RAPID AIRBORNE PATHOGENS DETECTION SYSTEM USING DISPOSABLE IMPACTION CARTRIDGE

K. Takenaka¹, S. Togashi¹, and R. Miyake²

¹Hitachi, Ltd., JAPAN and ²University of Tokyo, JAPAN

ABSTRACT

We have developed a rapid airborne pathogens detection system using disposable cartridge to diagnose infected patients safely, easily and rapidly for preventing pandemic. This system with a sampling bag filled with patient’s breath automatically handles the process in the cartridge which are consisted of collecting pathogens on the collection plate of the cartridge by the impaction technique, labeling fluorescent dyes to pathogens, removing free dyes from the collection plate, and detecting fluorescence of each pathogen. As result of the performance evaluation of this system, this system could detect E.Coli (3.0x10⁴ to 1.5x10⁵ particles/L) spread in the sampling bag within 10 min.

KEYWORDS: Disposable cartridge, Impaction, Fluorescence detection, Airborne pathogen

INTRODUCTION

There are growing concerns about a pandemic of respiratory infections such as tuberculosis, SARS and avian flu. It is very important to diagnose infected patients as soon as possible for preventing pandemic. And diagnostic techniques which enable anyone to make diagnoses of these infections safely, easily and rapidly are needed. Techniques in micro TAS that various chemical or biochemical processes can be done in a disposable micro device will be solutions for these needs. For example, disposable micro devices where waterborne pathogens were detected automatically by the fluorescent stain method or the adenosine triphosphate method were reported at µTAS2010 [1][2]. However, few devices where airborne pathogens were collected from air, labeled and detected were reported. In this paper, we report that we have developed a system using a disposable device we call cartridge where pathogens in breath or air are collected by the impaction technique and detected by the fluorescence method automatically and rapidly.

AIRBORNE PATHOGENS DETECTION SYSTEM

Figure 1 shows a conceptual diagram of breath test using this system. The system set with a sampling bag filled with patient’s breath and a cartridge can detect automatically pathogens in the sampling bag within 10 min. This system can be used by not just medical professionals but anybody because breath sampling is non-painful for patients and non-medical act.

Figure 1: Conceptual diagram of breath test for respiratory infection

Figure 2: Configuration diagram of airborne pathogen detection system
This system consists of the sampling bag, the cartridge, the fluorescence detector, the aspiration pump and valves for the flow control of breath and reagents (Figure 2). And this automates following detection process in the cartridge. This detection process are consisted of collecting pathogens on a collection plate of the cartridge by the impaction technique, labeling fluorescent dyes to pathogens, removing free dye from collection plate, and detecting fluorescence of pathogens (Figure 3). This system can do the detection process in 10 min (collecting:1min, labeling: 5~7min, washing: 1~2min, detecting: <1sec).

Figure 3: Detection process of airborne pathogen detection system

Figure 4 shows the picture of this system and the picture and assembly drawing of the cartridge. The functional features of the cartridge are following. The cartridge consisted of a multi-hole plate, a glass collection plate coated with anti-pathogen antibodies and a spacer works as an impactor of pathogens, a flow-cell for reagents and a window for fluorescent detection. Pathogens flow through an air inlet and nozzles and impact on the collection plate at 100 m/s by the inertial force. After that, stocked reagents in reservoir flow through the gap between the multi-hole plate and the collection plate into the waste reservoir to label pathogens with fluorescent dyes and remove free fluorescent dyes. Finally, fluorescence of pathogens under trapped on the collection plate are detected by the fluorescence detector.

Construction features are following. Pillars in a washing reservoir and inverted U-shaped channels of reservoir prevent reagents from flowing by the pressure difference (15 kPa) between the inside and the outside of the cartridge in collecting pathogens. And the multi-hole plate (100 μm thick) with 109 nozzles (pore diameter 70 μm), which was made from a light-permeable PET with the Tea-CO₂ laser, is less deformed and produces little autofluorescence and reflected light disturbing the fluorescence detection of pathogens.

Figure 4: Airborne pathogen detection system and Disposable cartridge
EXPERIMENTAL

We evaluated the collection efficiency of the cartridge and the detection performance of this system using the sampling bag where pathogens were spread. We used E.Coli bacteria (BacTrace Escherichia O157:H7 positive control, KPL) as substitutions of airborne pathogens such as tuberculosis and Hilite Fluore (Anaspec) conjugated anti-E.Coli antibodies (Anti-E.coli O157:H7, Goat-Poly, KPL) as fluorescent dyes.

An experimental procedure is shown on the following. Firstly, we spread E.coli in the sampling bag (3L) with an ultrasonic nebulizer (E-U07, OMRON), filled dry gas into this bag and left this bag for 5 minutes to evaporate micro water droplets. Secondly, we measured the number concentration of E.coli with a particle counter (237B, Hach Ultra). Finally, we set this bag and the disposable cartridge in the airborne pathogen detection system, and got this system started.

RESULTS AND DISCUSSION

Figure 5 shows this cartridge can collect most pathogens in the sampling bag because the collection efficiency is more than 97%. The collection efficiency C was given by the equation

$$C = \frac{N_{in} - N_{out}}{N_{in}},$$

where $N_{in}$ is the number concentration of E.Coli in the sampling bag and $N_{out}$ is the number concentration of E.Coli in the air passing through the cartridge. Both number concentrations were measured by the particle counter.

Figure 6 shows the detection performance of this system. Four photos in this graph, which were taken for reference after measurements, are fluorescent images of E.coli on a part of the collection plate under a nozzle. Figure 6 shows that the relative intensity of the fluorescence were correlated with the number concentration of E.Coli and this system can detect pathogens whose number concentration was $3.0 \times 10^4$ to $1.5 \times 10^5$ particles/L. These number concentration was equivalent of one of tuberculosis in tubercular patients’ bless.

CONCLUSION

It is very important to diagnose infected patients as soon as possible for preventing pandemic. And diagnostic techniques which enable anyone to diagnoses of these infections safely, easily and rapidly are needed. In this paper, we developed a system which could collect pathogens from breath or air in a disposable cartridge, label fluorescent dyes to collected pathogens and detect fluorescence from these automatically and rapidly. And this system with the cartridge can detect E.Coli ($3.0 \times 10^4$ to $1.5 \times 10^5$ particles/L) spread in the sampling bag.

This system is suitable for use in not only medical facilities but non-medical facilities (airports or schools) because breath sampling is non-painful for patients and non-medical act. And this is expected to contribute to the border control of infected patients or prevention of group infection for preventing pandemic.

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REFERENCES


CONTACT
*K. Takenaka, tel: +81-29-353-3289; kei.takenaka.tf@hitachi.com

Target value (>90%)

Figure 5: Collection efficiency of cartridge

Figure 6: Detection of substitution pathogens