Supplementary Information

Rapid monitoring of indoor airborne influenza and corona virus with high air flowrate electrostatic sampling and PCR analysis

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1. Design-changed HAFES

Our sampler was modified from the original sampler developed by Kim et al. (2021) based on the Deutsch-Anderson equation expressed as follows (Hinds, 1999);

$$\boldsymbol{\eta} = 1 - \exp\left(-\frac{\boldsymbol{V_{TE}} A_i}{Q}\right) \quad \text{(S1)}$$

where V_{TE} is the terminal electrical velocity, A_i is the area of the collecting plate, and Q is the air flow rate of sampler. The terminal electrical velocity V_{TE} is defined as follows;

$$\boldsymbol{V_{TE}} = \frac{\boldsymbol{n(t)} eEC_c}{3\pi\mu d_p} \quad \text{(S2)}$$

where *n(t)* is the particle charge number, *e* is the charge of an electron (1.6×10^{-19}C), *E* is the electric field, C_C is the slip correction factor, μ is the air viscosity, and d_p is the particle diameter. The particle charge number *n(t)* is the calculated as follows;

$$\boldsymbol{n(t)} = \left(\frac{3\varepsilon}{\varepsilon+2}\right)\left(\frac{Ed_p2}{4K_E e}\right)\left(\frac{\pi K_E e Z_i N_i t}{1+\pi K_E e Z_i N_i t}\right) + \frac{d_p kT}{2K_E e^2} ln\left[1 + \frac{\pi K_E d_p \bar{C}_i e^2 N_i t}{2kT}\right] \quad \text{(S3)}$$

where ε is the relative permittivity of particle ($\cong 80$), *T* is the temperature, K_E is the electrostatic constant of proportionality (9×10^9 Nm^2/C^2), Z_i is the mobility of air ions (0.00015 m^2/Vs), $\bar{C}_i$ is the mean thermal speed of the ions (240m/s), *k* is the Boltzmann constant (1.38×10^{-23} m^{2}kg/s^{2}K), and N_i is the concentration of ions. The N_i is calculated as follows;

$$\boldsymbol{N_{ion}} = \frac{2\pi\varepsilon\mu L(V-V_0)^2}{eZ_i E A_i d^2} \quad \text{(S4)}$$

where I_{Corona} is the corona current, *L* is the discharge wire length, *V* is the applied voltage, V_0 is the corona onset voltage, and *d* is the distance between the discharge wire and the collecting plate.

2. Preparation of virus stock

In this study, HCoV-229E (Korea Bank for Pathogenic Viruses, Korea), H1N1 (A/California/4/2009), and the MS2 bacteriophage (KORAM Lab-Tech, Korea) were used the laboratory tests.

HCoV-229E stocks (8.0×10^6 plaque-forming units (PFU) per milliliter (PFU/mL)) were purchased from the Korea Bank for Pathogenic Viruses (Seoul, Korea).

H1N1 influenza virus stocks (1.5×10^7 PFU/mL) were prepared following the method presented in Piri et al. (2021). To culture H1N1 influenza virus stocks, Madin-Darby canine kidney (MDCK) cells were got from the Korea Cell Line Bank (Seoul, Korea) for use as virus hosts. MDCK cells were preserved in 75- and 175-cm^2 cell-culture flasks (SPL Life Sciences, Korea) with minimum essential medium (MEM) and Earle's Balanced Salts Solution (MEM/EBSS; Hyclone, USA). All MEM/EBSS medium contained 10% fetal bovine serum (FBS; Gibco, USA) and 1% antibiotic-antimycotic solution (Gibco, USA) for MDCK cell culture. Up to the cells reaching 90% confluence (every 3–4 days), they were incubated at 37 °C in 5% CO_2 and 95% relative humidity atmosphere. MDCK cells were washed by 1x PBS buffer (Biosesang, Korea) and the culture media were removed. Then, the cells in the 75- and 175-cm^2 cell-culture flasks were infected by 3 and 6 mL FBS-antibiotic-antimycotic-free MEM/EBSS containing the H1N1 influenza virus.

The initial virus was delivered from BioNano Health-Guard Research Center (Daejeon, Korea). The infected cell was incubated for 45 min at 37 °C on a rocking shaker to allow virus internalization. After that, the cell was washed with 1x PBS buffer. Next, MEM/EBSS solution with 0.25% trypsin (Hyclone, USA) was put into the flask. The flasks were incubated at 37 °C

for 2 days in 5% CO_2 and 95% relative humidity condition. After 48h, the H1N1 viruses were sampled and centrifuged at 1200 rpm at 4 °C (for 10 min). A cultured H1N1 virus concentration was 1.5×10^7 PFU/mL. The H1N1 virus stocks were placed at − 80 °C for further experiments.

Bacteriophage MS2 stocks were prepared following the method presented in Park & Hwang (2014). The concentration of stocks was 107 PFU/mL. Escherichia coli strain C3000 (ATCC 15597) was selected as the host bacteria for culturing Bacteriophage MS2 stocks. The C3000 stock was melted on an ice pack. 0.1 mL of C3000 stock was mixed with 10 mL of tryptic soy broth (TSB). The bacteria mixture solution was incubated in shaking incubating for 24h at 37 °C. The Bacteriophage MS2 stock was melted on an ice pack. 0.1 mL of the viral solution was extracted. The viral solution was mixed with 0.3 mL of the cultured C3000 solution and 29 mL of soft tryptic soy agar (TSA) including 8 g/L of agar. The mixed agar solution was spilled into a Petri dish and incubated overnight at 37 °C. After 24 h, the surface of the agar was washed off with 10 ml of phosphate buffer solution (PBS). The washed solution was centrifuged for 20min at 5000G. The Bacteriophage MS2 stocks were placed at − 80 °C for further experiments.

3. Desalting process

Since each virus stock inevitably contains by-products such as MDCK cells and MEM, generated during the virus preparation process explained above, laboratory tests were carried out after removing these materials (desalting process). Laboratory tests were also performed without removing these materials. The desalting process was conducted as described in the manual by the manufacturer (PD SpinTrap G-25, Cytiva). The PD SpinTrap G-25 protocol for desalting is as follows:

First, PD SpinTrap G-25 preparation. (1) The medium is suspended by vortexing, the screw cap lid is loosened, and the bottom closure is removed using the plastic bottom cap removal tool. (2) The column is placed in an appropriately sized collection tube. (3) The storage solution is removed by centrifugation for 1 min at 800 G.

Second, Equilibration. (1) Equilibration is carried out by adding 400 μl equilibration buffer. (2) Centrifugation is carried out for 1 min at 800G, the flow-through is discarded, and the collection tube is replaced. (3) This procedure is repeated four more times (five times total).

Third, Sample application. (1) The used collection tube is replaced with a new clean collection tube for sample collection. (2) The sample (100–180 μL) is slowly applied to the middle of the packed bed.

Fourth, elution is carried out by centrifugation at 800G for 2 min. The cleaned products are now available in the collection tube.

4. Size distributions of H1N1 aerosols with and without desalting

Fig. S1 presents the H1N1 aerosol size distributions with and without desalting. The size distributions were obtained using a scanning mobility particle sizer (SMPS, TSI, USA) and an aerodynamic particle sizer (APS 3321, TSI). The SMPS is composed of a classifier (3080, TSI Inc., USA) with a Differential Mobility Analyzer (DMA, 3081, TSI Inc., USA), an aerosol charge neutralizer (Soft X-ray Charger 4530, HCT, Republic of Korea), and a condensation particle counter (CPC, 3775, TSI Inc., USA). The SMPS measures the concentration of particles with a mobility diameter of 20.9–661.2 nm using the principle of charged particle motion in an electric field. Using a double-crest optical system, the APS real-time measures the concentration and aerodynamic diameter of particles between 0.5 − 20 μm. Fig. S1-A presents the SMPS data. In the case of the desalted sample, the concentration decreased to 10% of the original sample concentration. Fig. S1-B shows the APS data. In the case of the desalted sample, the concentration decreased to 0.3% of the original sample concentration. These results confirm that all micro-sized aerosols were removed through the desalting process. In the case of nano-sized aerosols, more than 90% of the aerosols were removed, but the mean diameter did not change.

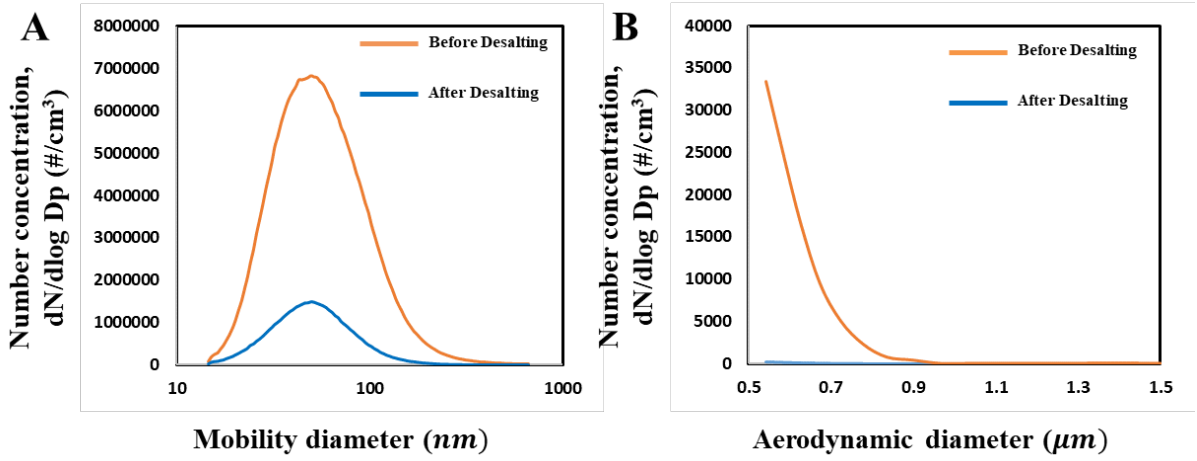

Figure S1. Aerosol size distributions with and without desalting process (A) SMPS measurement (B) APS measurement

5. Real-time qRT-PCR assays

All RNA extraction and real-time qRT-PCR processes were conducted as described in the manual by the manufacturer. Coronavirus and H1N1 influenza viruses were assessed using a PowerChek™ Pandemic H1N1 Real-time PCR Kit (KogeneBiotech, Korea) and Coronavirus 229E/OC43/NL63/HKU1 Real-time PCR Kit (64000F, Kogenebiotech, Korea). The total volume of the PCR assay was 20 µL which was a mixture of 5 µL of virus sample and 15 µL of PCR reaction mixture. The RNA extraction process was performed within 2 hours. PCR was performed using a Thermo Scientific™ PikoReal™ Real-Time PCR System (TCR0096, USA). The thermal cycling protocol for real-time qRT-PCR was as follows: a Uracil DNA Glycosylase (UDG) was activated at 50 °C for 30 min; initial denaturation was carried out at 95 °C for 10 min; 40 cycles of denaturation were carried out at 95 °C for 15 s; and annealing and extension was carried out at 60 °C for 1 min. Real-time qRT-PCR assays were performed in triplicates. Fig. S2 presents data of Ct value vs. RNA copies/mL.

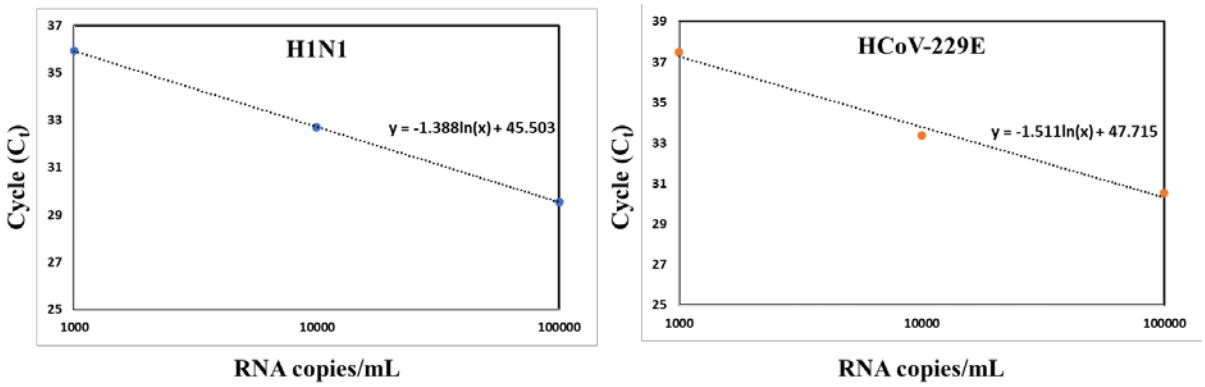

Figure S2. Ct value vs RNA copies/mL

6. Collection efficiency of the sampler for standard test aerosols (NaCl)

The size distribution of NaCl aerosols was measured by scanning mobility particle sizer (SMPS, TSI, USA). Fig. S3 presents the experimental set up for the size distribution measurement. The corona currents under various applied voltages were also measured. Fig. S4 presents the size distribution of NaCl aerosols generated from the atomizer. The NaCl particles had a wide range of sizes from 20.9 to 661.2 nm.

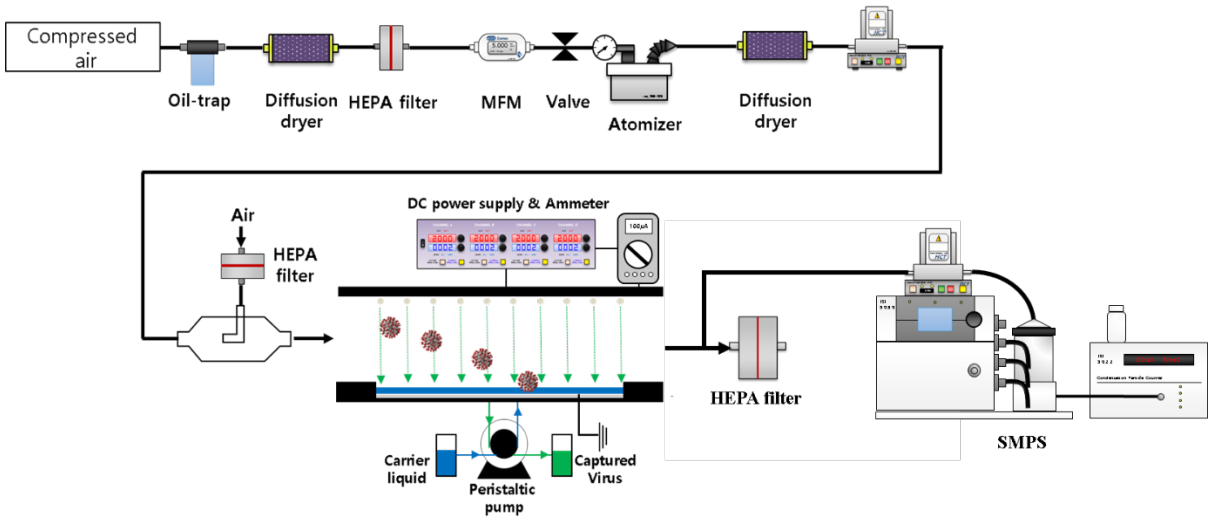

Figure S3. Experimental setup for collection performance of our electrostatic air sampler for NaCl aerosols

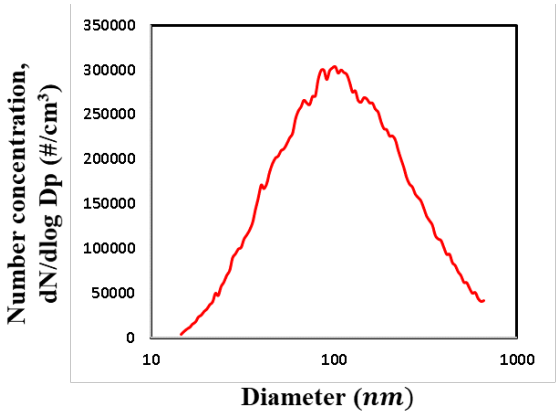

Figure S4. Size distribution of NaCl aerosols.

7. Virus damage during the atomization and drying processes.

Fig. S5 presents the experimental schematic for determining virus concentration in atomizer used in this study.

The RNA damage (D_{virus}), caused by the atomization and drying processes (99% and 99.7% for H1N1 and HCoV-229E, respectively), was determined using the following equation:

$$D_{virus} = 1 - \frac{1mL \times C_{Capture}}{0.5mL \times C_{atomizer}} \quad \text{(S5)}$$

where $C_{atomizer}$ is the concentration of the viral solution in the atomizer (RNA copies/mL) and $C_{capture}$ is the concentration of the virus sample solution (RNA copies/mL).

The original HCoV-229E viral stocks (8.0×10^6 PFU/mL each) were prepared from the Korea Bank for Pathogenic Viruses (Seoul, Korea) (see Section 2 of the Supplementary Information). For H1N1 influenza, original viral stocks with a virus concentration of 1.5 × 107 PFU/mL were prepared following the methodology presented by Piri et al. (2021). After diluting each viral stock solution, the diluted viral solution was poured into the atomizer. The concentration of the viral solution in the atomizer ($C_{atomizer}$, RNA copies/mL) was obtained by PCR analysis for H1N1 or HCoV-229E. The C_t value was chosen to be 19 for an H1N1 solution in the atomizer, and 18.3 for an HCoV-229E solution in the atomizer. Corresponding PFU concentrations (PFU/mL) were determined by matching the C_t value with the C_t value obtained by serial dilution of the original viral stock for each species (8.0×10^6 PFU/mL for HCoV-229E, 1.5×10^7 PFU/mL for H1N1). Using $\frac{\text{RNA Copies}}{PFU} \cong 400$ (H1N1) and $\frac{\text{RNA Copies}}{PFU} \cong 15{,}000$ (HCoV-229E) (Sohni, 2021), $C_{atomizer}$ for H1N1 and HCoV-229E were calculated

to be 3.03×10^7 RNA copies/mL (C_t = 19) and 3.54×10^8 RNA copies/mL (C_t = 18.3), respectively.

The viral solution in the atomizer was aerosolized using 2 L/min of clean compressed air. After aerosolization, the aerosolized virus particles were captured using a gelatin filter (225-9551, SKC Inc., USA), inserted into the SKC button sampler (225-360, SKC Inc., USA). The sampling time was 1 min as recommended by Jaschhof (1992). First, the collection efficiency of a gelatin filter in SKC button sampler for airborne virus was measured by scanning mobility particle sizer (SMPS, TSI, USA). Fig. S6 presents the experimental set up for collection efficiency measurement. The viral solution contained in an atomizer was aerosolized by using compressed air that passed through the atomizer. Then the virus aerosols moved towards the gelatin filter. The size distribution of viral aerosols was measured by scanning mobility particle sizer (SMPS, TSI, USA). The collection efficiency of gelatin filter was calculated by the following equation;

$$\eta_{collection} = 1 - \frac{C_{after\ gelatin\ filter}}{C_{before\ gelatin\ filter}} \quad \text{(S6)}$$

where $C_{before\ gelatin\ filter}$ is the aerosol concentration measured at a location upstream the SKC button sampler (number of particles per cubic centimeter of air) and $C_{after\ gelatin\ filter}$ is the aerosol concentration measured at a location downstream the SKC button sampler (number of particles per cubic centimeter of air). Fig. S7 shows the size distributions for H1N1 virus aerosols before and after the gelatin filter. As shown in Fig. S7, the collection efficiency is higher than 99% for particles larger than 80nm (virus size).

Next, it was observed that the volume of the viral stock solution decreased by 0.5 mL after 1 min of atomization. Therefore, the number of viral RNA copies collected on the gelatin filter

for 1 min was theoretically 0.5mL x $C_{atomizer}$. The gelatin filter loaded with viral particles was dissolved in 1 mL of DI water. Next, the concentration of the virus sample solution ($C_{capture}$, RNA copies per mL) was obtained by PCR analysis for H1N1 or HCoV-229E. $C_{capture}$ values of H1N1 and HCoV-229E were calculated to be 1.515×10^5 RNA copies/mL (C_t = 26.5) and 5.21×10^5 RNA copies/mL (C_t = 27.7), respectively. Therefore, the D_{virus} was 99.0% for H1N1 and 99.7% for HCoV-229E.

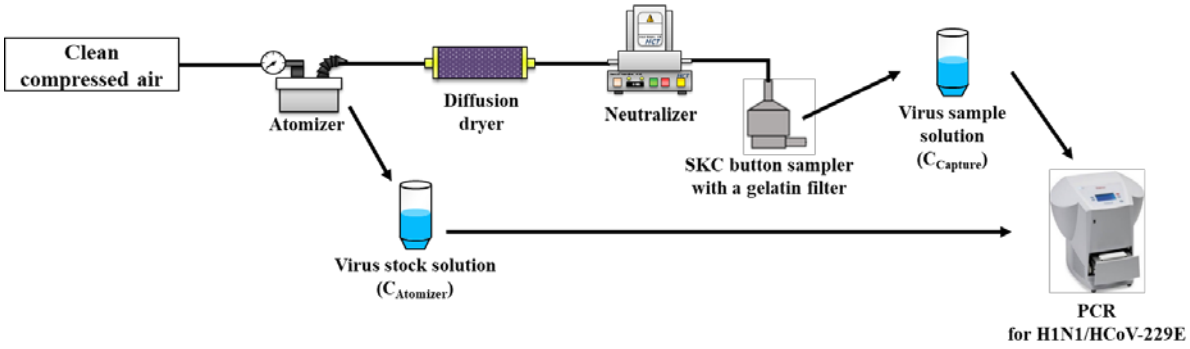

Figure S5. Experimental schematic to determine virus concentration in atomizer solution for simulating a viral pandemic scenario in air (3000 RNA copies per cubic meter of air)

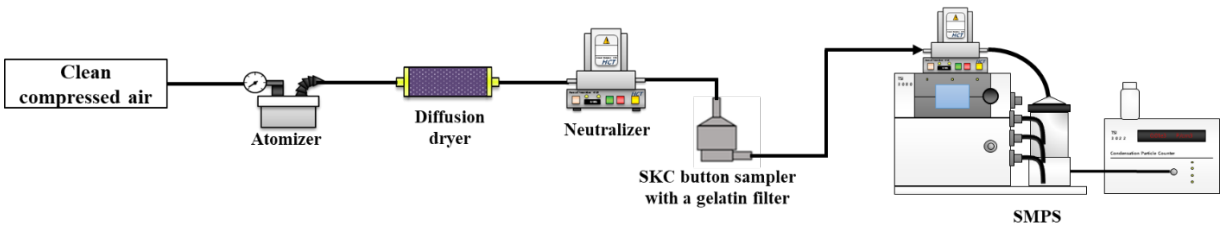

Figure S6. Experimental setup for collection performance of SKC button sampler with a gelatin filter for H1N1 virus aerosols

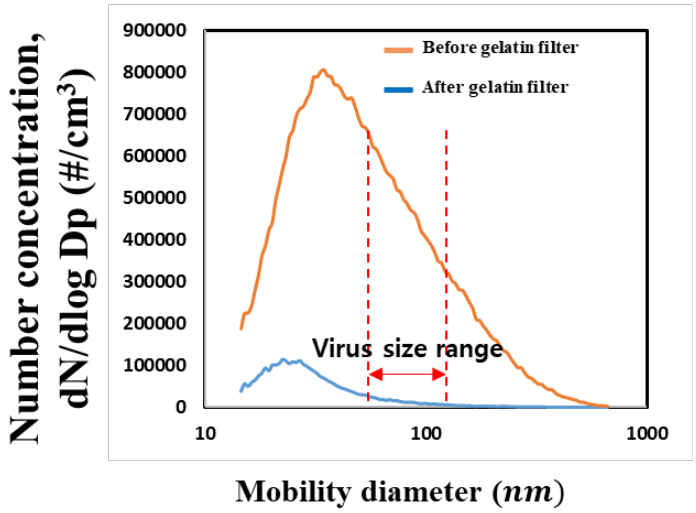

Figure S7. Size distributions for H1N1 virus aerosols before and after the gelatin filter

8. The number of particles exiting the sampler measured by CPC during sampling

A condensational particle counter (CPC) was located at the outlet of the sampler to measure the number of particles exiting the sampler. The collection efficiency of the sampler was calculated using the following equation;

$$\eta_{collection} = 1 - \frac{C_{on}}{C_{off}} \quad \text{(S7)}$$

where C_{on} and C_{off} are number concentrations of airborne particles measured at a location downstream the sampler when the power for corona discharge is on and off.

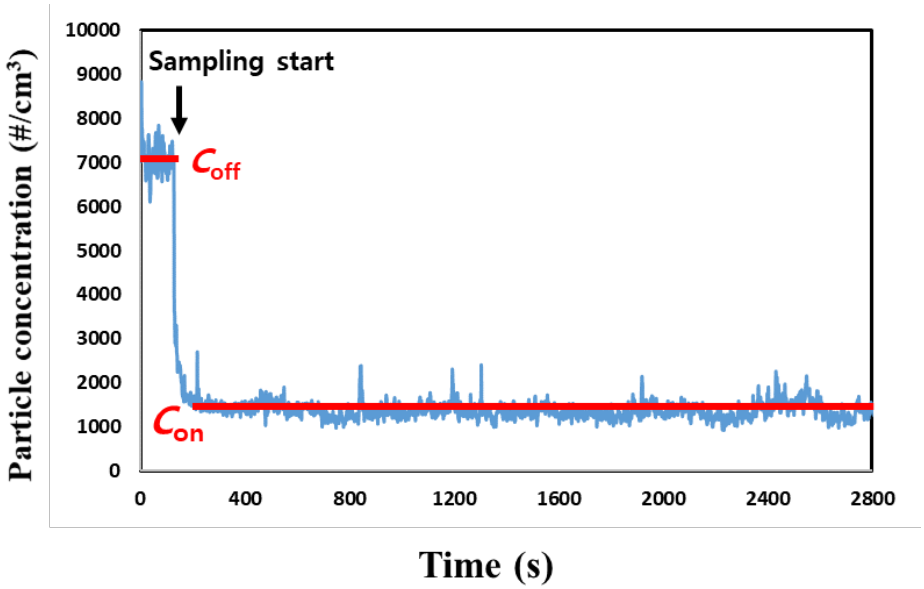

Figure S8. The number of particles exiting the sampler measured by CPC during sampling

9. Size distributions of MS2 bacteriophage and HCoV-229E aerosols

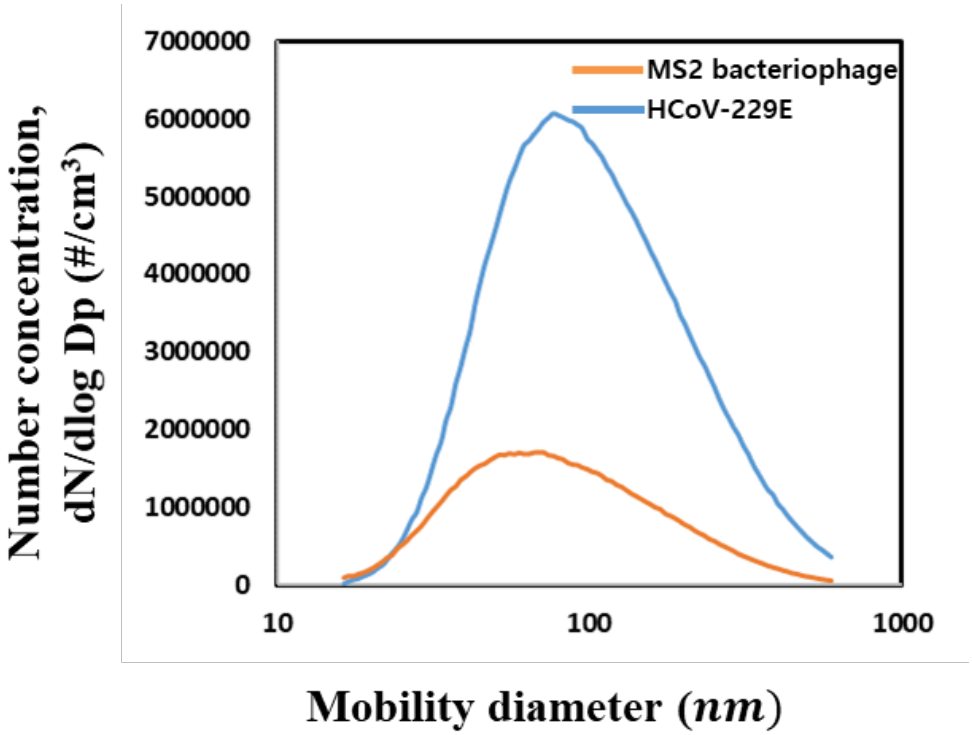

Figure S9. Size distributions of MS2 bacteriophage and HCoV-229E aerosols

10. Size distributions of tryptic soy broth (TSB) and minimum essential medium (MEM) aerosols

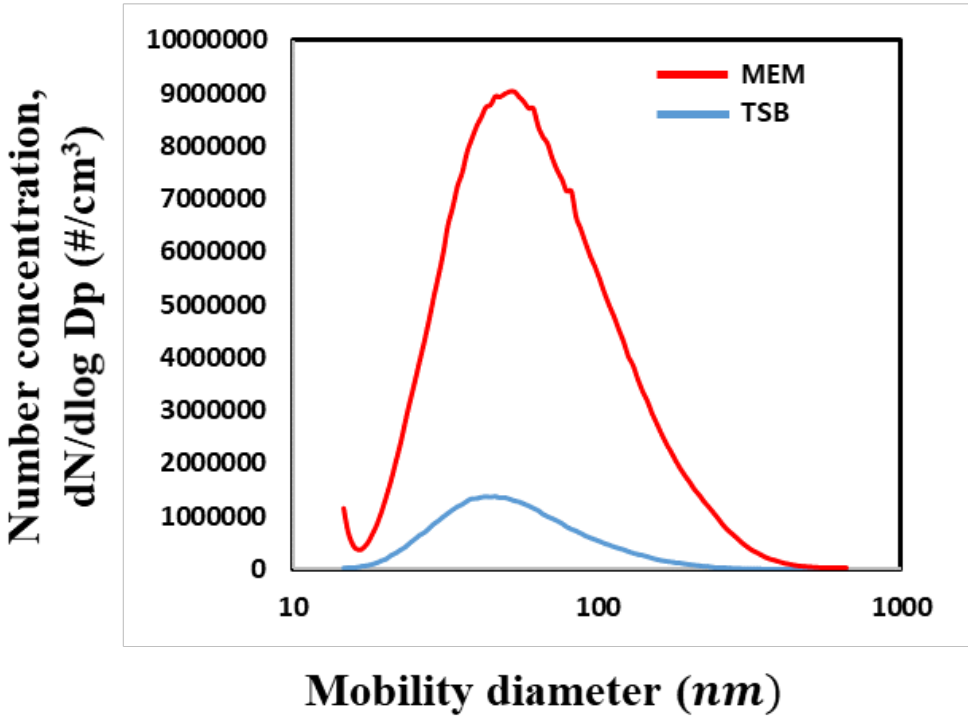

Figure S10. Size distributions of tryptic soy broth (TSB) and minimum essential medium (MEM) aerosols

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