DEVELOPMENT OF A BIOAEROSOL EXPERIMENTAL SYSTEM FOR VIRUS STABILITY MEASUREMENT

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Abstract. Respiratory infections, such as SARS, MERS, and COVID-19, have highlighted the importance of studying the transmission mechanism in indoor environments. Virus stability affected by temperature, humidity or particle size has remained inadequately investigated. A bioaerosol experiment system is developed in this study for virus stability measurement. Virus-laden droplets are generated by a Collision nebulizer, and afterwards enter a mixing chamber, where is filled with conditioned air. After complete evaporation, droplet nuclei go through an impactor, and only those smaller than the cut-off size (1μm, 2.5μm or 10μm) can finally enter Goldberg Drum to suspend for a designed period for later sampling and stability measurement. Temperature (20°C-40°C) and relative humidity (30%-80%) can be well controlled throughout the experiment. According to the virus inactivation mechanism, the sampling process is divided into the initial stage involving droplet evaporation and the subsequent aerosol suspension stage. Decay coefficients obtained by this method can be used for evaluation of virus exposure risks in indoor environments and for the development of efficient engineering control strategies.

1 Introduction

Since the outbreak of Covid-19 in December 2019, its main transmission routes are considered to be droplet transmission and contact transmission. It was not until May 2021 that the CDC in the United States recognized the aerosol transmission route [1]. However, the relative risk of various modes of transmission remains inconclusive, especially the aerosol transmission. In aerosols, fine particles are the main components, and viruses need to attach to the fine particles to spread. Some studies have pointed out [2] that the concentration of PM2.5 in the air also has a significant impact on the spread of COVID-19, which is due to the long suspension time of fine particles (diameter < 5 μm) in the air. The location where aerosols with different particle sizes are inhaled will be different. The particles larger than 10 μm are almost completely deposited in the nasopharynx, and about 10% of the particles in the range of 2-5 μm are deposited in the bronchial area, while particles smaller than 2 μm are mainly deposited in the alveolar tissue. When the size of biological particles is in the range of 1-2 μm, about 50% of the particles deposited in the alveoli. And the smaller the particle size is, the greater the deposition occurs [3]. Different particle sizes directly affect the sedimentation position of particles in the respiratory system, and particle size also affects the amount of virus attachment and pathogenicity [4]. At the same time, environmental temperature and humidity are also important factors affecting the activity of the virus. How to quickly and accurately quantify the transmission characteristics of virus aerosols and recognize the law of activity decay is extremely important.

Goldberg first proposed in 1958 to use a rotating drum-type aerosol holding chamber instead of a static aerosol chamber to research virus activity in virus aerosols, and initially proposed the theoretical basis for aerosol suspension in a rotating drum [5], but his experiments The system is relatively large and cannot achieve stable control of temperature and humidity.

Kormuth et al. independently built an experimental system to study the activity of virus aerosols and researched the activity of influenza virus aerosols [6]. In the system, a Nebulizer was used to generate aerosols, and the aerosols were then introduced into the drum. In the aerosol holding chamber, the humidity was adjusted by the flow rate of the drying airflow (0-10L/min, RH<10%), and the temperature was controlled by adjusting the ambient temperature. Kormuth used this experimental system to test the TCID50 of H1N1 influenza virus at seven relative humidity (23%, 33%, 43%, 55%, 75%, 85%, and 98%) and pointed out that compared to other factors, humidity was not a decisive factor on virus activity. It also mentioned that for the control of the spread of airborne diseases, it is recommended to increase the air exchange rate and sterilization treatment, rather than adopting humidity control to achieve the result of inactivation. The experimental system flow in the other literatures [7-9] is basically the same as that built by Kormuth, but there are differences in the selection of atomization device.

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and the adjustment strategy of temperature and humidity. To sum up, the current aerosol experimental system for virus activity research pays more attention to the effect of temperature and humidity on virus aerosols but ignores the impact of drastic changes in temperature and humidity during droplet evaporation on virus activity and the effect of particle size on virus aerosols.

Based on the current bioaerosol experimental system, many scholars have carried out research on different coronavirus bioaerosols under different temperature, humidity, and atomization time conditions. Ijaz et al. [7] studied the virus half-life of HCoV-229E bioaerosol at 6°C and 20°C under different conditions from low humidity to high humidity. The experimental results showed that the increase of temperature was beneficial to inhibit the virus activity to a certain extent. However, the change of humidity shows a trend of rising first and then falling, which only shows that the influence of humidity is complicated, and more experimental data need to be set to draw a more complete conclusion. Doremalen et al. [8] took MERS-CoV as an example and studied the virus activity under different humidity at 20°C. Pyanko et al. [9] also studied the inactivation time of MERS-CoV. The experimental conditions were close to the general indoor conditions and the outdoor temperature and humidity conditions in the Middle East in summer. Under the condition of 24% RH, 4.7% of the virus still survived in the air, correspondingly at 25°C and 79% RH, the virus survival rate exceeded 63%. Doremalen [10] and others also compared the half-life of SARS-CoV-1 and SARS-CoV-2 under the same temperature and humidity. They found that the survival time was very close, which indicated that for viruses with similar structures, the mechanism of the influence of temperature and humidity may be consistent.

Therefore, in order to systematically characterize the influence of temperature, humidity, and aerosol particle size on virus activity, a bioaerosol experimental system for virus stability measurement was developed in this study. According to the virus inactivation mechanism, the sampling process is divided into an initial phase involving droplet evaporation and a subsequent aerosol suspension phase. The attenuation coefficients obtained by this method can be used to assess virus exposure risks in indoor environments and develop effective engineering control strategies.

### 2 Experiment system

The bioaerosol experimental system is shown in Figure 1. The compressed air filtered by HEPA filter passes into the 3-jet Collison nebulizer (BGI, Inc, USA) at a flow rate of 6L/min. The air in the constant temperature and humidity box (DHT, China) flows at 24L/min. Adjust air mixes with the airflow from the nebulizer in the mixing chamber to ensure sufficient evaporation, and then goes through the impactor to achieve particle size screening. Only droplet nuclei smaller than the cut-off size (1μm, 2.5μm, or 10μm) can pass, and finally enter the Goldberg Drum for long-term suspension. After stabilization, the sampler samples at 4L/min flow rate for 1min at 0, 5, 15, 30, 45, 60min. During the sampling process, the make-up airflow was provided by the constant temperature and humidity box, and the airflow temperature and humidity were kept consistent with the experimental conditions. The main part of the experimental system is placed in a biosafety cabinet to avoid virus leakage and infection, and the environment in the biosafety cabinet is temperature-controlled to minimize temperature fluctuations in the Goldberg Drum. In addition, sampling ports are set at the inlet and outlet of the mixing chamber, which can be used to explore the virus activity attenuation coefficient \(k_2\) in the droplet evaporation stage. During the whole experiment, the fluctuations of temperature and humidity in Goldberg Drum were ±0.5°C and ±2%, respectively.

![Schematic diagram of bioaerosol experimental system](image)

**Fig. 1.** Schematic diagram of bioaerosol experimental system

### 3 Results

#### 3.1 Aerosol particle size distribution

A hand-held laser particle counter (Particle Counter 9306-v2, TSI) is used for particle measurement at the nebulizer outlet, the mixing chamber outlet, and the impactor outlet in the experiment. The PBS solution (Phosphate buffered saline, which is always used in virus test as Dilution reagent) was used as a Nebulized reagent and the results of the aerosol particle size distribution are shown in Figures 2(a) and 2(b).

It was found that the concentration of 0.3 μm and 0.5μm particles at the outlet of the nebulizer were five times as that of the outlet of the mixing chamber due to the extremely short evaporation time and dilution effect of the adjust air. And The increased concentration of 1μm particles is probably due to the evaporation of 3-5 μm diameter particles. Plus, the settlement effect and evaporation effect leads to the size distribution of the 3.5, 10μm.
The concentration of the aerosol

Amount of the aerosol

The number of 0.3-0.5μm particles at the exit of the impactor did not change significantly, but the number of 1μm, 3μm, 5μm, and 10μm particles decreased significantly by 52.6%, 85.1%, 97.5%, and 99.3%, respectively. The measured collection curve of the impactor can be seen in Figure 3.

3.2 Temperature and humidity characterization

Because the humidity of the high-pressure gas tank is extremely small, the temperature is close to room temperature, and the temperature control of the high-pressure gas is dangerous. Therefore, the aerosol system can adjust the temperature and humidity of the air flow and the quality of the liquid that escapes atomization in the atomizer. Body temperature and humidity, and all lines are insulated, while the impactor and Goldburg Drum are placed in temperature-controlled compartments. Therefore, it is first necessary to calibrate the atomization escape of the nebulizer, as well as the stability of the output airflow from the constant temperature and humidity box to the mixing chamber.

During the long-term operation, the mass of the atomizer and the pipe section connected to the mixing chamber were weighed every 5 minutes for one hour, and the rate of liquid entering the mixing chamber was 0.135 g/min. Therefore, we can calculate the moisture content entering the mixing air.

Then, we tested the stability of the adjust airflow, which could be seen in Table 1. The test content includes different conditions of 10℃-40℃, 20%-80%RH to show its regulation stability.

Table 1. Stability of the CTHB

<table>
<thead>
<tr>
<th>Setting point</th>
<th>Relative Error</th>
<th>Fluctuation</th>
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<tbody>
<tr>
<td></td>
<td>Temperature/℃</td>
<td>RH/%</td>
</tr>
<tr>
<td>10.0℃</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>25.0℃</td>
<td>0.8</td>
<td>3.0</td>
</tr>
<tr>
<td>40.0℃</td>
<td>0.3</td>
<td>0.0</td>
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<tr>
<td>10.0℃</td>
<td>1.0</td>
<td>0.2</td>
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<tr>
<td>25.0℃</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>40.0℃</td>
<td>0.0</td>
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<tr>
<td>10.0℃</td>
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<td>25.0℃</td>
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<tr>
<td>40.0℃</td>
<td>1.3</td>
<td>0.4</td>
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</table>

For example, the temperature and humidity of the adjust airflow are set at 40℃, 20%RH, and the flow rate is 24L/min. Actually, when entering the mixing chamber, the temperature and humidity of the regulated airflow are 40.1℃(±0.2℃) and 20.0%RH(±0.5%).

The nebulization amount of PBS solution in the nebulizer is 0.135g/min. According to the measurement results, the temperature and humidity at the outlet of the mixing chamber are 27.3℃, 48.3%RH, while the temperature and humidity inside the drum are 27.3℃, 50.3%RH. The variance is already represented on the graph.

Within one hour, the temperature inside the drum increased by 0.1℃, the humidity decreased by 0.8%, and the theoretical calculation value was 1.3%. The temperature inside the drum rises and the relative humidity decreases, but part of the droplet nuclei evaporates further, so the measured value is smaller than the theoretical change. Although slight temperature changes can cause changes in humidity, the fluctuations in temperature and humidity are within an acceptable range and will not have a huge impact on virus activity.
4 Conclusion

A bioaerosol experiment system is developed in this study for virus stability measurement. We tested the overall operation of the system, the particle size control effect(<1μm). Meanwhile, the temperature and humidity control is very stable. Plus, by changing the temperature and humidity parameters of the airflow, the temperature(30%-80%) and humidity(20-50°C) in the Goldberg Drum can be changed, and virus stability experiments can be carried out for multiple factors to study the decay law of virus activity in steady-state.

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References

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