Supplementary Information

Supplementary Methods and Materials

Bacterial cell density estimation

Total bacterial counts were performed using epifluoresent microscopy. 10mL of impinger liquid from each sample was used for enumeration of bacterial cells. Cells were fixed with 2% paraformaldehyde and stained with DAPI (4'6- diamidino-2-phenylindole) at a final concentration of 1µg/mL for 30 minutes at room temperature. The stained samples were filtered on 25mm black polycarbonate filters (Whatman, Maidstone, Kent, UK) with a pore size of 0.2µm. Microscopy analyses were performed on a Zeiss Axiophot 2 fluorescence microscope at 1000x magnification using a 0.1mm square counting grid. For each sample, cells were enumerated in thirty grids evenly spaced on the filter. Bacteria were discriminated from other stained particles by shape and size, and bacteria in aggregates were enumerated when possible. The average number of cells per grid was used to calculate the number of cells on the entire filter, which was the number of cells in 10mL of impinger liquid. From this, the total number of cells collected by each impinger was determined. The total cell number was divided by the total volume of air collected by each impinger during sampling to calculate the concentration (density) of airborne bacterial cells.

DNA extraction

30mL of impinger liquid from each sample was vacuum filtered onto sterile 0.2µM polycarbonate filters (47mm) and transferred to 0.70mm garnet bead tubes (MoBio Laboratories, Carlsbad, CA). Filters were incubated with 500µL of Zhou Buffer (100µM Tris HCl/100µM NaEDTA/100mM Na_2HPO_4/1.5M NaCl/1% CTAB) and 15µL lysozyme

(170mg/mL) for 30 min at 37˚C, 225 r.p.m. 25µL proteinase K (40mg/mL) and 8.25µL 20% SDS were added to each tube and samples were incubated for an additional 30 min at 37˚C, 225 r.p.m. 100µL 20% SDS were added to each tube and samples were incubated at 65˚C for 10 min. Samples were then subjected to two freeze-thaw cycles between liquid N_2 and a 65˚C water bath to complete cell lysis. DNA was extracted from solution by a modified phenol/chloroform/isoamyl alcohol method. 500µL phenol/chloroform/isoamyl alcohol (25:24:1) were added to solution, tubes were bead beaten for 2 minutes, centrifuged at 10,000 x g for 5 minutes at 4˚C, and the supernatant was transferred to a new tube. Samples were washed twice by adding 1mL phenol/chloroform/isoamyl alcohol, mixing by inversion, centrifuging at 10,000 x g for 5 minutes at 4˚C and transferring the supernatant to a new tube. Samples were washed twice more with 1mL chloroform/isoamyl alcohol using the same protocol. Two volumes cold 100% ethanol were added to the supernatant of the final wash, mixed by inversion and stored at -20˚C overnight. The following day samples were centrifuged for 30 minutes at 16,000 x g, 4˚C and liquid was removed. 1mL 70% ethanol was added to each sample, which were then centrifuged for 30 minutes at 16,000 x g, 4˚C. Liquid was removed and samples were air-dried in a laminar flow hood for 30 minutes. After resuspending in 200µL sterile water, extractions were cleaned using the Genomic DNA Clean and Concentrator kit (Zymo Research, Orange, CA) following manufacturer's protocols with the following modifications: binding buffer was added at 2:1 ratio and run through column twice, and DNA was eluted in 2 additions of 20µL warm sterile water.

Bacterial 16S gene amplification

Bacterial 16S gene fragments were amplified using universal bacterial 16S primers 27F and 338R modified for use with the GS FLX Titanium platform (454 Life Sciences, Branford, CT). Forward primers were labeled with a sample-specific barcode (17), while all samples were amplified with a common reverse primer. 25μL PCR reactions consisted of 12.5μL Failsafe PCR 2x Premix E (Epicenter; Interscience, Markham, Ontario, Canada), 1μL each of forward and reverse primers (20 μM), 0.5 μL (2.5U) Platinum Taq (Invitrogen), and 10μL DNA template. PCR conditions were as follows: initial denaturation at 94˚ for 3 min, 25 cycles of 94˚C for 45 sec, 50˚ for 1 min, and 72˚ for 1 min, and a final elongation at 72˚ for 7 min. Samples were amplified in duplicate and a negative control run for each reaction. Negative controls included both a no template control (to rule out PCR contamination) and an extraction control where a filter with sterile water passed through it was treated like the rest of the samples through the DNA extraction process and PCR steps (to rule out contamination during DNA extraction). PCR products of all samples were pooled and cleaned with the Ultra Clean PCR Clean-Up Kit (MoBio Laboratories, Carlsbad, CA) according to manufacturer's protocol. Flow-through was saved and run through an additional spin column to ensure all DNA was captured. DNA from both columns was combined for a final library concentration of 18.75ng/mL. A 1.5μg library was submitted to the EnGenCore sequencing facility (Colombia, SC) for pyrosequencing with the Lib-L unidirectional kit (454 Life Sciences, Branford, CT).

Architectural description of study site

Building 97 at Providence Milwaukie Hospital was selected for this study, in part, because it has two critical attributes: individually controllable heating, ventilating, and air conditioning (HVAC) zones, and operable windows. It is common in older hospital

buildings with operable windows to have mechanical system control only at the scale of the whole building or by floor, but not by individual rooms or small groups of rooms. Newer hospital buildings with more sophisticated HVAC control systems often have patient rooms without operable windows. We sampled from patient rooms selected because they were served by the same air handler, shared the same orientation of operable windows, were of similar size, had a similar furniture layout, and did not interfere with daily operations of the hospital or its staff (Figure S1).

Figure S2 shows the typical patient room layout for both window and mechanically ventilated rooms and can be referred to for the following descriptions of the window and mechanical ventilation cases. Figure S3 diagrams airflow in the window ventilation case. In this case, ventilation air is supplied directly from the outside through a window and removed through the HVAC return air system, bathroom exhaust system, and at times through the window. Two ceiling registers exhaust the room: a larger return register near the door to the corridor and a second exhaust register in the bathroom, the door to which was left open for all cases. The window aperture, depending on interior and exterior air movement, also acted as an exhaust location at times. A 1500W thermostatically controlled portable oil reservoir electric resistance heater was deployed in these rooms to maintain thermal conditions comparable to the mechanically supplied cases. The heaters were placed adjacent to the windows.

The HVAC system continued to supply air to the corridor and other rooms as it would normally, however, doorways were sealed to prevent air movement between the patient room and adjacent hallways. The HVAC supply, which normally serves the room, was diverted out the window and away from the opening by means of a temporary flexible

duct. Figure S4 shows the outside wall of the rooms used for testing. One operable window is shown open with a flexible duct diverting the HVAC supply air out of the building. This strategy was used as opposed to simply sealing off the supply register because obstructing this air path would change the balance of the HVAC system which was also serving other rooms, some of which were being sampled simultaneously under normal mechanical ventilation. Diverting the HVAC supply air in the window ventilated rooms allowed for this simultaneous sampling.

Figure S5 diagrams airflow in the mechanical ventilation cases. In these cases the building HVAC system was allowed to operate normally so that ventilation air was supplied by the HVAC system and removed by the room's return air system and the bathroom exhaust. The operable windows remained closed, and as in the window ventilated cases, doorways were sealed. Mechanical air supply to the room occurred at the ceiling plane near the window through a linear ceiling diffuser.

Both supply and return are connected via ducts to a roof top HVAC unit where air is exchanged with outdoor air. Figure S6 is a diagram of the roof top air-handling unit. The configuration shown was used during testing. Shown diagrammatically along the upper horizontal is the return air path. Reading right to left across the return air path: air leaves the room, it is driven by a fan that is on and drawing a certain amount of power, and 100% of the air is exhausted to the outside. The short vertical connector between the two horizontals is shown closed, and denotes there is no mixing of exhaust air with supply air, though that potential exists in the system. The lower horizontal diagrammatically represents the air path of supply air into the building. Reading left to right across the supply air path: air is brought into the roof top unit, it passes through a prefilter, and then

the air passes by a coil to be heated or cooled if needed. The air is driven by a fan through a final filter, and then travels through long duct runs that are divided, turn corners, and change size. The air ultimately reaches the room and returns again to the outside through the exhaust air path described above.

Outdoor samples were collected immediately adjacent to the air intake for the building's HVAC system (Figure S7). A tent was erected to prevent rain entering the samplers.

Environmental measurements

Air changes per hour were calculated for patient rooms taking into account room volume, and air speed and volume flowing into the room through the window (window ventilated rooms) or diffuser (mechanically ventilated rooms). Figure S8 shows the experimental setup for one of the patient rooms. For the window supply condition one anemometer was placed over the bed near the air samplers while two others were at the quarter points of the window next to the telltales. A grid of telltales (small flags that flutter with air movement) was placed within the frame of the open window and video-recorded to identify airflow direction through the window over the entire opening, as it is possible for air to flow in and out of the window simultaneously. For every one-minute period, it was determined whether airflow direction was into or out of the room for both the upper and lower halves of the opening to allow for calculation of airflow rate into the room.

For the mechanical supply condition one anemometer was placed over the bed near the air samplers while another was in the flow just below the mechanical supply. Figure S9 illustrates the anemometer on a stand, its sensor placed below the linear ceiling diffuser in a mechanically ventilated room. This location was sampled throughout the test and a

separate airspeed profile test was later referenced to determine air speed and volume into the room through the diffuser. Due to variations in flow of the mechanical slot diffuser, prior to the testing in the room the velocity at 24 locations along the slot diffuser was taken to develop the volumetric flow given a single velocity measurement.

Supplementary Tables

Table S1. Metadata on time and location of sampling and airborne bacterial cell density in samples from Providence Milwaukie Hospital.

Sample	Environment	Room	Collection time	Collection date	Bacterial cell density (cells/m3)
M1	Indoor - Mechanical	229	11:30	02/27/10	1420000
M2	Indoor - Mechanical	229	13:00	02/27/10	937000
M3	Indoor - Mechanical	231	16:00	02/27/10	607000
M4	Indoor - Mechanical	231	17:00	02/27/10	1200000
M5	Indoor - Mechanical	235	12:20	02/28/10	2580000
N1	Indoor - Window	235	11:30	02/27/10	723000
N3	Indoor - Window	229	16:00	02/27/10	867000
N4	Indoor - Window	229	17:00	02/27/10	625000
N5	Indoor - Window	231	12:20	02/28/10	969000
O1	Outdoor	Roof	11:30	02/27/10	572000
O2	Outdoor	Roof	13:00	02/27/10	607000
O3	Outdoor	Roof	16:00	02/27/10	502000
O5	Outdoor	Roof	12:20	02/28/10	2050000

Supplementary Figure Legends

Figure S1. Floor plan of Providence Milwaukie Hospital indicating patient rooms selected for microbial community sampling.

Figure S2. Diagram of typical patient room layout and location of sampling equipment at Providence Milwaukie Hospital.

Figure S3. Supply flow diagram for window ventilated rooms at Providence Milwaukie Hospital selected for microbial community sampling.

Figure S4. Exterior view of Providence Milwaukie Hospital.

Figure S5. Supply flow diagram for mechanically ventilated rooms at Providence Milwaukie Hospital selected for microbial community sampling.

Figure S6. Air handling diagram for patient rooms at Providence Milwaukie Hospital.

Figure S7. Outdoor microbial community sampling equipment setup on roof of Providence Milwaukie Hospital near building air intake.

Figure S8. Window ventilated patient room with sampling equipment.

Figure S9. Supply airflow measurement equipment in a mechanically ventilated patient room.

Supplementary Figures

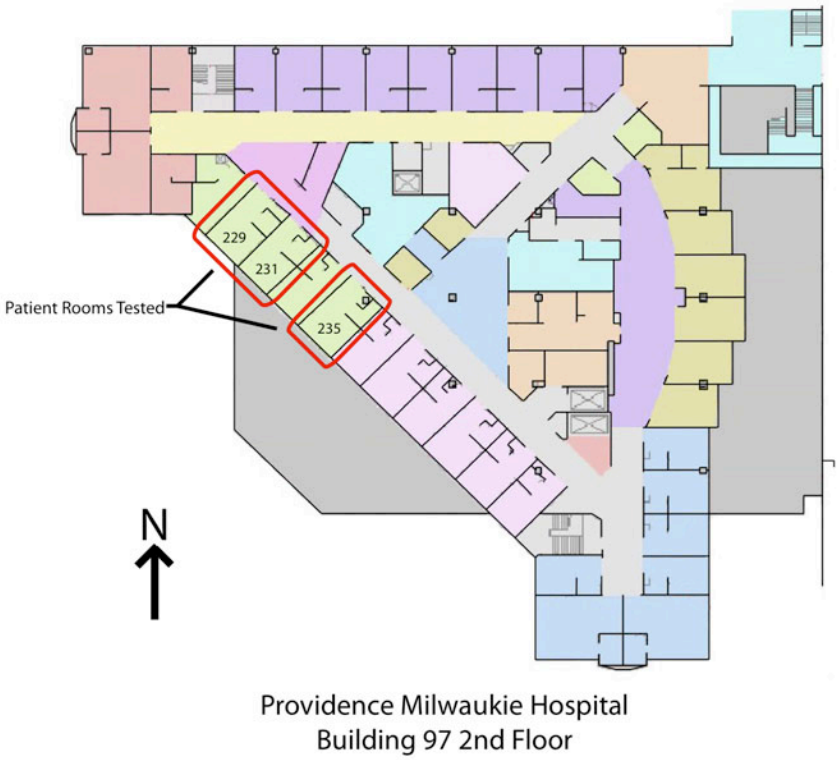

Figure S1

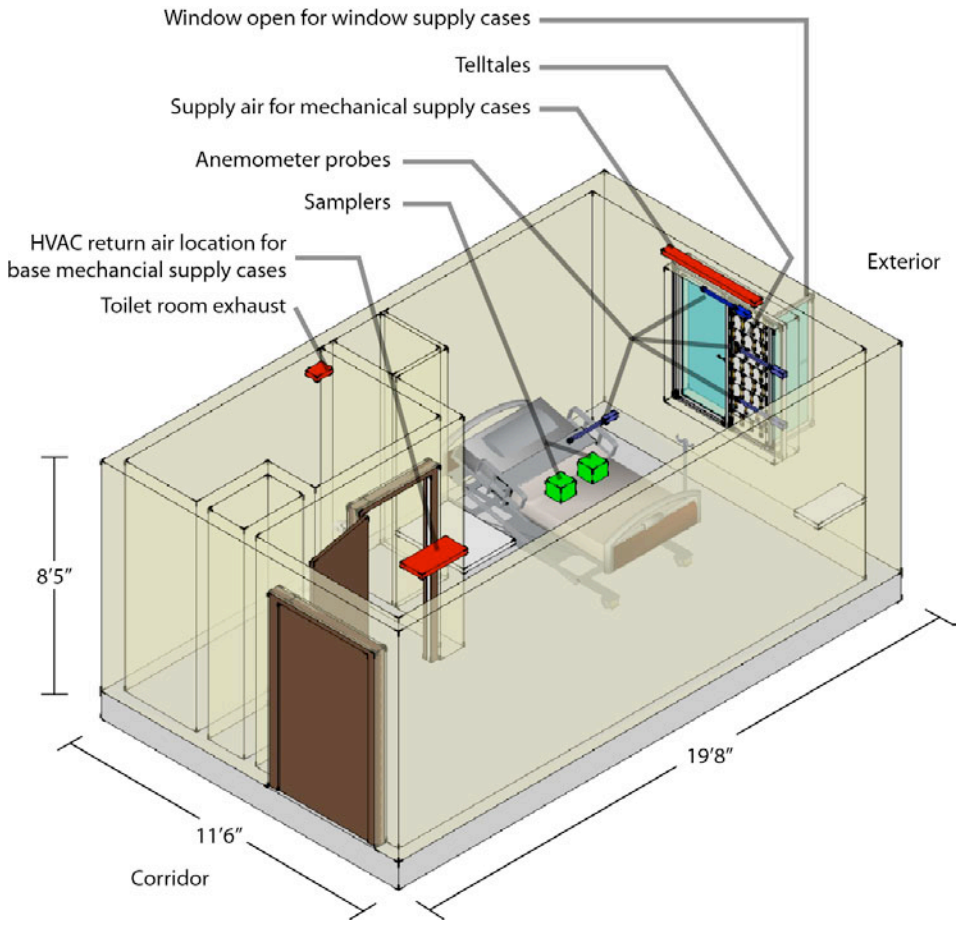
Window open for window supply cases
Telltales
Supply air for mechanical supply cases
Anemometer probes
Samplers
HVAC return air location for base mechancial supply cases
Toilet room exhaust
Exterior
8′5″
19′8″
11′6″
Corridor

Figure S2

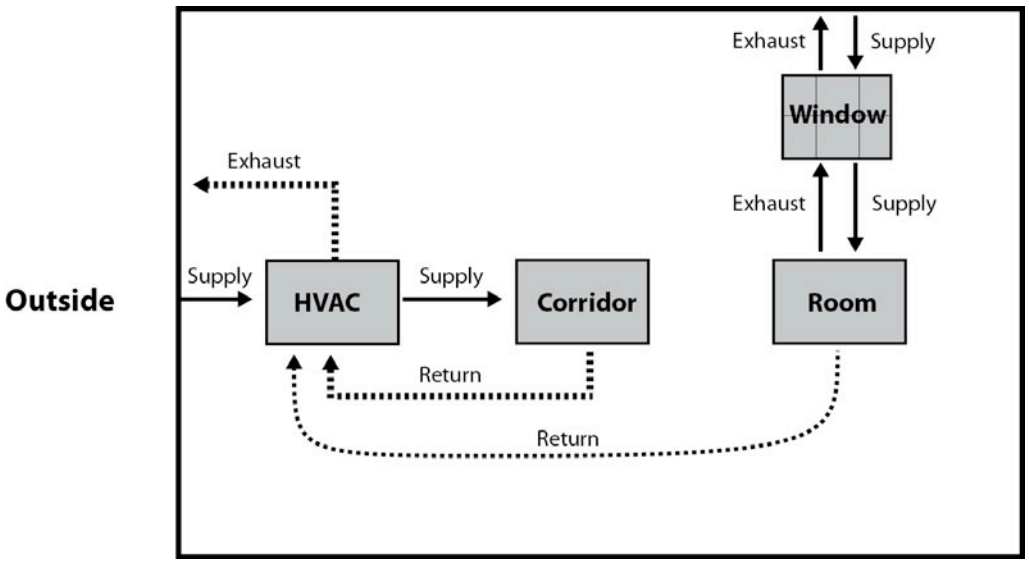
Outside
Exhaust
Supply
Window
Exhaust
Supply
Exhaust
Supply
HVAC
Supply
Corridor
Room
Return
Return

Figure S3

Figure S4

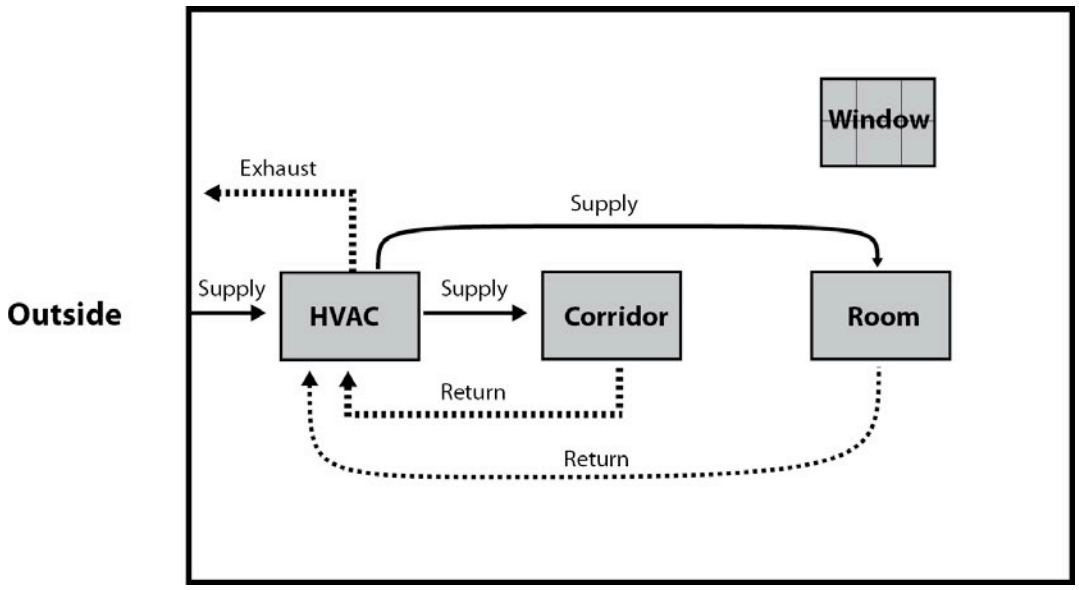
Window
Exhaust
Supply
Outside
Supply
HVAC
Supply
Corridor
Room
Return
Return

Figure S5

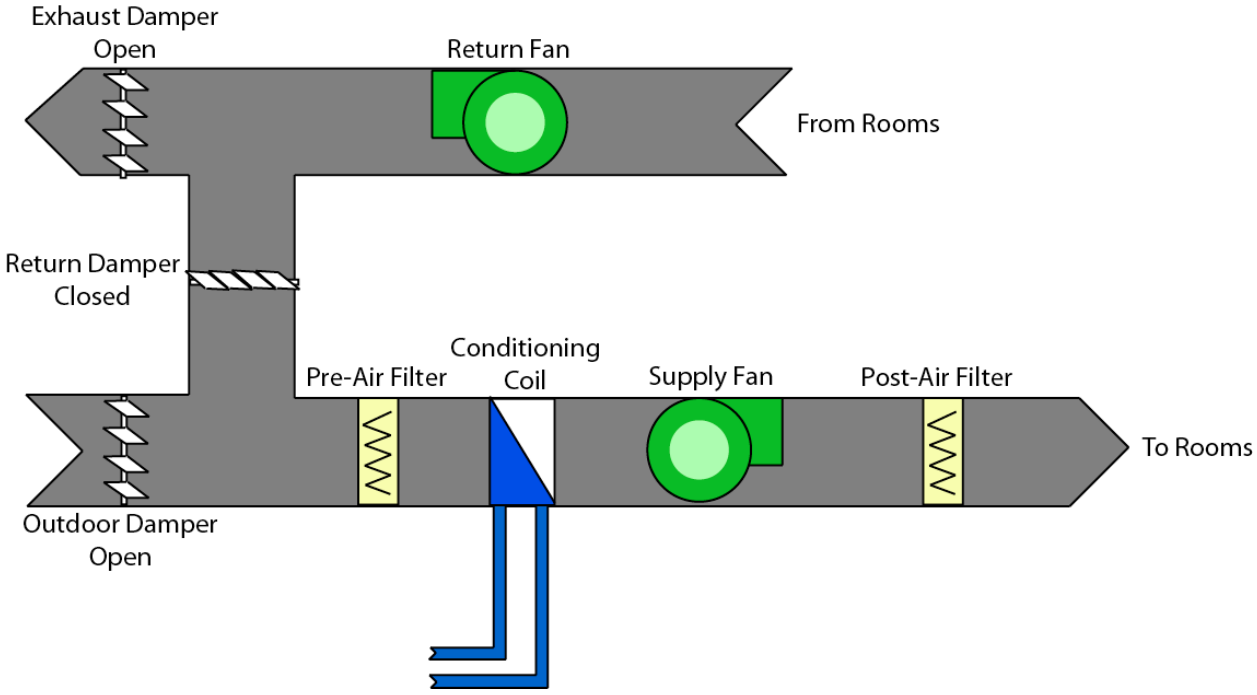
Exhaust Damper
Open
Return Fan
From Rooms
Return Damper
Closed
Conditioning
Coil
Pre-Air Filter
Supply Fan
Post-Air Filter
To Rooms
Outdoor Damper
Open

Figure S6

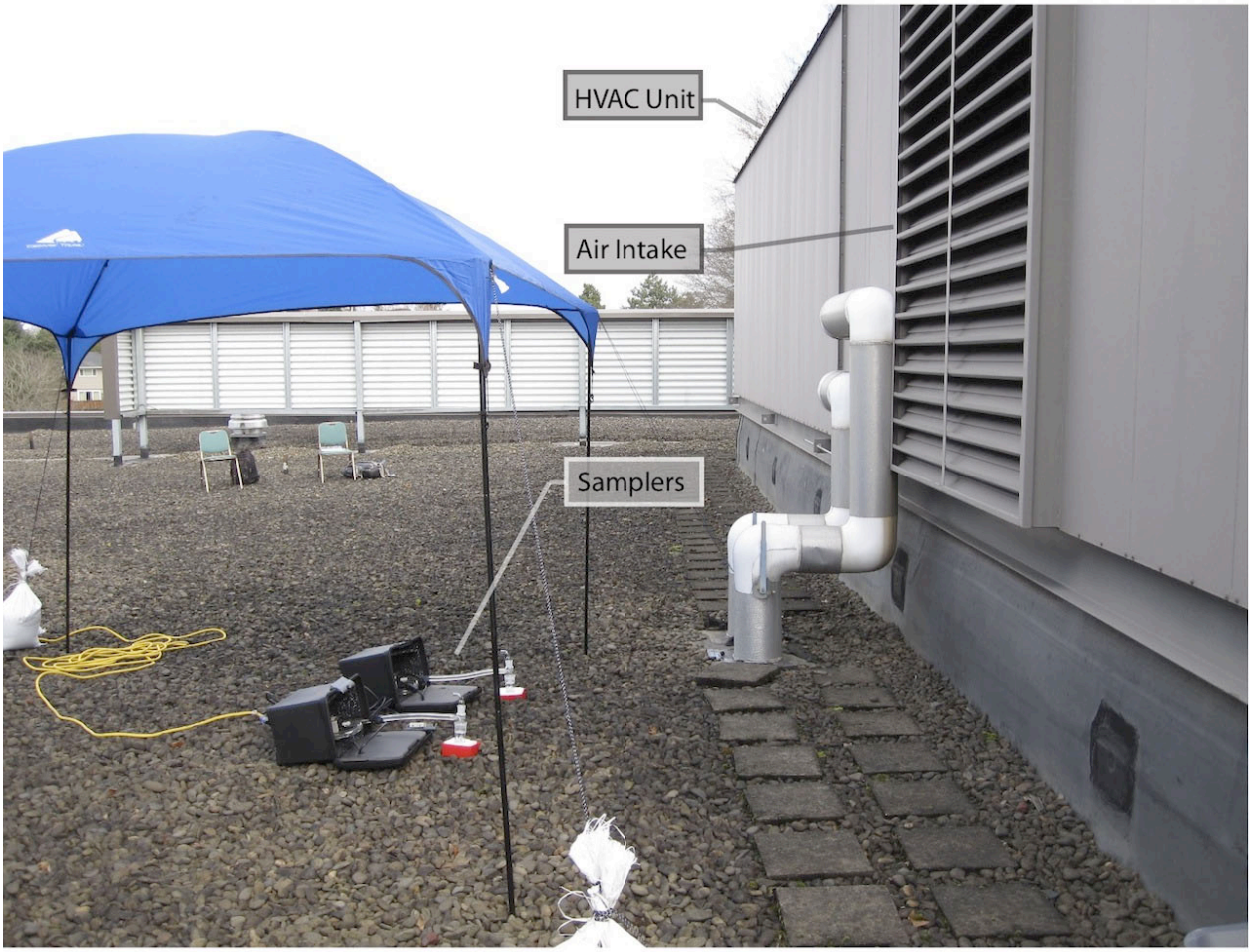

Figure S7

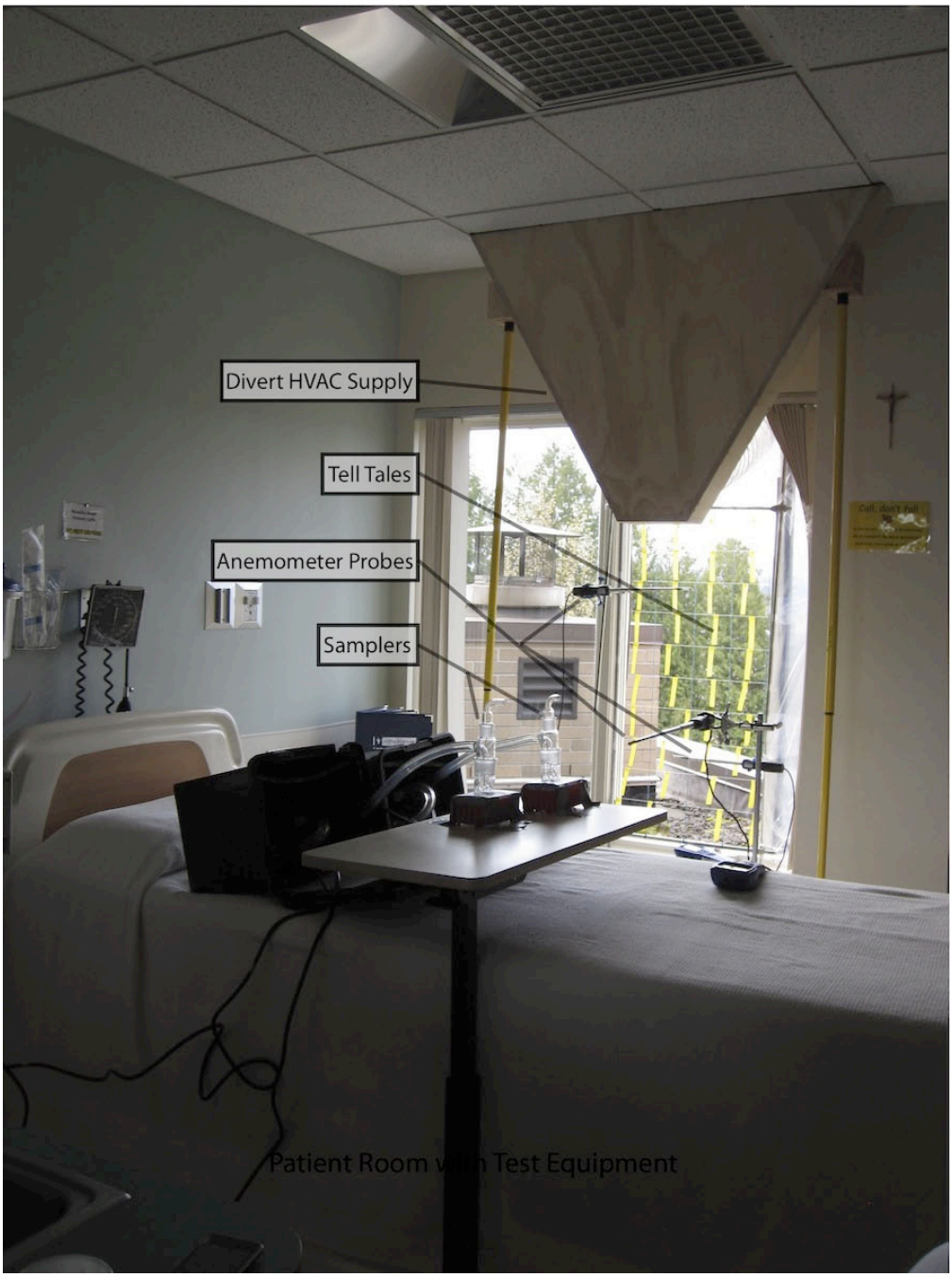
Divert HVAC Supply
Tell Tales
Anemometer Probes
Samplers
Patient Room with Test Equipment

Figure S8

Figure S9