Rapid Detection System of Airborne Pathogens
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Abstract—We developed new processes which can collect and detect rapidly airborne pathogens such as the avian flu virus for the pandemic prevention. The fluorescence antibody technique is known as one of high-sensitive detection methods for viruses, but this needs up to a few hours to bind sufficient fluorescence dyes to viruses for detection. In this paper, we developed a mist-labeling can detect substitution viruses in a short time to improve the binding rate of fluorescent dyes and substitution viruses by the micro reaction process. Moreover, we developed the rapid detection system with the above “mist labeling”. The detection system set with a sampling bag collecting patient’s breath and a cartridge can detect automatically pathogens within 10 minutes.

Keywords—Viruses, Sampler, Mist, Detection, Fluorescent dyes, Microreaction.

I. INTRODUCTION

There are growing concerns about a pandemic of a lethal infection such as avian flu. McKibbin and Sidorenko estimated that the avian flu pandemic had the potential to reduce the total global gross domestic product by 40 trillion dollars less than now [1]. A vaccine is one of the available preventative methods against well-known infections such as normal flu; however, it cannot prevent a new infection such as avian flu from spreading in the early phase of the epidemic because it takes a long time to supply enough vaccines to the people at risk. There is presently no method for preventing a new infection from spreading at that time.

To address this problem, we thought that we could prevent the spread of an infection using a rapid and high sensitive system which can collect and detect airborne viruses or bacteria rapidly because it is easy to provide safety measures rapidly such as a warning of an infection before people are affected with the infection by inhalation of airborne pathogen.

For the purpose of developing this system, the authors previously developed a virus sampler using an impaction technique [2]. This sampler can collect more than 80% of particles with equivalent size to that of airborne virus particles (>3.0 μm) discharged from infected patients [3]. The next technical target concerning the sampler is to detect the collected viruses rapidly. For example, gene amplification such as a polymerase chain reaction (PCR) is commonly known as a rapid method for detecting pathogens such as viruses and bacteria. Although gene amplification is a highly sensitive detection method with a theoretical lower detection limit equivalent to a single virus’s gene, it requires a few hours and considerable effort to carry out complicated processes such as eluting the collected viruses with an aqueous solution, extracting genes from the viruses, and amplifying genes.

With that requirement in mind, we previously focused on fluorescence methods, like fluorescent immunoassays [4], to bind fluorescent dyes to objective substances and optically detect the fluorescence emitted from fluorescent dyes binding to those objective substances. In other words, binding fluorescent dyes to collected viruses as markers makes detecting collected viruses easy. Although this method is known as one highly sensitive detection method for viruses, it needs up to a few hours to bind sufficient fluorescence dyes to viruses to be detected [5].

In the current study, we theoretically devised and evaluated a new method, called “mist labeling,” for shortening the time for binding fluorescent dyes to viruses. Compared with the conventional method (which executes the binding reaction in a millimeter-sized well filled with an aqueous solution of fluorescent dyes and viruses), mist labeling makes micrometer-sized aqueous droplets containing fluorescent dyes collide the viruses on the collection plate by an impaction technique and executes the binding reaction in the droplets. Moreover, we developed the rapid detection system with the above “mist labeling”. The detection system set with a sampling bag collecting patient’s breath and a cartridge can detect automatically pathogens within 10 minutes.

II. MIST LABELING

A conceptual schematic showing how fluorescent dyes (binding substance) bind to viruses (objective substances) on the surface of a plate is shown in Fig. 1. The fluorescent dyes in the liquid move toward the objective substances on the plate by Brownian motion, and they form complexes with the viruses by a binding reaction after arrival.

![Fig. 1 Conceptual schematic of fluorescent dyes binding to viruses on a plate](image-url)
To make fluorescent dyes bind to viruses rapidly, it is necessary to shorten the diffusion time in which the fluorescent dyes move to the objective substances and to accelerate the binding reaction between the fluorescent dyes and viruses on the plate. To make binding substances bind to objective substances on the plate rapidly, it is possible to flow liquid containing a binding substance in a micro channel along the bottom of which an objective substance is fixed [6].

A binding reaction in a micro channel is improved in time by the microreaction effect for the following reasons. Firstly, the length between the binding substances in liquid and the objective substances at the channel bottom is shortened, thereby reducing the diffusion time. The time it takes for the binding substances to diffuse to objective substances (hereafter “diffusion time”). Secondly, the volume of the reaction space is decreased, thereby creating a high concentration of objective substances at the bottom and accelerating the binding reaction.

For example of the microreaction effect in a micro channel, Honda et al. evaluated the time of an antibody-antigen reaction, namely, the time until an antigen-antibody reaction reaches equilibrium state, by using a micro channel (100 micrometer depth) and a microtiter plate cell (3 millimeter depth). They showed that the reaction time was reduced from four hours in a microtiter plate cell to 10 min in a micro channel [6]. On the basis of that result, it is expected that using a micro channel will shorten the reaction time of the binding reaction between fluorescent dyes and viruses on a plate; however, it is difficult to apply a micro channel to detection of airborne viruses. That is, it is hard to collect airborne viruses efficiently at the bottom of a micro channel. In response to that difficulty, we have devised a new detection method as shown in Fig. 4 for collecting airborne viruses on the surface of a collection plate and binding fluorescent dyes to the collected viruses rapidly by utilizing the microreaction effect.

III. DETECTION SYSTEM

A conceptual schematic of breath test using this detection system is shown in Fig. 3. The system set with a sampling bag collecting patient’s breath and a cartridge can detect automatically pathogens within 10 minutes. Everybody can use this system because breath sampling is non-painful for patients and non-medical act.
Sampling bag

Pathogens (≥ φ0.3 μm)

Dye mist ( φ5 μm)

Nebulizer

Disposable cartridge

Fig. 4 Configuration schematic of airborne pathogens detector

Collecting pathogens using impaction

Labeling fluorescent dyes to pathogens

Removing free fluorescent dyes

Detecting fluorescence from pathogens

Pathogen

Airflow (100m/s)

Dye mist

Fluorescent dye

Free fluorescent dye

Excitation light

Fluorescence detector

Fluorescence

Fig. 5 Detection process of airborne pathogens using detection system

Air pillar

Reagent Air hole

Air hole Waste reservoir

Washer reservoir

Nozzle

60 mm

670 μm

70 μm

Number of nozzles: 109
Diameter: 70μm

Enlarged view of multi-hole plate

Multi-holeplate Collection plate

Multi-holeplate (100μm)

Multi-holeplate ( Light-permeable PTFE)

Nozzles

670 μm

300μm

300μm

A-A Cross-section view of cartridge

Assembly drawing of cartridge

Fig. 6 Disposable cartridge
This detection system consists of the sampling bag, the cartridge, the fluorescence detector, the aspiration pump and valves for the flow control of breath and reagents as shown in Fig. 4. Moreover, these automatic four steps (collecting pathogens, labeling fluorescent dyes, removing free dye, and detecting fluorescence) are shown in Fig. 5.

The assembly drawing of the disposable cartridge is shown in Fig. 6. The functional feature of the cartridge are that the part 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 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. Pathogens are detected under trapped on the collection plate.

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 micrometer thick) with 109 nozzles (pore diameter 70 micrometer), which was made from a light-permeable PET (Polyethylene Terephthalate) with the Tea-CO₂ laser, is less deformed and produces little autofluorescence and reflected light disturbing the fluorescence detection of pathogens.

IV. EXPERIMENTS AND RESULTS

We evaluated the collection efficiency of the cartridge and the detection performance of this system using the sampling bag where pathogens were nebulized. We used E. coli bacteria as substitutions of airborne pathogens such as tuberculosis and HiLite Fluore™647 conjugated anti-E. coli antibodies as fluorescent dyes.

Fig. 7 shows the cartridge can collect most pathogens in the sampling bag. In Fig. 7, the number concentration of E. coli was defined as one of particles counted by the particle counter. And four photos, which were taken for reference after measurements, are fluorescent images of E. coli on a part of the collection plate under a nozzle.

Fig. 8 shows that the relative intensity of the fluorescence was correlated with the number concentration of E. coli and this system can detect pathogens whose number concentration was 3.0×10⁴ to 1.5×10⁵ particles/L. This number concentration was equivalent of one of tuberculosis in tubercular patients’ bless.

REFERENCES