneumoniae; *MPV*, metapneumovirus; *PIV*, parainfluenza virus; *RSV*, respiratory syncytial virus; *RV*, rhinovirus; *SARS-CoV-2*, SARS-coronavirus-2
reference R2. The positive reference R1 is a mixture of 13 positive QCs at a concentration of $1.0 \times 10^4$ copies·mL$^{-1}$, while the negative reference R2 is the sample preservation solution. In a 6-day test, the R1 and R2 references were tested by two operators using three batches of microchips on individual instruments in different laboratories, with the test repeated 10 times by one operator using microchips of the same batch every day. All of the R1 tests were positive, while all of the R2 tests were negative. As shown in Fig. 4, the intra-batch variation in Ct values of the targets in R1 ranged from 0.9 to 2.66%, and the inter-batch variation ranged from 0.08 to 0.69%. This result suggests that the Onestart assay gives highly reproducible detection results.

### Clinical sample testing

The ability of the Onestart system to detect clinical samples was verified by analyzing 93 throat swab samples (ESM Table S5). The viral loads of these samples were determined

---

**Table 2** Detection results (Ct value) of P1–P6 reference sample mix

| Pathogen          | Reference sample mix |
|-------------------|----------------------|
|                   | P1       | P2       | P3       | P4       | P5       | P6       |
| SARS-CoV-2        | 27.8±0.9 | –        | –        | –        | –        | –        |
| CoV 229E          | –        | 28.2±1.0 | –        | –        | –        | –        |
| CoV OC43          | –        | –        | 29.7±0.7 | –        | –        | –        |
| CoV NL63          | –        | –        | –        | 29.7±0.9 | –        | –        |
| CoV HKU1          | –        | –        | –        | –        | 29.2±0.8 | –        |
| Flu A/H1N1 pdm 2009 | 28.2±1.0 | –        | –        | –        | –        | –        |
| Flu A/H1N1        | –        | 28.7±1.4 | –        | –        | –        | –        |
| Flu A/H3N2        | –        | –        | 31.5±1.1 | –        | –        | –        |
| Flu A/H5N1        | –        | –        | –        | 30.0±0.9 | –        | –        |
| Flu A/H7N9        | –        | –        | –        | –        | 28.4±0.6 | –        |
| Flu B             | 27.3±0.9 | 29.4±0.7 | –        | –        | –        | –        |
| ADV               | 28.4±0.7 | 28.8±1.1 | 29.2±0.7 | 30.6±1.2 | 27.9±1.5 | 30.3±1.0 |
| PIV               | –        | –        | 29.4±1.0 | 28.0±0.6 | 31.0±1.5 | –        |
| RSV               | –        | –        | 27.6±1.2 | 28.3±1.0 | –        | –        |
| MPV               | –        | –        | –        | –        | 28.9±0.7 | –        |
| EV/RV             | 28.0±0.9 | 27.9±1.0 | 29.3±1.0 | 29.1±1.3 | 29.7±1.0 | 29.0±0.8 |
| *M.pne*           | 30.6±0.6 | –        | –        | –        | –        | –        |
| *C.pne*           | –        | 29.2±0.8 | –        | –        | –        | –        |

---

**Fig. 4** Variations in Ct value in the analysis of the positive references using the Onestart system

**Fig. 5** Ct values of sample diluents with target concentration around LOD$_{95}$
with real-time PCR. Following the determined viral loads, these samples were serially diluted to a target concentration of $1.0 \times 10^3$ copies·mL$^{-1}$. The dilutions were analyzed with the Onestart microfluidic chips of three batches. When the target is presented at a concentration higher than $1.0 \times 10^3$ copies·mL$^{-1}$, all the targets’ detection rates were 100%. With target concentration around LOD$_{95}$, 89 out of 93 dilutions were determined to be positive (Fig. 5). Only one case in each of the CoV 229E, CoV HKU1, Flu B, and RV groups were not identified.

### Comparison with existing systems

The Onestart system is designed to detect multiple targets in a sample in resource-limited settings rather than the central clinical laboratory with a large sample size. The single run time of the Onestart assay is 1.5 h. To meet the requirements of point-of-care testing, we will continue to reduce the turnaround time. When a large number of samples need to be analyzed, several Onestart analyzers can be stacked and used simultaneously. In this way, users can choose the number of instruments to meet their requirements for different throughput.

Rapid and accurate point-of-care nucleic acid diagnosis is conducive to the efficient management of clinical decisions in the field setting. Recently, microfluidic diagnostics have made considerable advances in realizing point-of-care nucleic acid diagnosis [44]. However, some microfluidic devices still need liquid transferring or device displacement to complete the whole nucleic acid detection process, which is not desirable for the applications under field conditions [45]. Isothermal amplifications (such as LAMP) were also used in some microfluidic nucleic acid detection assays, thus simplifying temperature control and thus reducing instrument size [29, 46]. However, these isothermal amplification assays generally cannot achieve good nucleic acid quantification, making them difficult to distinguish pathogenic microorganisms from resident ones. Moreover, primer design for isothermal amplifications is usually challenging, making it no easy to design a large panel to detect multiple pathogens. To address these problems, we developed the fully automatic Onestart system for rapid identification of respiratory tract infection pathogens under field conditions.

The Onestart system is compared with the existing multiplex respiratory pathogen detection systems in terms of run time, run size, number of targets, and integration (Table 3). The Onestart system has all the reagents required preloaded in the microchip, so that requires minimal hands-on time and avoids the risk of misoperation. Running cost for the Onestart RP was about $100 per run, which is significantly reduced compared to the existing products [47].

### Conclusions

In summary, the Onestart system is capable of detecting multiple respiratory pathogens in a fully integrated manner. This system was successfully applied to the testing of batched clinical samples and showed advantages of ease of operation and reduced turnaround time. The Onestart system is not limited to use in specialized laboratories or by skilled operators and thus is a powerful tool for pathogen detection in resource-limited settings. Future work will be directed towards expanding the detection panel to provide more efficient and flexible solutions for the diagnosis, treatment, and prevention of infectious diseases.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s00216-021-03171-4.

### Funding

This work was supported by the NSFC (Grant No. 81871726, 21804044), Guangdong Natural Science Foundation (2020A1515010935, 2020A1515010754), Guangzhou Science and Technology Program (201904010413, 201904010237), the Fundamental Research Funds for the Central Universities (2019MS134, 2019PY14), and the Science Foundation of Guangzhou First People’s Hospital (PT81871726, PT21804044). The authors gratefully acknowledge their financial support.

### Compliance with ethical standards

#### Ethical approval

The pharyngeal swab specimens were approved by the Research Ethics Committee of Guangzhou First People’s Hospital (IRB Table 3  Comparison of available diagnostic assays for multiplex respiratory pathogens

| Platform          | Tests                             | Run time | Maximum run size | Number of targets | NA extraction integrated |
|-------------------|-----------------------------------|----------|------------------|-------------------|--------------------------|
| Onestart          | Respiratory Panel                 | 1.5 h    | 1                | 21                | Yes                      |
| FilmArray [28]    | Respiratory Panel                 | 1 h      | 1                | 21                | Yes                      |
| The Luminox MAGPIX [3] | NxTAG Respiratory Pathogen Panel | ~4 h     | 96               | 22                | No                       |
| The Panther Fusion [43] | Respiratory assay              | ~2.5 h   | 32               | 10                | Yes                      |
| Rotor-Gene Q [48] | Qiagen ResPlex II V2.0            | ~3 h     | 22               | 16                | No                       |
| GeneXpert [35]    | Xpert Flu/RSC XC                  | 20 min   | 1–80 (depending on instrument models) | 3 | Yes |
number: K-2020-068-01), and the study was performed in accordance with the relevant guidelines.

Conflict of interest The authors declare that they have no conflicts of interest.

References
1. Charlton CL, Babady E, Ginocchio CC, Hatchette TF, Jerris RC, Li Y, et al. Practical guidance for clinical microbiology laboratories: viruses causing acute respiratory tract infections. Clin Microbiol Rev. 2019;32(1):1–49.
2. Denny FW. The clinical impact of human respiratory virus infections. Am J Respir Crit Care Med. 1995;152(4 Pt 2):S4–S12.
3. Zhang N, Wang L, Deng X, Liang R, Su M, He C, et al. Recent advances in the detection of respiratory virus infection in humans. J Med Virol. 2020;92(4):408–17.
4. DeGeorge KC, Ring DJ, Dalrymple SN. Treatment of the common cold. Am Fam Physician. 2019;100(5):281–9.
5. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, et al. A systematic review of antibiotic prescription associated with upper respiratory tract infections in China. Medicine. 2016;95(19):e3587.
6. Koulenti D, Song A, Ellingboe A, Abdul-Aziz M, Harris P, Gavey E, et al. Infections by multidrug-resistant gram-negative bacteria: what’s new in our arsenal and what’s in the pipeline? Int J Antimicrob Agents. 2019;53(3):211–24.
7. Januja NZ, Mahmood B, Dharma VK, Sathiakumar N, Khan ML. Use of biomass fuel and acute respiratory infections in rural Pakistan. Public Health. 2012;126(10):855–62.
8. Lambkin-Williams R, Noulin N, Mann A, Catchpole A, Gilbert E, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362(18):1708–19.
9. Janus A, Akessell Z, O’Neill N, Khan M, Aggra R. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020;76:71–6.
10. Pandey CM, Augustine S, Kumar S, Kumar S, Nara S, Srivastava S, et al. Microfluidics based point-of-care diagnostics. Biotechnol J. 2018;13(1):1700047.
11. Mauk MG, Liu C, Song J, Bau HH. Integrated microfluidic nucleic acid amplification, isothermal amplification, and amplicon quantification. Microarrays (Basel). 2015;4(4):474–89.
12. Chang T, Yeh YT, Gulino K, Zhang Y, Sabestien A, Chou TW, Zhou B, et al. A rapid and label-free platform for virus capture and identification from clinical samples. Proc Natl Acad Sci U S A. 2020;117(2):895–901.
13. Luka G, Ahmad A, Najaran H, Alocilia E, DeRosa M, Wolthers K, et al. Microfluidics integrated biosensors: a leading technology towards lab-on-a-chip and sensing applications. Sensors (Basel). 2015;15(12):30011–31.
14. Mauk MG, Liu C, Song J, Bau HH. Integrated microfluidic nucleic acid isolation, isothermal amplification, and amplicon quantification. Microarrays (Basel). 2015;4(4):474–89.
15. Lambkin-Williams R, Noulin N, Mann A, Catchpole A, Gilbert E, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362(18):1708–19.
16. Janus A, Akessell Z, O’Neill N, Khan M, Aggra R. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020;76:71–6.
17. Pandey CM, Augustine S, Kumar S, Kumar S, Nara S, Srivastava S, et al. Microfluidics based point-of-care diagnostics. Biotechnol J. 2018;13(1):1700047.
18. Mauk MG, Liu C, Song J, Bau HH. Integrated microfluidic nucleic acid amplification, isothermal amplification, and amplicon quantification. Microarrays (Basel). 2015;4(4):474–89.
19. Lambkin-Williams R, Noulin N, Mann A, Catchpole A, Gilbert E, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362(18):1708–19.
20. Janus A, Akessell Z, O’Neill N, Khan M, Aggra R. World Health Organization declares global emergency: a review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020;76:71–6.
21. Pandey CM, Augustine S, Kumar S, Kumar S, Nara S, Srivastava S, et al. Microfluidics based point-of-care diagnostics. Biotechnol J. 2018;13(1):1700047.
22. Mauk MG, Liu C, Song J, Bau HH. Integrated microfluidic nucleic acid isolation, isothermal amplification, and amplicon quantification. Microarrays (Basel). 2015;4(4):474–89.
38. Lafleur LK, Bishop JD, Heiniger EK, Gallagher RP, Wheeler MD, Kaufman P, et al. A rapid, instrument-free, sample-to-result nucleic acid amplification test. Lab Chip. 2016;16(19):3777–87.

39. Sciuto EL, Petralia S, Calabrese G, Conoci S. An integrated biosensor platform for extraction and detection of nucleic acids. Biotechnol Bioeng. 2020;117(5):1554–61.

40. Martin ET, Kuypers J, Wald A, Englund JA. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. Influenza Other Respir Viruses. 2012;6(1):71–7.

41. Schjelderup Nilsen H-J, Nordbø SA, Krokstad S, Døllner H, Christensen A. Human adenovirus in nasopharyngeal and blood samples from children with and without respiratory tract infections. J Clin Virol. 2019;111:19–23.

42. Wanunu M, Cao Q, Mahalanabis M, Chang J, Carey B, Hsieh C, et al. Microfluidic chip for molecular amplification of influenza A RNA in human respiratory specimens. PLoS One. 2012;7(3):e33176.

43. Stellrecht KA, Cimino JL, Wilson LI, Maceira VP, Butt SA. Panther fusion(R) respiratory virus assays for the detection of influenza and other respiratory viruses. J Clin Virol. 2019;121:104204.

44. Yew CT, Azari JR, Li F, Pingguan-Murphy B. Electrospin-coating of nitrocellulose membrane enhances sensitivity in nucleic acid-based lateral flow assay. Anal Chim Acta. 2018;1009:81–8.

45. Ye X, Xu J, Lu L, Li X, Fang X, Kong J. Equipment-free nucleic acid extraction and amplification on a simple paper disc for point-of-care diagnosis of rotavirus A. Anal Chim Acta. 2018;1018:78–85.

46. Zhang H, Xu Y, Fohlerova Z, Chang H, Iliescu C, Neuzil P. LAMP-on-a-chip: revising microfluidic platforms for loop-mediated DNA amplification. Trends Anal Chem. 2019;113:44–53.

47. Loeffelholz MJ, Pong DL, Pyles RB, Xiong Y, Miller AL, Bufton KK, et al. Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens. J Clin Microbiol. 2011;49(12):4083–8.

48. Deng J, Ma Z, Huang W, Li C, Wang H, Zheng Y, et al. Respiratory virus multiplex RT-PCR assay sensitivities and influence factors in hospitalized children with lower respiratory tract infections. Virol Sin. 2013;28(2):97–102.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Enqi Huang is currently a postgraduate student in Clinical Laboratory at Guangzhou Medical University. Her research focuses on developing microfluidic technologies for diagnosis of infectious diseases.

Yu Wang currently works as a postdoc at Guangzhou First People’s Hospital, South China University of Technology. His research focuses on developing droplet microfluidic assays for rapid antibiotic susceptibility testing.
Na Yang has a background of clinical pathologist, with years of research experience in cancer biology. She is currently a Senior Group Leader in the Department of Laboratory Medicine, Guangzhou First People’s Hospital. Her research focuses on translational medicine from molecular mechanism to clinical diagnosis and treatment.

Bowen Shu currently works as an associate researcher in the Department of Laboratory Medicine, Guangzhou First People’s Hospital, South China University of Technology. His research focuses on point-of-care molecular diagnostics, μTAS, and novel biosensing strategies for single-molecule detection.

Guohao Zhang is the Director of Beijing Baicare Biotechnology Co. Ltd. Baicare researches, manufactures, and markets microfluidic IVD products, which are widely used in China, Europe, and other regions.

Dayu Liu is a Professor in the Department of Laboratory Medicine, Guangzhou First People’s Hospital, South China University of Technology. His research focuses on developing microfluidic chip technologies for diagnosis of cancer and infectious diseases.