RAPID AIRBORNE VIRUS DETECTION USING MIST-LABELING BASED ON MICRO REACTION PROCESS

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ABSTRACT

We have devised a new method "mist-labeling" binding fluorescent dyes to viruses in less time for a rapid detection of airborne viruses such as avian flu. The mist-labeling is the method that makes micro size mists (ϕ 5 nm) containing fluorescent dyes run into viruses collected on a plate using airflow through a small nozzle, and improves the binding rate of fluorescent dyes and viruses by a micro reaction process.

We achieved that the detection rate of substitution viruses in 5 min reaction time is over 90 % by the mist-labeling compared to less than 10 % by the common method.

KEYWORDS

Rapid detection, airborne microbe, mist, fluorescence, pandemic prevention.

INTRODUCTION

There are growing concerns about a pandemic of a lethal infection such as avian flu. Vaccine is one of methods against infections however this can't prevent an infection spread in early phase of the epidemic because it takes long time to supply enough vaccine. We think that we can prevent an infection spread using a rapid and high sensitive system which can collect airborne viruses or bacteria and detect them (Figure 1) because it is easy to provide measures rapidly such as a movement restriction.

We achieved the system which can collect viruses ($>\phi$ 0.3 μm) and detect them in hours by a gene amplification [1]. However, a gene amplification needs processes to elute genes of virus in water and long time to amplify genes. We studied how to bind fluorescent dyes to collected viruses by a fluorescence antibody technique and to detect viruses optically for a simple and rapid detection. The fluorescence antibody technique is known as one of high-sensitive detection methods for viruses, but this needs

Figure 1. Conceptual diagram of virus detection system

up to a few hours to bind sufficient fluorescence dyes to viruses for detection [2]. In this paper, we have developed a mist-labeling that can detect substitution viruses in a short time to improve the binding rate of fluorescent dyes and substitution viruses by the micro reaction process.
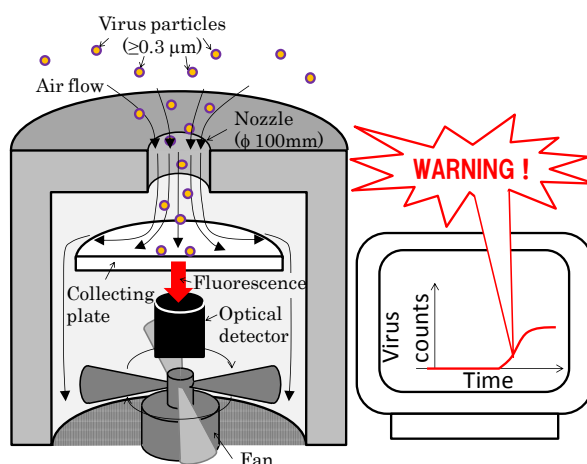
MIST-LABELING

Figure 2 shows processes of the mist-labeling and a common method. A feature in processes of the mist-labeling is that this uses an impaction technique, which makes particles run into a collecting plate by an inertial force a high-velocity airflow through a small nozzle, in three processes consisted of collecting viruses on a collecting plate, binding fluorescent dyes to viruses and removing free fluorescent dyes, while a common method uses the impaction technique in only collecting viruses.

The mist-labeling makes micro size mists containing fluorescent dyes run into viruses on a collecting plate by the impaction technique in binding fluorescent dyes to viruses. And the mist-labeling has following advantages. (1) This can collect airborne viruses, bind fluorescent dyes to viruses and remove free fluorescent dyes continuously. (2) This can collect viruses and mists on a small area of collecting plate under a nozzle and make mists run into viruses effectively. (3) This can improve the binding rate of viruses (antigen) and fluorescent dyes (antibody) by the micro reaction process without a micro flow-cell.

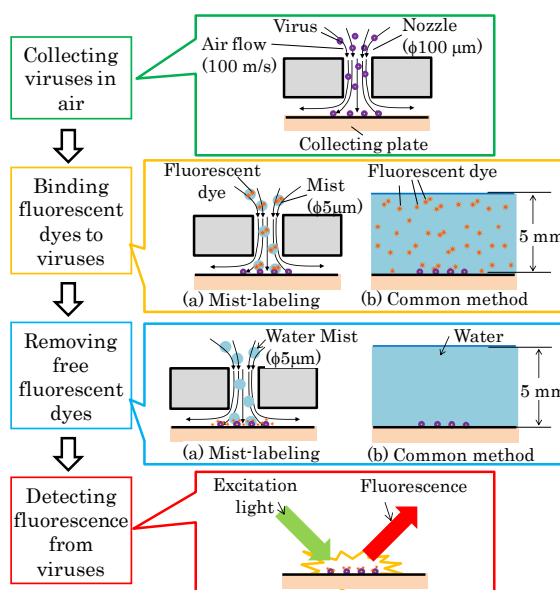The fluorescent dye's diffusion time in this is shorter than that in a common method as a reaction space is smaller

Figure 2. Process the mist-labeling and the common method

(Figure 3). Moreover, the binding reaction between fluorescent dyes and viruses proceeds more as virus concentration is higher with a minimization of a reaction space.

Figure 4 shows a diffusion time of a fluorescent dye (ϕ 5 nm). Following the theory of brownian motion, the diffusion length during a time interval t as

$$\langle x \rangle^2 = 2Dt \quad , \qquad (1)$$

where D is a diffusion coefficient of a fluorescent dye.

The diffusion time is shortened from 2.5×10^5 (sec) in common method to 2.5×10^{-1} (sec) in mist-labeling.

Secondly, figure 5 shows a kinetic analysis of an influenza virus's antigen-antibody binding reaction referring to Katakura [3] and Gopinath [4].

Antigen-antibody reaction is reversible reaction. The reaction rate of antigen-antibody complex AB is shown as

$$\frac{d[AB]}{dt} = k_{on}[A][B] - k_{off}[AB] \quad , \qquad (2)$$

where k_{on} is association rate constant and k_{off} is dissociation rate constant, [A] is antibody concentration and [B] is antigen concentration, and solving equation (2) gives,

$$[AB] = \frac{\alpha\beta\{1 - \exp(\gamma k_{on}t)\}}{\beta - \alpha\exp(\gamma k_{on}t)} \quad , \qquad (3)$$

where t is reaction time,

$$\alpha = (p+a)/2 \quad , \qquad (4)$$

$$\beta = (p-a)/2 \quad , \qquad (5)$$

$$a = (p - 4q)^{1/2} \quad , \qquad (6)$$

$$p = [A]_{t=0} + [B]_{t=0} + k_{on}/k_{off} \quad , \qquad (7)$$

$$q = [A]_{t=0}[B]_{t=0} \quad , \qquad (8)$$

$[A]_{t=0}$ is initial antibody concentration and $[B]_{t=0}$ is initial antigen concentration.

The time until a binding rate exceeding 80% is shortened from 540 (sec) in common method to 30 (sec) in mist-labeling.

EXPERIMENT

We used a virus sampler to evaluate the mist-labeling (Figure 6). This sampler has more than 80 % collection efficiency for more than ϕ0.3 μm particles [1]. This consists of a multi-hole plate with 308 nozzles (ϕ100 μm) made by an electron beam and a collecting plate consists of a PDMS sheet and a cover glass. An outlet of the sampler was connected to a vacuum cleaner (CV-SR9, Hitachi) in use and air containing viruses flow through nozzles at 100 m/sec.

We used biotinylated beads (ϕ0.2 μm, Invitrogen) as substitution viruses. These beads emit green fluorescence (peak wavelength: 510nm) to find easily. And we used Dylight650 streptavidin (Thermo Scientific) as fluorescent dyes. These dyes are preparations of streptavidin biotin-binding protein that are tagged Dylight650 emitting red fluorescence (peak wavelength: 650nm).

In the process of collecting biotinylated beads, water mists containing biotinylated beads were spread in air with an ultrasonic nebulizer (NE-U17, OMRON) (particle size distribution: ϕ1~8 μm, nebulization rate: 1 ml/min) and the sampler collected biotinylated beads after the evaporation of water mists. In the process of binding fluorescent dyes to biotinylated beads, an inlet of the sampler was connected directly to an outlet of another ultrasonic nebulizer (NE-U17) and the sampler collected mists containing fluorescent dyes before the evaporation of mists.
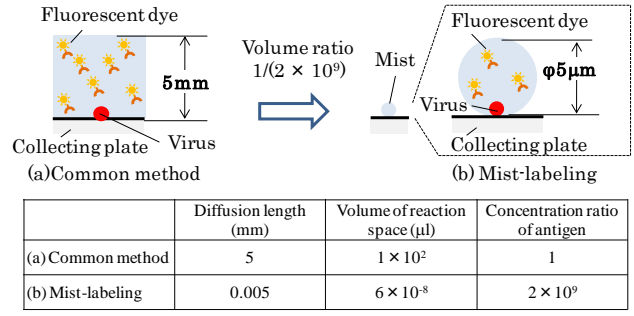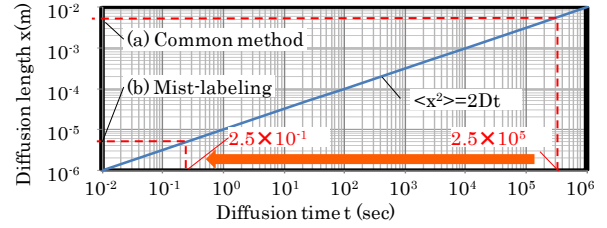

Figure 3. Comparison of reaction spaces

	Diffusion length (mm)	Volume of reaction space (μl)	Concentration ratio of antigen
(a) Common method	5	1×10^2	1
(b) Mist-labeling	0.005	6×10^{-8}	2×10^9

Figure 4. Diffusion time of an fluorescent dye

Initial antibody concentration $[A]_{t=0}$; 1.0×10^{-8} (mol/l), combination rate constant k_{on}; 2.3×10^5 (mol/lsec), dissociation rate constant k_{off}; 6.6×10^{-4} (/sec), initial antigen concentration $[B]_{t=0}$; 8.3×10^{-17} (mol/l) in (a) common method, 1.7×10^{-7} (mol/l) in (b) mist-labeling.
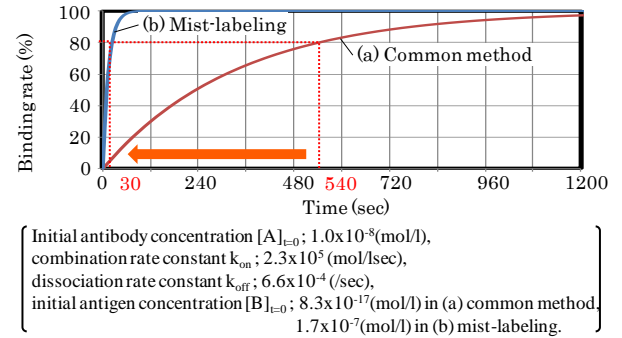
Figure 5. Kinetic analysis of an influenza virus's antigen-antibody binding
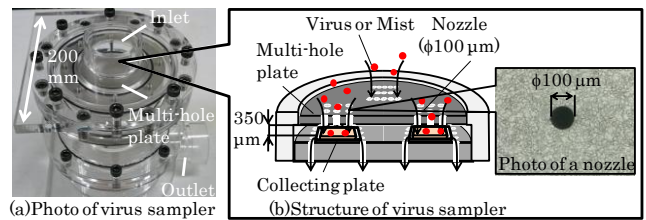

Figure 6. Virus sampler

RESULT AND DISCUSSION

Figure 7 shows trinary images of biotinylated beads captured with a fluorescent microscope (IX71, ORYMPUS) and a cooled CCD (ORCA-ER, HAMAMATSU PHOTONICS). We evaluated the detection rate of biotinylated beads in the mist-labeling and the common method. The detection rate was defined as the rate of the number of biotinylated beads emitting fluorescence of Dylight650 to the total number of biotinylated beads. Figure 7 shows that the detection rate in mist-labeling is over 90% while this in common method is less than 10%.

Figure 8 shows the reaction time dependence of the detection rate of biotinylated beads in using 1.2 µg/ml Dylight650 streptavidin. Figure 9 shows the Dylight650 streptavidin concentration dependence of that in 5 min reaction time. Non-biotinylated beads were to check an absence of non-specific binding between a bead's surface and Dylight650 streptavidin.

Figure 8, 9 show that the mist-labeling improves the detection rate considerably because this binds more Dylight650 streptavidin to biotinylated beads in a short reaction time and the low concentration than the common method.

CONCLUSION

Although there are growing concerns about a pandemic of a lethal infection such as avian flu, we can't prevent an infection spread in early phase of epidemic with existing prevention methods such as vaccine. We have devised a new method "mist-labeling" binding fluorescent dyes to viruses in less time for the purpose of development of a system which can collect and detect airborne viruses rapidly. The mist-labeling is the method that makes micro size mists (ϕ5 µm) containing fluorescent dyes run into viruses collected on a plate using airflow through a small nozzle, and improves the binding rate of fluorescent dyes and viruses by a micro reaction process. We achieved that the detection rate of substitution viruses in 5 min reaction time is over 90 % by the mist-labeling compared to less than 10 % by the common method. We think the mist-labeling has a large potential of contributing to the pandemic prevention.
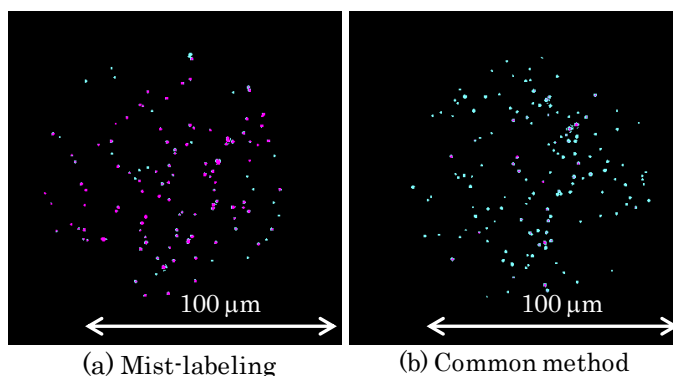

(a) Mist-labeling (b) Common method

Blue points : biotinylated beads emitting no fluorescence of Dylight650
Red points : biotinylated beads emitting fluorescence of Dylight650
Reaction time : 5 min
Concentration of Dylight650 streptavidin : 1.2 µg/ml

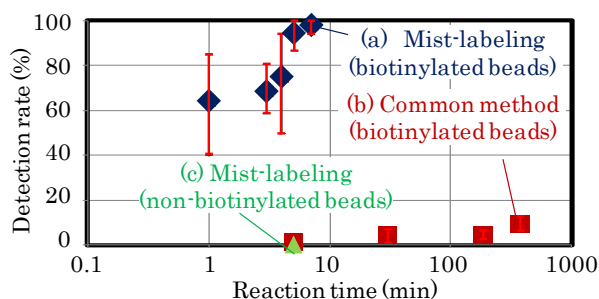*Figure 7. Trinary images of biotinylated beads*

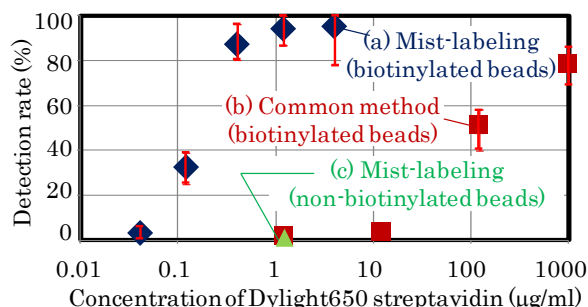*Figure 8. Reaction time dependence of the detection rate of biotinylated beads*

Figure 9. Concentration dependence of the detection rate of biotinylated beads

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