Supporting Information

An Integrated Bioaerosol Sampling/Monitoring Platform: Field-Deployable and Rapid Detection of Airborne Viruses

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Materials and methods

Materials

Mouse monoclonal anti-MS2 IgG (TC-7033), rabbit polyclonal anti-MS2 IgG (TC-7004), and ELISA kit for MS2 bacteriophage detection (TC-4042) were purchased from Tetracore, Inc., Rockville, MD, USA. Glass fiber pad (Grade 8964, Ahlstrom-Munksjö, Helsinki, Finland) and polyester pad (Grade 6613, Ahlstrom-Munksjö, Helsinki, Finland) were purchased from Bore Da Biotech, South Korea. HEPES buffer (1M, pH 7.2-7.5) was obtained from Gibco. Polystyrene beads (L5155), phosphate buffered saline (PBS), polyvinylpyrrolidone (PVP), bovine serum albumin (BSA), Tween 20, and sucrose were obtained from Sigma-Aldrich, St Louis, MO, USA. N-succinimidyl S-acetylthioacetate (SATA) and sulfo-SMCC (sulfosuccinimidyl4-(N-maleimidomethyl) cyclohexane-1-carboxylate) were purchased from Thermo Fisher Scientific, Waltham, MA, USA.

Fabrication of artificial aerosolization chamber

For analysis of aerosols, a closed system maintaining a constant temperature and humidity is required.[1] We identified this closed system, which is connected to an aerosol loading system, as the artificial aerosolization chamber. The overall schematic representation of the artificial aerosolization chamber is shown in **Figure 1A** and **Figure S1A**. The chamber was constructed with acrylic plates. A pass box functioning in transport of materials during the experiment is additionally attached to this chamber body. The chamber also consists of a nebulizer and compressed air line for producing aerosol, as well as a vacuum pump and control box with a thermometer and a relative humidity (RH) meter connected to a heater and humidifier, respectively. All temperature and humidity settings are automatically adjusted by the control box. When the chamber temperature is lower than the set temperature, the heater turns on. When the humidity in the chamber is lower than the set humidity, the humidifier provides additional vapor. When the humidity is higher than the setting, compressed air is injected into the chamber. A vacuum pump is operated to maintain pressure in the artificial aerosolization chamber system during aerosol loading experiments. The overall system is fully closed, and loaded aerosols are uniformly circulated by fan. A UV lamp in the chamber is used for sterilization of tested virus particles. After 10 min of UV radiation, most bacteria and viruses are completely destroyed.[2] Eliminations of loaded MS2 virus in the chamber was confirmed by real-time PCR and ELISA tests (data not shown).

Air sampler design and simulation

The air sampler was designed as a portable instrument for on-site sampling and detection of airborne viruses. The compact-sized air sampler ($25 \times 13 \times 10$ cm^3) contains an electronic circuit

board, blow fan, mass flowmeter, and customized structure for the airborne pathogen sampling/monitoring kits (**Figure S1B**).

Three-dimensional solid modeling was conducted by AutoCAD software, and was imported into COMSOL Multiphysics numerical software, using the Finite Element Method. The total number of 240,378 tetrahedron meshes for each domain was empirically constructed using the Delaunary triangulation method. The simulation considered turbulent flow since the Reynold number belonged to a transitional stage of 1,500-3,500, but did not take into account the transient state, since we empirically discovered that flow streams reached a stable state in a short time.

MS2 virus sample preparation

Escherichia coli bacteriophage MS2 (ATCC 15597-B1) and *Escherichia coli* strain C-3000, (ATCC 15597 (*E. coli*)) were purchased from American Type Culture Collection (ATCC, Manassas, VA). MS2 was recovered in the solution phase and cultured in *E. coli* grown in specific medium made with a mixture of tryptone, yeast extract, sodium chloride, glucose, calcium chloride, thiamine, and different concentration of agars (0-1.5%), following the ATCC protocol. MS2 solution was injected with *E. coli* host cell solution in the log phase (after 6 h incubation) and incubated at 37°C for 24 h inside an incubator, with shaking at 200 rpm. The MS2 virus particles were separated from the solution by centrifugation at 1000 rpm for 25 min and filtration through a 0.22 μm syringe filter. The MS2 virus solution was stored at 4°C until use in experiments. For plaque-forming unit (PFU) counting, MS2 solutions were serially diluted 10-fold in liquid broth and counted by culture technique using a double layer of agar. Diluted MS2 solutions were plated

on the top 0.5% agar layer containing host *E. coli* cells. After a 24 h incubation, number of plaques in the medium were counted and used to calculate PFU concentration of MS2.

Preparation of avian influenza viruses (AIVs) and clinical samples

AIV H1N1 (A/NWS33/1933) was provided by Konkuk University. H1N1 was propagated in 10-day-old specific pathogen-free (SPF) embryonated eggs. After a 72h incubation, the allantoic egg fluid was harvested. Amount of virus in stock was titrated to $10^{8.167}$ EID_{50}/ml. Positive clinical samples were collected from 2-week-old SPF chickens challenged with 10^6 EID_{50}/ml of H9N2 (A/Korean Native Chicken/K040110/2010). Cloacal swab samples were collected at 3 dpc (days post challenge) and 5 dpc. Swab samples were examined using real-time reverse transcription polymerase chain reaction (rRT-PCR) directed at the M gene of AIV.[3] Avian paramyxovirus type 4 (APMV), Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) were propagated in 10-day-old SPF embryonated chicken eggs. All animal experiments performed in this study were supervised, reviewed, and approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University (permit number: KU17034).

Aerosol loading in the chamber

MS2 virus was diluted in 10 mM PBS to a final concentration to 10^9 PFU/mL. AIV H1N1 aerosol samples were prepared at a concentration of $10^{2.5}$ to 10^5 EID_{50}/mL. Cloacal swab samples were collected from chickens experimentally infected with H9N2 viruses. Samples were then diluted 50-fold in 10 mM PBS for aerosolization. They were titrated in the range of $10^{6.121}$ to $10^{4.782}$ EID/mL in sampling solution. Viral aerosols were generated with a commercially available aerosol generator (3-jet collision nebulizer, CN24, CH technologies), where the desired viral suspension

was nebulized into a myriad of droplet aerosols at an airflow of 5 L/min. The produced bioaerosols were directly imported into the chamber, which acted as a simulated bioaerosol environment. RH and temperature within the chamber were maintained within a range of 50-55 % and 25°C, respectively. The chamber was cleaned with UV treatment for 10 min between each nebulization event.

Aerosolized virus sampling

The effect of sampling flow rate and sampling time on virus aerosol sampling efficiency was studied using an MS2 viral suspension at a concentration of 10^9 PFU/mL in 10 mM PBS. The results were evaluated based on amount of MS2 viruses captured by the air sampler. The aerosolized MS2 collection was operated between 10 to 100 L/min of sampling flow rate with fixed sampling time (10 min) or was operated at 100 L/min of sampling flow rate for 10-40 min. Aerosolized AIV samples were collected at 75 L/min for 30 min.

For control tests, sedimentation collection of aerosolized MS2 viruses was tested by measuring integrated kit exposure to the outside environment of the chamber (zone 1) and inside the aerosolization chamber without MS2 virus (zone 2) and with MS2 virus (zone 4). Aerosol sampling was conducted at 100 L/min for 10 min in the absence (zone 3) and presence (zone 5) of aerosolized MS2 in the chamber.

Antibody-conjugated nanoprobe preparation and characterization

Synthesis of nanoprobes (NIR-to-NIR UCNPs) and conjugation of antibodies with nanoprobes was performed according to previous publications,[4] with slight modifications. Transmission electron microscopy (TEM) conducted with a Talos F200x instrument (FEI Co., USA) was used to

characterize nanoprobe morphologies at an accelerating voltage of 200 kV. A Zetasizer Nano ZS instrument (Malvern Co., UK) was used to determine size distributions and zeta potentials of the nanoprobes. The upconversion PL emission spectra were recorded using a flame spectrometer (Ocean Optics, Inc., USA) under external excitation at 980 nm with an infrared diode laser (Changchun New Industries Optoelectronics Tech. CO., China). An XRD-7000 diffractometer characterized XRD patterns of the nanoprobes.

Fabrication of the integrated sampling/monitoring kit

A conjugate pad was pre-treated with 2.0% (w/v) bovine serum albumin (BSA), 2.0% (w/v) Tween 20, 2.5% (w/v) sucrose, and 0.3% (w/v) PVP in distilled water. After drying the conjugated pad at 35°C in a vacuum oven, 20 μL of antibody-conjugated nanoprobes in 10 mM HEPES buffer was dropped onto the pre-treated conjugated pad. The sample pad (the sampling pad for aerosol), conjugate pad, nitrocellulose membrane, and absorbent pad were mounted, in that order, on a plastic adhesive backing pad with 2 mm overlap between adjacent pads. The test and control lines for the MS2 sampling/monitoring kit were separately generated by applying 1 mg/mL rabbit anti-MS2 IgG and 1 mg/mL goat anti-rabbit IgG, respectively. The lines for the AIV sampling/monitoring kit were separately generated by applying 1 mg/mL anti-AIV nucleoprotein IgG and 1 mg/mL goat anti-mouse IgG, respectively.

Assays using the fabricated sampling/monitoring kit

Loading buffer (lysis/transfer solution) was prepared by mixing one part of commercial lysis buffer (Median Diagnostic, Korea) with three parts of 10 mM HEPES buffer, and then adding 2% Tween 20 to the total volume. The MS2 stock solution was diluted in loading buffer to produce a final

concentration ranging from 10^9 to $10^{6.5}$ PFU/mL. The AIV H1N1 stock solution was diluted in loading buffer to produce a final concentration ranging from 10^6 to 10^2 PFU/mL. The NIR nanoprobe signal was read using the NIR-to-NIR LFA platform previously developed by our group, which is a portable NIR signal reader for the LFA kit (**Figure S1B**).[4] Diluted virus solution samples (100 μL) were injected into the sample pad and the NIR signal was analyzed after 20 min. After collecting the aerosolized virus samples, 100 μL of loading buffer was dropped onto the sampling pad. The MS2 viruses or nucleoproteins from AIVs were identified within 20 min using the NIR signal reader.

Reverse transcription quantitative real-time PCR (RT-qPCR) of MS2 virus

Primers for reverse transcription quantitative real-time PCR (RT-qPCR) were designed to target sequences on the MS2 complete genome from the NCBI database. The DNA sequence of the forward primer was 5′-TCGGTATACACCAAGACTCC–3′, and 5′-GCTATGTAGCGACCACTGTC–3′ for the reverse primer. A RT-qPCR mixture containing dNTPs, Taq polymerase, reverse transcriptase, and reaction buffer was made using the One-step RT qPCR SYBR Green with low ROX kit (Enzynomics, Daejeon, South Korea). The reaction solution contained forward and reverse primer (100 nM each), 1X commercial RT-qPCR reaction mix, 1X enzyme mix, and 2 μL of purified MS2 RNA from aerosol sampling. The thermal profile used was: 50°C hold for 30 min; 95°C for 10 min; and 40 cycles of 95°C for 5 s and 60°C for 30 s. MS2 viruses harvested from cells were separated by centrifugation and serial diluted in PBS to a concentration between 10^{10} and 10^4 PFU. RNA of MS2 viruses in each solution was extracted

using the Qiagen viral RNA extraction mini prep kit (Qiagen, Valencia, CA). RNA samples from all solutions containing MS2 virus were analyzed by RT-qPCR and real-time curves were obtained for concentrations ranging from 10^4 to 10^{10} PFU. MS2 RNA was quantified using the same PCR primers. All real-time PCR reactions were run on a CFX96 real-time PCR instrument (Bio-Rad) and RNA was quantified by determining the relationship between RNA quantity and Cq value using CFX96 software (**Fig. S4**).

Enzyme-linked immunosorbent assay (ELISA) for MS2 detection

Wells of a 96-well microplate were coated with monoclonal anti-MS2 IgG to a concentration of 5 μg/mL in PBS buffer at 4°C overnight. Wells were blocked with 5% skim milk in PBST (blocking/dilution buffer) for one hour at room temperature under shaking conditions. After washing wells four times with PBST, serial dilutions of MS2 in blocking/dilution buffer were added to wells and incubated for one hour. Wells were washed four times with PBST and blotted on a paper towel to remove excess buffer. A detector antibody, polyclonal anti-MS2 IgG, was added to each well at a concentration of 5 μg/mL, and plates were incubated for one hour. Horseradish peroxidase-conjugated anti-rabbit IgG was added to each well and plates were incubated for one hour, followed by four washes in PBST. ABST peroxidase substrate was added to each well and incubated for 30 min. The optical density at 405 nm was read using a microplate reader.

Preparation of dried viruses in the pad and recovery test

MS2 virus solution (10^9 PFU/mL, 50 μL) was dropped onto the sample pad and dried at 35°C for 30 min. An LFA test was then performed using the dipstick method. The sample pad containing

dried MS2 was assembled to the LFA strip and then dipped into loading buffer containing nanoprobes for 20 min. For the solution sample test using LFA, the strip without the sample pad was dipped into the loading buffer containing MS2 viruses and nanoprobes. For comparison to ELISA, dried MS2 in the pad was eluted in 10 mM PBS buffer for 20 min, and amount of MS2 in the eluate was quantified. The recovery rate (%) was calculated as (amount of MS2 virus recovered from the sample pad / amount of MS2 virus in the solution) × 100

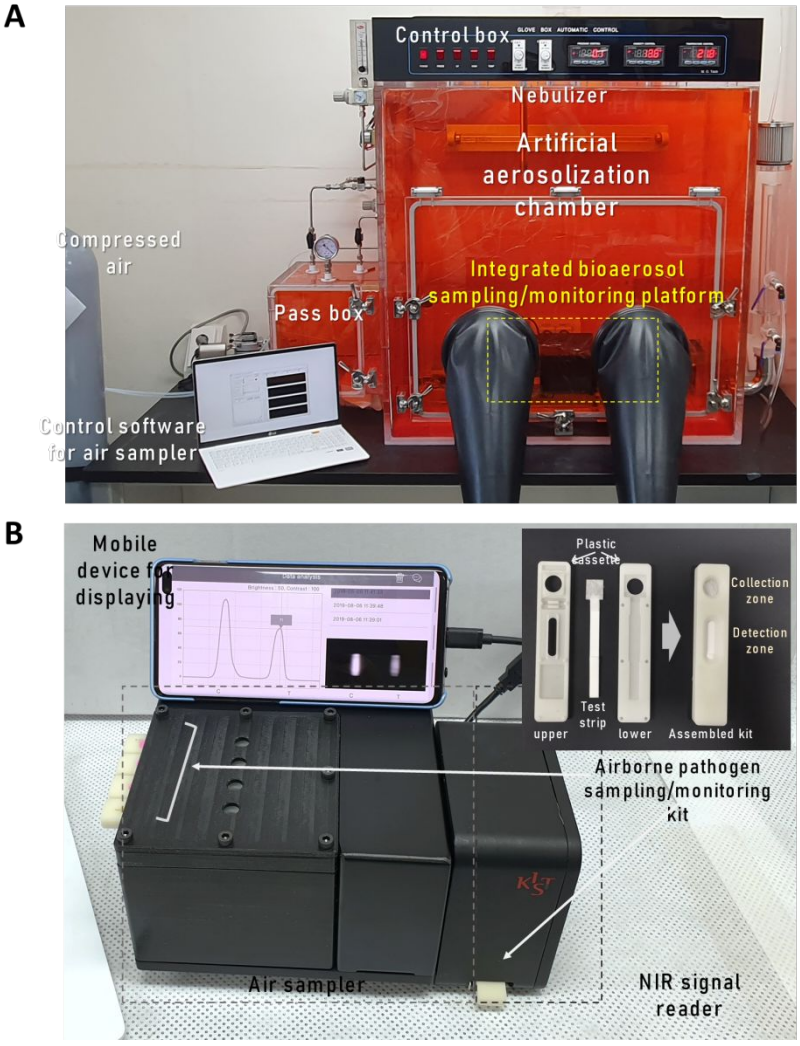


Figure S1. Digital images of the artificial aerosolization chamber and the integrated bioaerosol sampling/monitoring platform. (**A**) The artificial aerosolization chamber consists of a nebulizer and compressed air line for producing aerosol, and a control box with a thermometer and a relative

humidity meter. The integrated bioaerosol sampling/monitoring platform was placed inside the chamber and operated by an external control software via Bluetooth communication. (**B**) Integrated bioaerosol sampling/monitoring platform contains a compact size air sampler and a portable NIR signal reader, which connected to a mobile device with an application installed to display a NIR emission image of LFA. The airborne pathogen sampling/monitoring kits (inserted image) are placed in the air sampler during aerosol sampling, target transfer, and target detection processes, and then they transferred to the NIR signal reader.

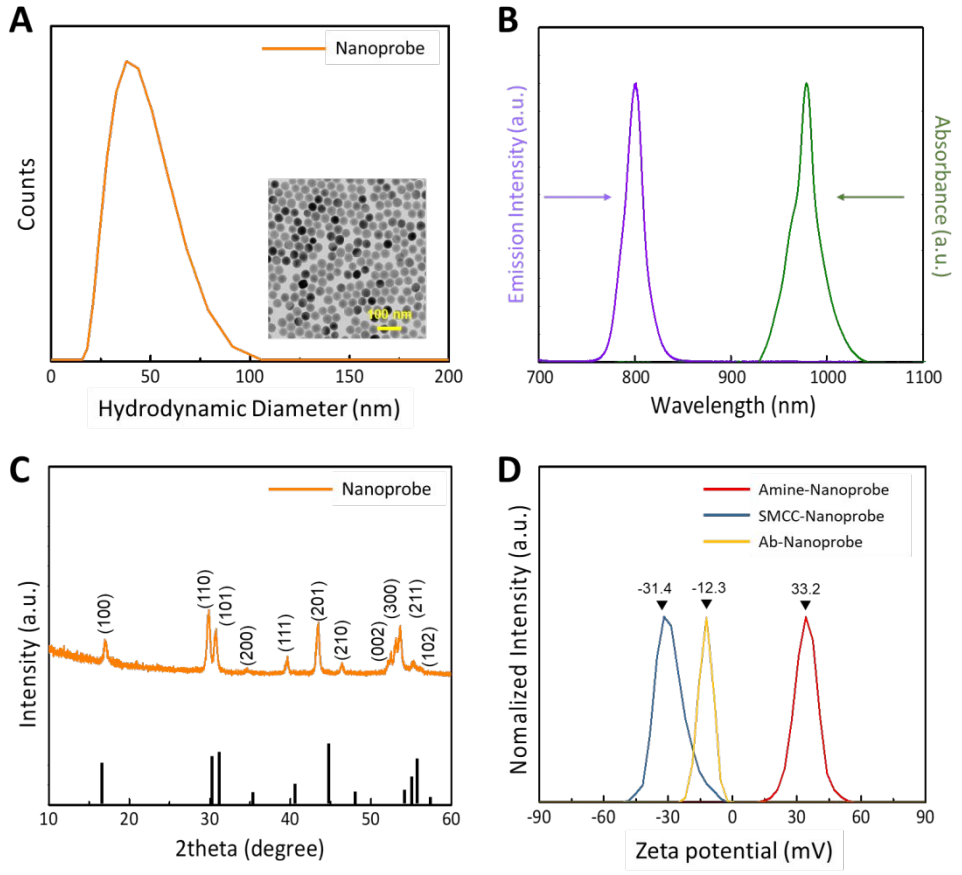


Figure S2. Characterization of nanoprobes incorporated into the integrated sampling/monitoring kit for airborne virus detection. (**A**) Particle size distributions by dynamic light scattering analysis (insert: TEM image), (**B**) NIR emission and absorbance spectrum of nanoprobes, (**C**) X-ray diffraction pattern of nanoprobes, and (**D**) Zeta-potentials of amine-nanoprobes, SMCC-nanoprobes, and antibody (Ab)-conjugated nanoprobes.

Characterization of nanoprobes: Transmission electron microscopy (TEM) images revealed that core/shell UCNPs (NaYF4: Yb, Tm@NaYF4) were monodispersed with an average diameter of about 30 nm. (**Fig. S2A**, inset). The absorbance (excitation) spectra at 980 nm and the emission spectra at 800 nm of UCNPs were shown in **Fig. S2B**. X-ray diffraction (XRD) patterns of the UCNPs agreed with the pattern of hexagonal beta-NaYF4 (JCPDS: 16-0334), which is a better upconverting host lattice structure than cubic alpha-NAYF4 due to the low phonon energy of the crystal lattice (**Fig. S2C**). Conjugation of antibodies with nanoprobes were performed via a reaction between sulfo-SMCC-treated nanoprobe and thiolated antibodies (**Fig. S2D**). An abrupt decrease in zeta potential was observed from 33.2 mV to -31.4 mV after the sulfo-SMCC modification. The negative zeta potential could be attributed to the reaction between the amine-terminal of NH2-nanoprobes and the NHS of SMCC. After antibody conjugation, the zeta potential increased from -31.4 mV to -12.3 mV, which indicated successful conjugation of antibodies with sulfo-SMCC-treated UCNPs [4].

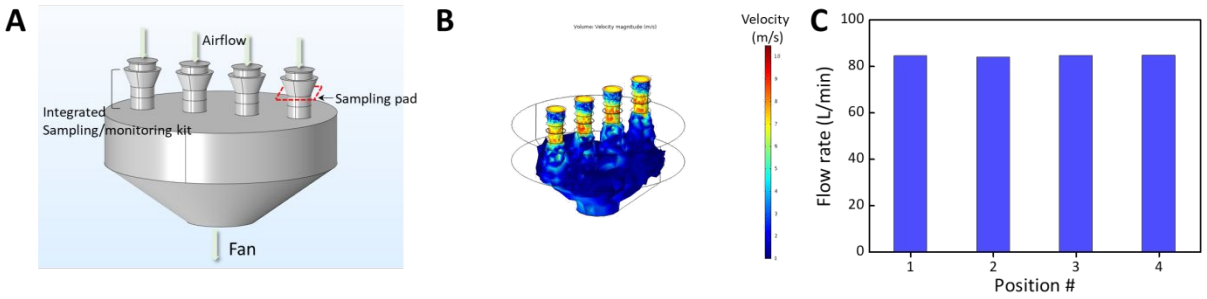
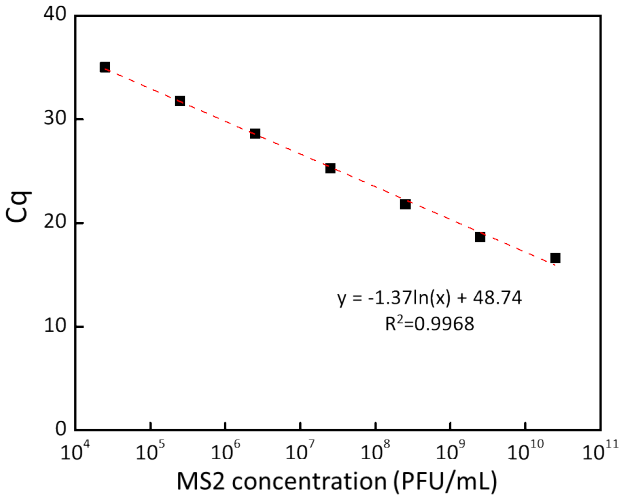


Figure S3. Computational fluid dynamics analysis for air flow velocity around and inside the air sampler. (**A**) Structure of the designed air sampler, (**B**) Air flow field, and (**C**) Calculated air flow rate at each of four collection zones at an operating flow rate of 100 L/min.



Test	Cq	PFU/mL
Negative	35.64	1.4 E+04
10^4	35.03	2.2 E+04
10^5	31.76	2.4 E+05
10^6	28.65	2.3 E+06
10^7	25.29	2.7 E+07
10^8	21.80	3.5 E+08
10^9	18.64	3.5 E+09
10^{10}	16.61	1.5 E+10

Figure S4. PCR calibration data for MS2 virus detection.

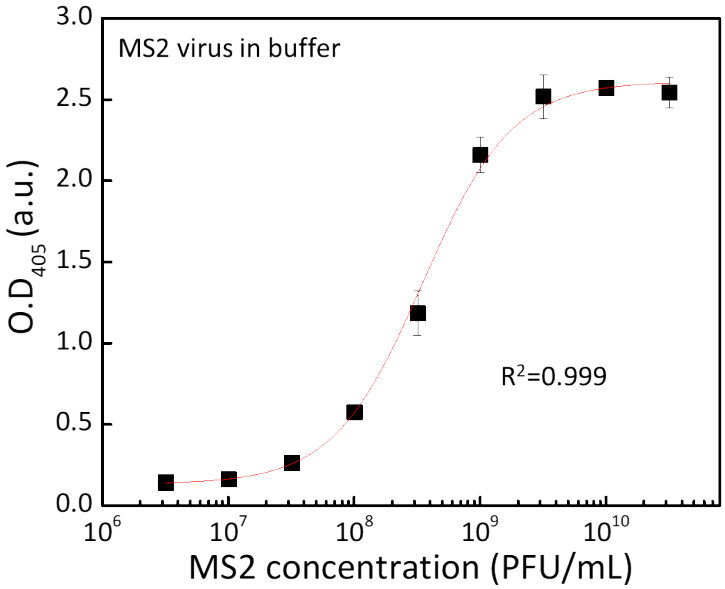
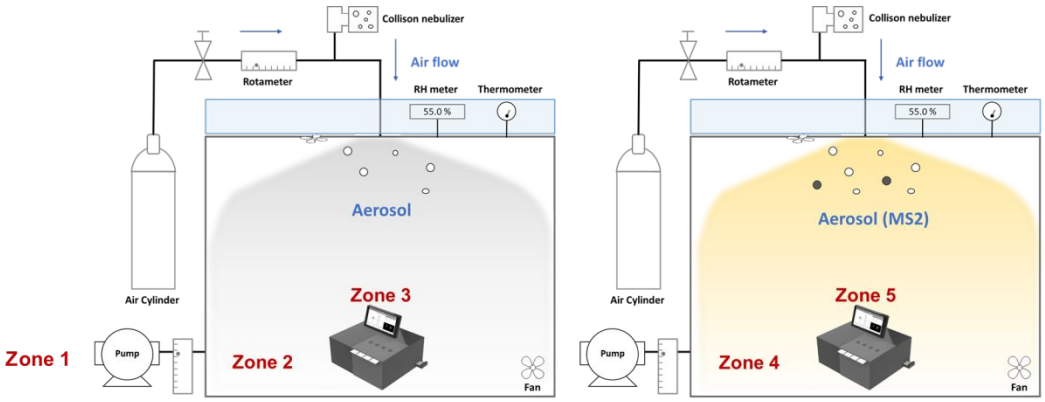


Figure S5. Calibration curve for detection of MS2 virus with the commercial ELISA method.



	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Chamber	Outside	Inside	Inside	Inside	Inside
MS2 virus	Absence	Absence	Absence	Presence	Presence
Sampler	Outside	Outside	Inside	Outside	Inside
	T C	T C	T C	T C	T C
NIR signal					

Figure S6. Control tests of the integrated sampling/monitoring platform for detection of MS2 virus under different artificial aerosolization environments. T and C mean test line and control line on the detection zone of the LFA strip, respectively.

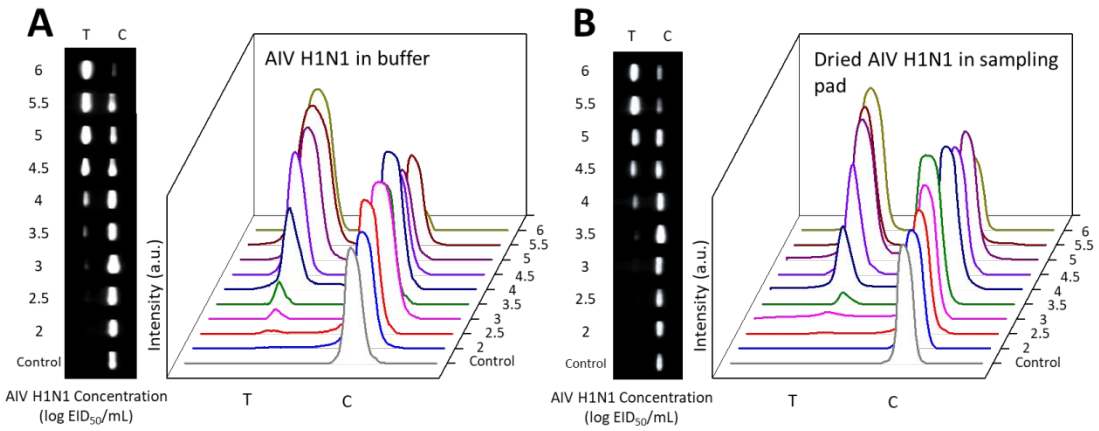


Figure S7. Detection of avian influenza virus (AIV) H1N1 in buffer (**A**) and dried AIV H1N1 in sampling pad (**B**) using the integrated sampling/monitoring kit.

Reference

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