Received: 29 April 2018 | Revised: 17 August 2018 | Accepted: 3 September 2018

DOI: 10.1111/ina.12506

ORIGINAL ARTICLE

WILEY

Impact of air-handling system exhaust failure on dissemination pattern of simulant pathogen particles in a clinical biocontainment unit

Jennifer Therkorn[1] | David Drewry III[1] | Thomas Pilholski[1] | Kathryn Shaw-Saliba[2,3] | Gregory Bova[4] | Lisa L. Maragakis[2,3,4] | Brian Garibaldi[2] | Lauren Sauer[2,3]

[1]Applied Biological Sciences, Johns Hopkins Applied Physics Laboratory, Laurel, Maryland

[2]Johns Hopkins University School of Medicine, Baltimore, Maryland

[3]Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland

[4]Johns Hopkins Health System, Baltimore, Maryland

Correspondence
Jennifer Therkorn, Johns Hopkins Applied Physics Laboratory, Laurel, MD.
Email: Jennifer.Therkorn@jhuapl.edu

Funding information
The National Health Mission Area at the Johns Hopkins University Applied Physics Laboratory

Abstract

Biocontainment units (BCUs) are facilities used to care for patients with highly infectious diseases. However, there is limited guidance on BCU protocols and design. This study presents the first investigation of how HVAC (heating, ventilation, air-conditioning) operating conditions influence the dissemination of fluorescent tracer particles released in a BCU. Test conditions included normal HVAC operation and exhaust failure resulting in loss of negative pressure. A suspension of optical brightener powder and water was nebulized to produce fluorescent particles simulating droplet nuclei (0.5-5 μm). Airborne particle number concentrations were monitored by Instantaneous Biological Analyzers and Collectors (FLIR Systems). During normal HVAC operation, fluorescent tracer particles were contained in the isolation room (average concentration = $1 \times 10^4 \pm 3 \times 10^3/L_{air}$). Under exhaust failure, the automated HVAC system maximizes airflow into areas adjacent to isolation rooms to attempt to maintain negative pressure differential. However, 6% of the fluorescent particles were transported through cracks around doors/door handles out of the isolation room via airflow alone and not by movement of personnel or doors. Overall, this study provides a systematic method for evaluating capabilities to contain aerosolized particles during various HVAC scenarios. Recommendations are provided to improve situation-specific BCU safety.

KEYWORDS

air-conditioning, clinical biocontainment unit, exhaust failure, fluorescent aerosol tracer, heating, isolation unit, pathogen transmission, ventilation

1 | INTRODUCTION

Even prior to the understanding of the infectious agent transport pathway(s), it was understood that the air of the indoor environment and the spread of infectious diseases were linked.[1] The concept of ventilation was first introduced to dilute the indoor pollutants produced by humans, and to provide comfort.[1] Following the 2003 SARS (severe acute respiratory syndrome) epidemic, a systematic literature review confirmed that there is an association between the ventilation system of the built environment and the potential for airborne dissemination of infectious pathogens.[2] Furthermore, in the face of increasing globalization, interconnected world travel, and climate change, the risk of infectious disease transmission is presently increasing, including emerging pathogens that cause severe respiratory syndromes, such as MERS-CoV (Middle East Respiratory Syndrome Coronavirus).[3-7] Thus, characterizing the role of indoor air

ventilation in the spread of airborne pathogens is of utmost importance for protecting the public's health.

Air ventilation in healthcare environments presents unique challenges due to the collocation of patients, visitors, and healthcare workers because some of them may be infectious and/or immunocompromised. Highly infectious diseases (HIDs) are defined as viral and bacterial infections that are easily transmissible, cause serious life-threatening illness, have no or few treatment options, and pose a threat to the general public.[8] Patients suspected or confirmed to be infected by HIDs require care in isolation rooms or specialized biocontainment areas in hospitals to prevent exposure of healthcare workers, visitors, and other patients to the person with the HID.[9] Standard and transmission-based isolation precautions to prevent contact with the patient and their droplet secretions (eg, mucus from sneezing or coughing) include the use of personal protective equipment (PPE), such as gloves, masks, gowns, and eye protection.[10,11] In addition to the use of PPE, airborne transmission-based precautions include special air-handling and ventilation systems, such as negative-pressure airborne infection isolation rooms (AIIRs), to prevent the dissemination of airborne droplet nuclei or small particles to areas where noninfected individuals are present, and to remove airborne pathogens by air filtration.[10,11]

When a patient infected with a respiratory pathogen coughs or sneezes, thousands of droplets are expelled, ranging in size from ~0.05 to 500 μm; the majority of these particles range in size from 10 to 100 μm.[12,13] The smaller range of droplets can rapidly desiccate to form droplet nuclei (ie, particles ≤5 μm in diameter) that can contain infectious pathogens.[11-14] Droplet nuclei are assumed to have such low settling velocities that this results in extended airborne suspension times, on the order of hours, resulting in particle transport over long distances (eg, >1 m).[12,15] This long-distance aerosol dissemination is mainly determined by airflow patterns in the built environment as generated by ventilation systems and movement of personnel.[15] Previous research has illustrated the potential for widespread airborne dissemination within a hospital driven by the building's air ventilation systems, including nosocomial outbreaks of MERS and SARS.[16,17]

One type of specialized healthcare environment, a clinical biocontainment unit (BCU), is designed to care for patients infected with HIDs and incorporates specialized environmental controls and isolation protocols to prevent pathogen transmission. In response to the 2014-2015 Ebola virus disease (EVD) outbreak, Johns Hopkins Medicine created a BCU by redesigning and renovating existing clinical space.[18] The Johns Hopkins BCU (JH-BCU), along with nine others around the United States, were funded by the Office of the Assistant Secretary for Preparedness and Response (ASPR) to serve as Regional Ebola and other Special Pathogen Treatment Centers (RESPTCs). These centers have enhanced capabilities to care for patients and offer fully contained routine laboratory diagnostics and testing of bio-specimens from patients infected with or suspected to be infected with high-consequence pathogens, such as viral hemorrhagic fevers and highly drug resistant *Mycobacterium tuberculosis* (TB).[18,19] Similar to other BCUs, the JH-BCU includes the capability to isolate airborne pathogen transmission via negatively pressurized patient rooms and a negative pressure laboratory space.[18]

Practical Implications

- A systematic method is provided for using fluorescent tracer particles to characterize the isolation capabilities of a clinical biocontainment unit (BCU) under two different HVAC (heating, ventilation, air-conditioning) operating conditions: normal operation and loss of negative pressure due to HVAC exhaust failure.
- During normal HVAC operation, there were no tracer particles detected outside of the patient isolation room suggesting that the patient room's negative pressure differential and exhaust vents operated sufficiently to maintain airborne isolation conditions.
- However, during HVAC exhaust failure, while the patient room still contained the majority of the contaminated air, ~6% of fluorescent tracer particles were detected in areas adjacent to the patient isolation room suggesting that the current protocols used by the HVAC automation system to increase airflow into these adjacent spaces during emergency loss of exhaust capabilities is insufficient to maintain complete airborne isolation conditions.
- Since it is hypothesized that particles were transported out of the patient room through cracks in the doors and door handles, it should be further investigated how to improve the seal on these doors to improve the airborne isolation capabilities.
- Further investigation is needed to identify safe BCU protocols that can be used during the emergency loss of negative pressure, such as required use of respiratory personal protective equipment in all areas of the BCU.

While general guidance was provided to aid RESPTCs by defining the minimum requirements needed for treating patients, such as private patient rooms with their own bathrooms, there was no specific guidance provided on engineering or HVAC (heating, ventilation and air-conditioning) design needs to maintain airborne pathogen isolation within those rooms.[20] Guidelines are available from the Centers for Disease Control and Prevention (CDC) and the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) for preventing transmission of airborne pathogens, such as TB, in healthcare settings,[11,21] but there are currently no guidelines specific to BCUs. Furthermore, there are no formal, mandated guidelines for BCU design, construction, and capabilities; therefore, specifications vary among the facilities, and there are no data to date examining the efficiency of any BCU environment to maintain isolation of airborne infectious particles.[8,22,23]

Recently, Drewry et al[24] presented a study to investigate the potential for healthcare worker (HCW) exposure to simulant airborne pathogens while working in the JH-BCU. The goal was to develop a safe and effective method that could be used by BCUs to evaluate potential failure points of PPE to reduce the risk of exposure to infectious particles. The objective of this study was to provide the first investigation of a BCU's ability to contain aerosolized simulant fluorescent pathogen particles under different

FIGURE 1 (A) An illustration of the Johns Hopkins Hospital biocontainment unit (JH-BCU) depicting room types. Red areas are the three patient rooms, labeled rooms 1-3, and the laboratory space for processing samples (room 4). There is a dedicated space for putting on personal protective equipment (PPE) (ie, donning) which is shared by patient rooms 1 and 2 (room 5), as well as a shared donning space between patient room 3 and the laboratory (room 6). Each of the red spaces have areas for removal of PPE (ie, doffing), labeled as the yellow rooms 7-10. Area 11 is a nurses' station. All green areas do not require the use of PPE according to the JH-BCU standard protocols while red areas do require PPE. For more details, see Garibaldi et al[18] (B) Overview of the JH-BCU space with the number of air changes per hour per room during normal HVAC (heating, ventilation, air-conditioning) operation, and the directional movement of personnel between spaces to minimize risk for contamination of clean zones (arrows). (C) Locations in the JH-BCU for the release of fluorescent tracer particles from the Collison nebulizer ("CN" in figure), and eight IBAC (Instantaneous Biological Analyzer and Collector, FLIR Systems) sensors for detecting and measuring fluorescent particle concentrations and dissemination pattern. The IBAC locations in order include: (1) patient room, (2) PPE doffing room, (3-6) hallway, (7) PPE donning room, and (8) nurses' station

HVAC operating conditions. These experiments were conducted to investigate the airborne isolation capabilities of a JH-BCU patient room in the absence of any HCW movement or activity; that is, particle dissemination via airflow patterns alone. Considering the number of BCUs currently in operation (there are currently 11), the lack of formal guidelines for BCU safety controls, and the ever-present threat of naturally or intentionally spread HIDs,[23] there is a critical need for systematic research addressing the safety of BCU facilities and protocols. The methodologies presented below provide a testing framework that can be used across multiple BCU facilities and other indoor air environments to evaluate dispersion patterns and minimize the risk for exposure to and transmission of airborne pathogens.

2 | TEST FACILITY, STUDY DESIGN AND METHODS

2.1 | Air-handling system of the JH-BCU

The air-handling system of the JH-BCU was designed based on the airborne precautions guidance documents of the CDC and ASHRAE for preventing transmission of airborne particles containing infectious microorganisms in general healthcare settings.[11,21] Figure 1A illustrates the different rooms and areas in the JH-BCU. Figure 1B presents the overview of the JH-BCU space, the number of air changes per hour (ACH) per room (black numbers) during normal operation, and the relative directional flow of personnel between rooms to minimize the risk for contamination of clean spaces (arrows). The main goal of the air-handling system design is to maintain the contaminated spaces of the JH-BCU (ie, the patient rooms and laboratory) at negative pressure relative to the rest of the JH-BCU and the general hospital; this is intended to prevent aerosol escape from these rooms and removes airborne pathogens by filtration.[9]

The JH-BCU uses the main hospital's building supply air, but the entire JH-BCU is operated under a high efficiency particulate air (HEPA) filtered exhaust system that is separate from the general exhaust system for the rest of the hospital building. The exhaust system has two fans to maintain negative pressure in the patient rooms and laboratory, and each of these fans can operate independently in the case that one fails. Only one fan runs at a given time to operate the connected total airflow to maintain the appropriate negative pressure of the unit. The second fan is on standby. The patient rooms and the laboratory space exceed the minimum recommendation of 12 ACH for adequately ventilated single rooms without controlled, directional airflow[11]; the patient rooms each have 15 ACH, and the laboratory space has 32 ACH. Ductless supplemental cooling/heating units have been installed in each patient room, laboratory, and the dirty side of the autoclave room. These units are connected to the emergency backup power and are designed to provide additional cooling/heating to the areas in case of failure of the BCU central cooling and heating systems. The general airflow with respect to the BCU is from areas of higher to lower pressure which maps as (high to low): the surrounding hospital > the entire JH-BCU > the main JH-BCU corridor > PPE donning and doffing spaces (rooms where PPE is put on and removed, respectively) > patient care rooms and laboratory.

The patient rooms and laboratory are maintained at a negative pressure differential of at least −5 pascal (Pa) to the adjacent areas (PPE rooms and hallway). This is continuously monitored by 15 room pressure sensors—one at each of the entrances to BCU rooms (Pressura Model 8631-HM-BAC Room Pressure Monitor, TSI Inc., Shoreview, MN, USA). These sensors alarm if the negative pressure differential is diminished below the minimum of 2.5 Pa.[21] The room pressure (ie, airflow direction) at each room's doorway are continuously monitored and the averages are recorded every 10 min in a continuous log by the sensors. The average air velocity at the doorways can also be recorded using the same pressure monitors. While the JH-BCU space is not in use, the room pressures and pressure sensors are checked quarterly and the airflows are checked annually. Additionally, the airflows are also checked as needed during the quarterly room pressure checks. According to CDC recommended guidelines for maintaining continuous negative pressure conditions and airflow patterns of protective environment rooms,[25] airflows are checked ideally on a daily basis using a visual indicator (eg, flutter strips or smoke tubes). These are guidelines only, not requirements. A full description of the design and configuration of the JH-BCU is detailed in Garibaldi et al.[18]

2.2 | Test scenarios

This study investigated the effectiveness of the JH-BCU to contain released simulant pathogen particles in an AIIR under two different scenarios: (a) normal HVAC operation; and (b) HVAC exhaust failure. Under normal operating conditions, it has been previously shown that the ventilation systems of hospital airborne isolation units may not be as effective as anticipated for removing airborne particles regardless of whether or not these units function in accordance with design specifications.[26-29] Furthermore, while the fans operating the JH-BCU HVAC exhaust can operate independently, and the fans and the air-handling system are connected to the emergency power of the hospital,[18] there have been recent occurrences and disasters which have resulted in catastrophic hospital power failures. For example, in June 2001, Memorial Hermann Hospital in Houston, TX, a level I trauma center, lost electrical power, communications, and running water during tropical storm Allison.[30] During the 2012 Super Storm Sandy, two hospitals in New York City were forced to evacuate, including Bellevue Hospital, after two days on backup generator power.[31]

In this study, three repeat trials were conducted for both HVAC test conditions. The exhaust failure trials were conducted by coordinating with the facility manager to turn off the JH-BCU HEPA exhaust system. As described above, the exhaust system has two fans; both fans were off during the exhaust failure trials. According to the exhaust failure standard operating protocol of the JH-BCU, this results in automatic shut off of the building supply air to the areas of the JH-BCU that are assumed to be contaminated (ie, the

three patient rooms, laboratory, and the dirty side of the autoclave room) in order to reduce further disruption of the pressure differentials. The air-handling system's automation system maximizes airflow to the clean areas of the JH-BCU by increasing supply air to these clean areas to push particles from the rest of the unit toward the contaminated areas. In addition, an emergency backup power supply provides power to ductless cooling/heating units in the patient rooms; these units can be operated by the staff to maintain air circulation and provide comfort. During the exhaust failure trial, the effect of turning off the ductless cooling/heating units in the patient room was also investigated to observe how this influenced particle dissemination and transport.

2.3 | Nebulization of fluorescent tracer particles

Figure 1C illustrates the locations in the JH-BCU where fluorescent tracer particles were released via Collison nebulizer and the locations for the real-time aerosol sensors. More details on the aerosol sensors are provided below. The particles were released next to the bed in a patient room (simulating the release from a typical patient location). To disseminate fluorescent tracer particles for each trial, a 25-mL suspension of optical brightener power (50 mg/mL, UV Blue D-282, DayGlo, Cleveland, OH, USA) and urea (250 mg/mL, U5378, Sigma-Aldrich, St. Louis, MO, USA) mixed in filtered, deionized water was nebulized from a 3-jet Collison nebulizer (138 kPa, 5 L/min; Mesa Laboratories, Inc., Butler, NJ, USA) for 5 min. Under normal HVAC operation, detection of a peak in fluorescent particle concentration above background particles was detected when at least 1 min of nebulization of the optical brightener suspension was conducted—hence the decision to run the Collison nebulizer for 5 min during each trial. The air pump providing positive pressure to the Collison nebulizer was operated via a remote switch so that the pattern of particle dissemination was not disturbed by test operator movement. The suspension was replaced in the Collison nebulizer before every new repeat trial. The temperature and relative humidity of the JH-BCU ranged from 20-22°C and 37%-40%, respectively, throughout testing.

The rationale for using the selected suspension to produce the tracer particles was fourfold. First, the use of optical brightener powder is a cost-effective option at a cost of $0.40/g of powder. Second, the nebulized particles are fluorescent allowing for feasible real-time tracing of the airborne particle number concentrations and dissemination patterns using fluorescent aerosol sensors as described below. Third, the optical brightener powder is a safe, nontoxic material approved for release into public environments by the U.S. Department of Homeland Security.[32] Fourth, the size of particles produced by nebulizing the suspension range from 0.5 to 5 μm, with the highest number concentration of particles being ~1 μm aerodynamic diameter (Figure 2). As defined by the World Health Organization, droplet nuclei are ≤5 μm in diameter.[14] Thus, the resultant particle number size distribution produced by nebulizing the suspension was representative of infectious respiratory aerosols that can be transmitted as droplet nuclei via the airborne route through breathing, talking, coughing, or sneezing.[13]

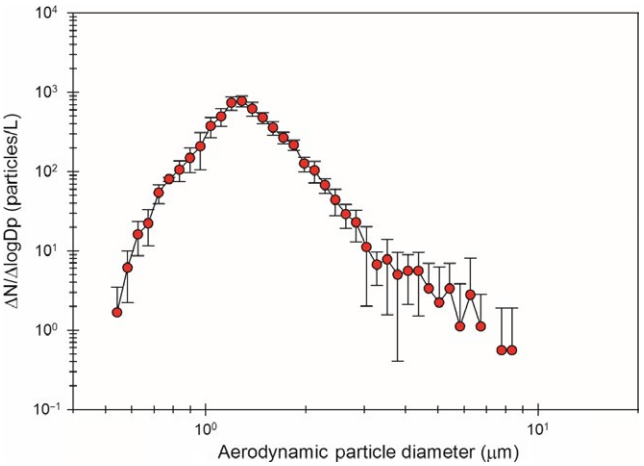

FIGURE 2 The airborne particle number size distribution of the optical brightener suspension post-nebulization using a Sono-Tek ultrasonic atomizer (Sono-Tek Corporation, Milton, NY). The data represent six averaged 20-sec sample measurements by UVAPS (Ultraviolet Aerodynamic Particle Sizer, Model 3314, TSI Inc., Shoreview, MN). Details for the chamber setup used to conduct these measurements are provided by Ratnesar-Shumate et al[33]

2.4 | Fluorescent tracer particles—particle number size distribution measurement

The particle number size distribution for the nebulized fluorescent suspension was investigated before conducting testing. For detailed explanation of the chamber setup used to conduct these measurements, see Ratnesar-Shumate et al[33] Briefly, the optical brightener/urea/water suspension was nebulized using a Sono-Tek ultrasonic atomizer (nozzle power setting = 3 watts, liquid injection rate = 100 μL/min) (Sono-Tek Corporation, Milton, NY, USA). The particles were allowed sufficient time to desiccate to a core particle size in aerosol capacitance chambers, well-mixed using a high chamber flowrate (2500 L/min), and sampled isokinetically to measure particle size and number concentration using a UVAPS (Ultraviolet Aerodynamic Particle Sizer, Model 3314, TSI Inc., Shoreview, MN, USA). While a Sono-Tek atomizer was used for chamber measurements, the Collison nebulizer was used during the JH-BCU experiments due to its ease of transport and setup. Also, since the experimental repeats conducted in the JH-BCU monitored particle dissemination for 20-min post-nebulization, chamber desiccation was used during the measurements of the suspension's particle number size distribution to mimic aerosol aging; thus, the nebulized particles were dried to a solid, core size to simulate droplet nuclei.[34] Further, as described below in results, nebulization of the fluorescent suspension by the Collison nebulizer during experiments at the JH-BCU was found to produce a particle number size distribution with 82.3% ± 0.8% of the particles in the 0.5 to 1.7 μm size range. In comparison, the particle number size distribution produced by the Sono-Tek atomizer resulted in 87.4% ± 2.5% of particles in the 0.5 to 1.7 μm size range. Therefore, the chamber measurements of the fluorescent suspension's particle number size distribution are

representative of the nebulized suspension during the experiments at the JH-BCU.

2.5 | Monitoring airborne concentrations and dissemination patterns of fluorescent tracer particles

After nebulizing the fluorescent tracer particles into the air of the patient room, a networked array of eight aerosol sensors, Instantaneous Biological Analyzers and Collectors (IBACs) (FLIR Systems, Inc. Elkridge, MD, USA), was used to track the concentrations and dispersion of particles in real time. The IBAC samples ambient air at 3 L/min and provides the total number concentrations for fluorescent particles (particle number per liter of air, particles/L) every second. The IBAC utilizes ultraviolet laser induced fluorescence (UV-LIF) at an excitation wavelength of 405 nm to discriminate fluorescent particles from ambient background particles.[35] The particles are binned into two size categories: 0.5-1.7 and 1.7-10 μm.[36] The IBACs used in this study were within calibration, and particle concentration, particle sizing, and fluorescence were confirmed to be within tolerance prior to conducting testing using fluorescent polystyrene latex spheres of known size (2 μm, Thermo Scientific, Waltham, MA, USA).

As illustrated in Figure 1C, the IBACs were located throughout the JH-BCU space to characterize if and to what extent particles disseminated into areas adjacent to the patient room. With the exception of the patient room where particles were released, staff and HCWs would not be wearing PPE and respiratory protection under normal operating conditions. The placement of the IBACs was informed by subject matter experts, including JH-BCU staff and HCWs, to represent areas of concern for where staff may be most likely present.

The IBACs were run continuously throughout all trials and control experiments. Following the five-min nebulization of tracer particles, the IBACs recorded fluorescent particle concentrations for 15 min after the Collison nebulizer was turned off. Therefore, each trial represents a total of 20 min. It was predetermined that this time was sufficient during normal HVAC operation for allowing the level of fluorescent particles in the JH-BCU to return to background levels before beginning the next trial. During the exhaust failure trials, the exhaust of the JH-BCU air-handling system was turned back on after the 20-min observation time and allowed to run for at least 20 min to clear the particles from the previous trial; the IBACs were used to ensure that conditions returned to background fluorescent particle levels before beginning the next test run.

3 | RESULTS AND DISCUSSION

3.1 | Background fluorescent particle concentration

Before releasing tracer particles and taking trial measurements, background particle levels were monitored in the JH-BCU by setting up the IBACs in their respective sampling locations and allowing to run for 1 h. This included time during which the investigators were active in the space setting up other instrumentation. During this time, the average background number concentration of fluorescent particles in the JH-BCU was 11 ± 4/L. This is lower but similar to previously reported average concentrations of fluorescent particles measured in occupied areas of various hospital types (30-60/L).[37]

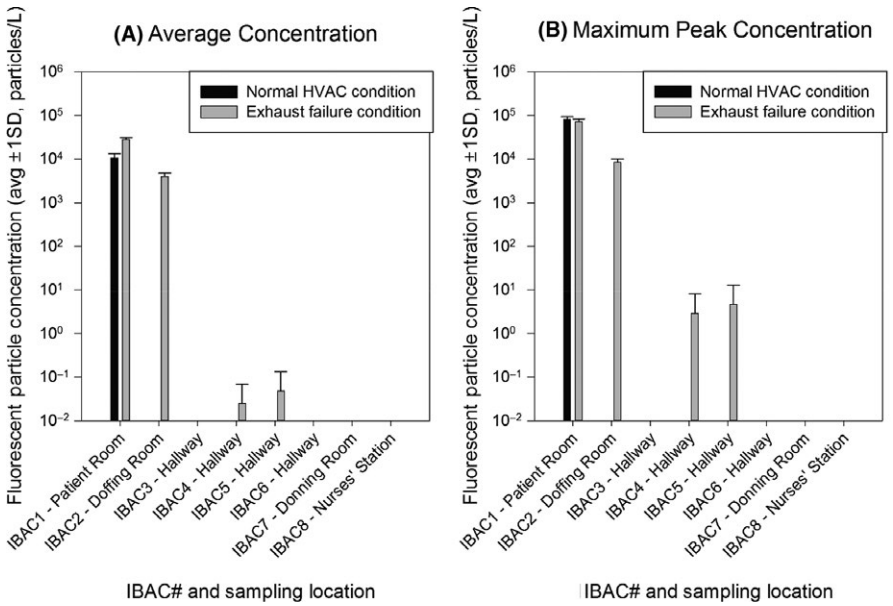

FIGURE 3 Average and mean maximum peak fluorescent particle concentrations (particles/L, average ± 1SD) measured by each of the Instantaneous Biological Analyzers and Collectors (IBACs, FLIR Systems, Elkridge, MD) throughout the Johns Hopkins biocontainment unit during the test scenarios. Test scenarios included normal HVAC (heating, ventilation, air-conditioning) operation (n = 3 repeat trials) vs. HVAC exhaust failure condition (n = 3 repeat trials). The average concentrations were recorded for 20 min for each trial: 5 min of fluorescent tracer particle nebulization time, and 15 min of post-nebulization observation time

Due to the presence of background fluorescent particles, including what is suspected to be biological particles and clothing/linen particles,[37-39] the maximum peak concentration of fluorescent particles detected during the background measurement period (81/L) was subtracted from all measurements taken during fluorescent tracer particle releases.

3.2 | Fluorescent particle concentrations and dissemination under different HVAC conditions: normal HVAC condition

Figure 3 presents the average and maximum peak fluorescent particle concentrations (particles/L, average ± 1SD) measured by each of the IBACs throughout the JH-BCU during two test scenarios: normal HVAC operation, and the HVAC exhaust failure condition. During normal HVAC conditions, the released particles were contained in the patient room with an average concentration of $1 \times 10^4 \pm 3 \times 10^3$/L, and a maximum peak concentration of $8 \times 10^4 \pm 1 \times 10^4$/L (Figure 3). Compared to previous research, the particle number size distribution for coughed droplet nuclei after flow through an aerosol desiccator has been reported as 6×10^5/L with a mean size by particle number between 1 and 2 μm.[40] Thus, the size range and concentration of the fluorescent tracer particles used in the present experiments are similar to values reported for droplet nuclei produced by actual coughing.

Across repeat trials, the peak number concentration occurred in the patient room within 2 min after the start of nebulization, and the fluorescent particle concentration began to decrease after the Collison nebulizer was stopped at minute five (Figure 4A). The particle sizes detected by the IBACs indicated that 82.3% ± 0.8% of the nebulized fluorescent particles were in the 0.5 to 1.7 μm IBAC size bin. The average airflow in the patient room and the donning room throughout normal HVAC operating conditions was 425 ± 3 m^3/h and 234 ± 2 m^3/h, respectively. It should be noted that the JH-BCU system does not measure average airflow in the doffing room.

The JH-BCU is designed to maintain the patient rooms and laboratory at a negative pressure differential compared to the other areas of the BCU of at least −5 Pa.[18] Under normal HVAC operating conditions, it was observed that the negative pressure differential threshold of -5 Pa was maintained between the patient room and donning space (Figure 5). However, the patient room to doffing space corridor had a measured pressure differential that was slightly higher than the desired value (−4 Pa ± 0.4 Pa) and the pressure differential between the doffing space and the hallway corridor was about half the JH-BCU target value for sufficient containment of airborne infectious particles (−2 Pa ± 0.1 Pa). It should also be noted that the average pressure differential measured between the doffing space and hallway corridor of −2 Pa is higher than the CDC recommended pressure differential for negative pressure areas of at least −2.5 Pa.[25]

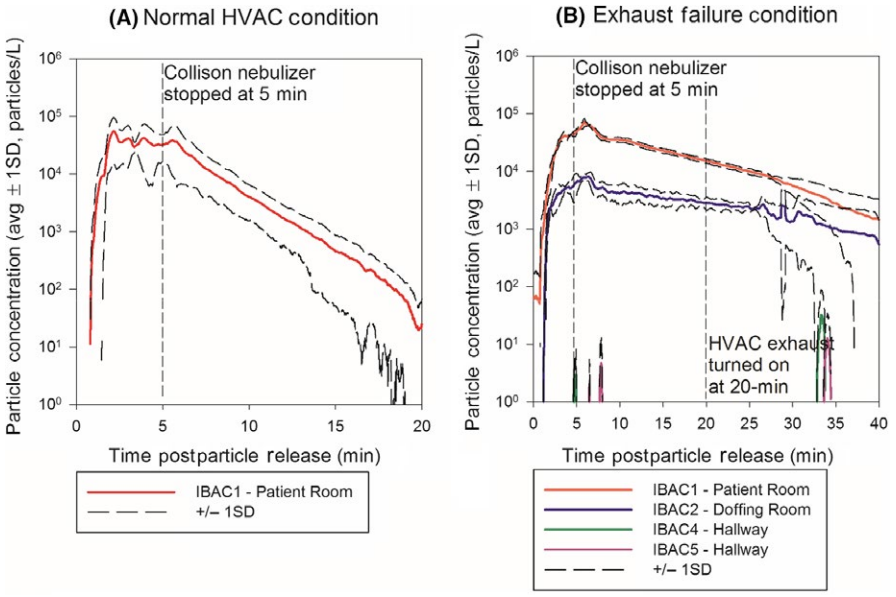

FIGURE 4 Average fluorescent particle concentration (particles/L, average ± 1SD) measured by each of the Instantaneous Biological Analyzers and Collectors (IBACs, FLIR Systems, Elkridge, MD) that detected fluorescent particle concentrations as determined after subtracting the fluorescent particle background concentration (ie, 81/L). Data are presented as concentration vs. time (min) post fluorescent tracer particle release for (A) normal HVAC (heating, ventilation, air-conditioning) operation (n = 3 repeat trials) and (B) HVAC exhaust failure condition (n = 3 repeat trials). The Collison nebulizer was run for 5 min and then turned off while the IBACs were run for 20 and 40 min, respectively, for the two test scenarios. For scenario (B), after 20 min, the HVAC exhaust system was turned back on to reduce fluorescent particle levels in the Johns Hopkins biocontainment unit (JH-BCU) back to background levels before beginning the next trial. The dotted line in both figures represents the time when the Collison nebulizer was turned off to stop fluorescent particle nebulization. The second dotted line in the Figure 4B represents the time when the HVAC exhaust system was turned back on to clear airborne particles in the JH-BCU in preparation for the next repeat test

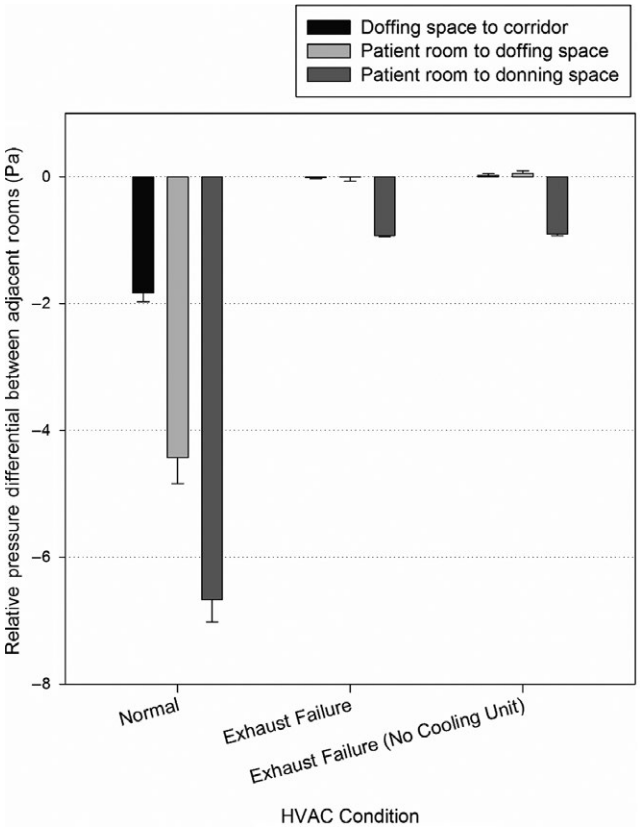

FIGURE 5 Relative pressure (Pa, average ± 1SD) recorded between rooms of the Johns Hopkins biocontainment unit (JH-BCU) vs HVAC (heating, ventilation, air-conditioning) test condition. The data represent continuous 10-min averages recorded by the JH-BCU pressure sensors throughout each HVAC condition (normal HVAC operation: n = 29, exhaust failure: n = 10, exhaust failure (no cooling/heating unit): n = 4)

The doffing space of the JH-BCU is divided into two halves based on PPE doffing protocol; the half of the room closest to the patient room egress is for the removal of PPE and the half closest to the hallway door should only be stepped into after the HCW has fully doffed their contaminated PPE. While the clean side of the doffing space is still considered potentially contaminated space according to JH-BCU protocols, HCWs are unprotected, as they have already doffed their PPE, including their respiratory protection. Regardless, during the given test scenario for which there were no HCWs present, there were no particles detected outside of the patient room suggesting that the lower pressure differential between the doffing space and hallway corridor may not result in the release of particles into the hallway space assuming that the doffing space acts as a buffer zone between the patient room and hallway.

The complete containment of particles achieved in the patient room suggests that the locations and ventilation rates of the patient room exhaust vents were sufficient to maintain airborne isolation conditions under normal HVAC operation. The locations of inlet and exhaust air vents in the patient room with respect to the patient bed are illustrated in Figure 6. The exhaust vents are each 31*20 cm and approximately 31 cm above the floor. The patient room has two supply air vents (23*23 cm) located above two corridor doorways. These doors are not used when a patient is present and are taped off to alert HCWs not to enter/exit through these doors.

Supply/outlet vent placement near patients has been suggested to be a better predictor for the removal of airborne bacteria in isolation rooms than air change rate alone.[41,42] For example, previous research has shown that ceiling level exhausts are more efficient than floor level exhausts for removing fine respiratory particles in hospital isolation rooms.[27] Huang and Tsao[41] suggest locating three exhaust vents (60*60 cm) arranged with one on the ceiling and two on both side walls of the patient's bed. However, practical limitations during the design and building process should also be considered; the inlet and outlet vent locations in the JH-BCU were selected in the patient room to be furthest from the doors and closest to the patient beds so that the net airflow of the patient room moves across the patient and then exits. It was also desired to locate the exhaust vents as far as practically possible from the donning and doffing room doors to remove particles away from these corridors to reduce the risk of particles entering into these spaces when the doors open.[43] Furthermore, in the present experiments in the JH-BCU under normal HVAC conditions, the exhaust vents appear to sufficiently remove released tracer particles.

3.3 | Fluorescent particle concentrations and dissemination under different HVAC conditions: HVAC exhaust failure condition

During the HVAC exhaust failure trials, the average recorded patient room airflow was reduced by 96% to 17 ± 5 m^3/h, while the average recorded donning room airflow was similar to normal HVAC operation (238 ± 3 m^3/h). The design of the JH-BCU air-handling system is such that the loss of exhaust capabilities results in maximization of supply airflow to the clean areas of the JH-BCU; this is meant to force the air, and therefore any present aerosols, toward the patient rooms and laboratory. Thus, shutting down the exhaust system resulted in reduced airflow in the patient room, but not in the donning room adjacent to the patient room.

As illustrated in Figure 3, the exhaust failure condition resulted in transport of particles from the patient room where the fluorescent particles were released into the doffing room and certain hallway locations (IBAC locations 2, 4, and 5 in Figure 1C). In the patient room, the average fluorescent particle number concentration was measured as $3 \times 10^4 \pm 2 \times 10^3$/L with a maximum peak concentration of $7 \times 10^4 \pm 1 \times 10^4$/L (Figure 3). This peak occurred after about 1 min from the start of the Collison nebulizer (Figure 4B). One minute after detecting the appearance of fluorescent particles in the patient room, a smaller peak of fluorescent particles was observed in the doffing space (Figures 3 and 4B; average = $4 \times 10^3 \pm 9 \times 10^2$/L, maximum peak = $9 \times 10^4 \pm 2 \times 10^4$/L). This is in contrast to the normal HVAC conditions during which the released fluorescent tracer particles were contained in the patient room.

These results suggest that approximately 6% of the fluorescent particles detected in the patient room were transported into the

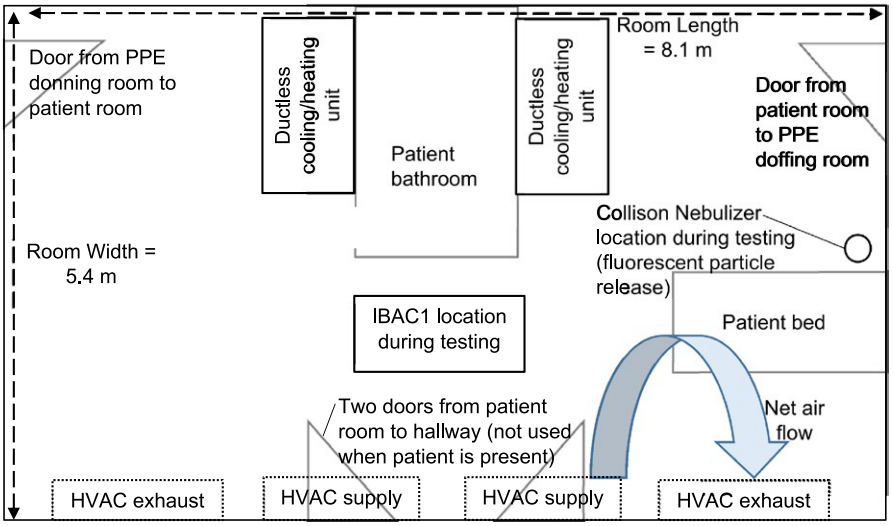

FIGURE 6 Layout plan for the patient room where the fluorescent tracer particles were released. The diagram indicates the following features: the relative locations for the doorways, direction of healthcare worker movement through these doors, patient bed and bathroom, HVAC (heating, ventilation air-conditioning) supply and exhaust vents, and the ductless cooling/heating units that staff utilize for comfort during an HVAC failure. The patient room has two exhaust vents which are located furthest from the doors and closest to the patient bed so that the net airflow of the patient room moves across the patient and exits while minimizing HCW exposure. This net airflow, illustrated by the blue arrow, has been qualitatively observed by the JH-BCU engineering team using smoke. The exhaust vents are each 0.3*0.2 m and approximately 0.3 m above the floor. The patient room has two supply air vents (0.2*0.2 m) located above two corridor doorways. These doors are not used when a patient is present and are taped off to alert HCWs not to enter/exit through these doors. Also depicted is the instrumentation used during experiments, including the IBAC sensor (FLIR Systems, Inc., Elkridge, MD) in the patient room (IBAC1) and the fluorescent particle release location from the 3-Jet Collison nebulizer (25 mL suspension, 138 kPa, 5 L/min; Mesa Laboratories, Inc., Butler, NJ). The room dimensions are as follows: length = 8.1 m, width = 5.4 m, height = 2.5 m (total room volume = 109.4 m^3)

doffing space, and <1% may have been transported to the hallway. The total volume of the patient room, doffing room, and corridor space is 109.4, 54.7, and 328.1 m^3, respectively. To estimate the percentage of the total number of particles that were transported out of the patient room, the average particle number concentrations detected by each of the IBACs throughout the exhaust failure trials were multiplied by their total room volume to estimate the number of particles in each location (ie, patient room, doffing room, and hallway). Assuming that the sum of all particle counts across the JH-BCU space was the total number of particles released, the percentages of the total that were transported out of the patient room and into the doffing space and hallway corridor were calculated. It was also assumed that the airborne particle number concentrations detected by the IBACs in each location was representative of that entire room, and that the rooms were well mixed. For the hallway, an average was calculated across the multiple IBACs located there.

Maximum peak particle counts in the hallway reached 5 ± 8/L and the presence of fluorescent particles was detected by the IBACs intermittently between 5 and 15 min from the start of the Collison nebulizer (Figure 4B). This represents a potential risk of airborne pathogen exposure for HCWs since respiratory PPE is removed in the doffing room and is not worn elsewhere in the JH-BCU, according to the JH-BCU protocols. For reference, Figure 1B illustrates which areas of the JH-BCU require the use of respiratory PPE (red zones), and Figure 1C illustrates the IBAC sensor locations. The yellow zones are where the PPE is removed, and the green zones are considered clean and do not require PPE. During the HVAC exhaust failure trials, tracer particles were detected outside of the patient room and in both yellow (doffing room, room #8 in Figure 1A, IBAC #2 in Figure 1C) and green (hallway, IBAC #4 and #5 in Figure 1C) zones.

Drewry et al[24] also noted the presence of simulant pathogen particles in the JH-BCU doffing room following simulated patient care and doffing procedures. The key difference between the present study and the study of Drewry et al[24] is that there were no HCWs in the present study; that is, particle dissemination was observed as a result of airflow patterns alone while manipulating HVAC condition. Therefore, the potential for HCW exposure to airborne pathogens is of concern in the doffing room due to multiple potential scenarios for this contamination. Thus far, between the present study and Drewry et al,[24] these identified scenarios include HVAC exhaust failure, re-aerosolization of particles deposited on the outside of HCW's PPE during doffing, and/or particle transport from the patient room after opening the door between the two spaces.

The potential risk for an individual to acquire an infection depends partially on the dose of the inhaled pathogen, which is governed by the airborne pathogen concentration, the individual's breathing rate, and exposure duration in the room.[15,44] For pathogens such as TB and variola (smallpox) virus, the infectious dose may be as low as a single microorganism.[44,45] While it may seem unlikely for a patient to present with smallpox, the potential for

malicious use of infectious agents through bioterrorism, including variola virus, must be considered.[46,47] Therefore, the identification for potential transport of the fluorescent tracer particles from the patient room to adjacent areas of the JH-BCU during HVAC failure warrants further investigation to identify methods and protocols that can be used during the emergency loss of negative pressure in the patient rooms. For example, during HVAC failure, the JH-BCU protocols can be updated to require the use of PPE and respirators in all areas of the unit. Future research will also investigate the potential for pathogen dissemination beyond the physical space of the JH-BCU into other areas of the hospital during different HVAC scenarios.

Because the IBACs that detected the presence of fluorescent tracer particles during the exhaust failure condition (IBAC locations 2, 4, and 5 in Figure 1C) were located near patient room doorways, it is suspected that the particles were transported from the patient room into adjacent areas around the doors and door handles. This includes the two doorways that directly connect the patient room and hallway corridor. Therefore, even though these doors are not used when a patient is present, the presence of the doorways alone may be a potential source for particle leakage out of the patient room under HVAC exhaust failure.

During the exhaust failure condition, an average pressure differential was maintained between the patient room and the donning space of about −1 Pa ± 0.01 Pa (Figure 5) and there were no fluorescent particles detected in the donning room. However, the donning space is also located on the opposite side of the room from where the tracer particles were released. On the other hand, the pressure differential between the patient room to the doffing space and the doffing space to the hallway corridor was close to zero (<−0.001 Pa to +0.05 Pa) (Figure 5). This suggests that the exhaust failure emergency protocol of increasing airflow to the areas adjacent to the patient rooms was not sufficient enough to create a negative pressure differential between the doffing room and patient room. It should be investigated if further increasing the airflow into the doffing space can reestablish the negative pressure differential between the doffing room and patient room. Also, since it is hypothesized that particles were transported out of the patient room through cracks in the doors, another recommendation to improve the airborne isolation capabilities of the patient rooms is to replace the hinged doors with sliding doors.[43]

Previous research has demonstrated that opening doors can act as a mechanism by which infectious particles can be transported from airborne isolation rooms into adjacent spaces, particularly for hinged doors that open outward.[15,48,49] However, the doors in the present study were kept closed throughout all trials. Nevertheless, the influence of door cracks has been previously investigated as a means for particle transport from outdoors to indoors with particle penetration factor estimates of 0.8-1.0 for 1 μm diameter particles through window and door frame cracks in different types of buildings; the average air velocity through these cracks was estimated to be 0.3-2.2 m/s.[50] This is supported by laboratory studies which have also shown a penetration factor of 0.8-1.0 for 1 μm particles through horizontal cracks (0.5 mm high, 102 mm deep, 433 mm wide) under an applied pressure of 2 Pa across connected chambers.[51] Even though the pressure differential between the patient room and adjacent JH-BCU areas was less than that described in previous chamber studies, an air velocity of 1 m/s was observed in the present study moving in the direction out of the patient room through the cracks of the door handles which could facilitate particle transport (Velocicalc, Model 9515, TSI Inc., Shoreview, MN, USA). Future research will further characterize the particle size-dependent penetration factors through the JH-BCU door handles under different HVAC operating conditions using monodispersed fluorescent particles of various sizes.

3.4 | Influence of the patient room ductless cooling/heating unit

As described above, an exhaust failure of the HVAC system in the JH-BCU automatically provides power to ductless cooling/heating units in the patient rooms; staff can operate these units to maintain air circulation and comfort. The effect of turning off the two ductless cooling/heating units in the patient room was investigated during one exhaust failure trial to observe how this influenced particle transport. The locations of these units in the patient room are depicted in Figure 6.

Figure 7 illustrates the time series plots for fluorescent tracer particle concentrations as measured by the IBAC sensors in the patient room (IBAC #1) and doffing room (IBAC #2) after turning off the ductless cooling/heating unit. In this test, the following factors were similar between the HVAC exhaust failure condition with and without the ductless cooling/heating unit turned on: (a) average concentrations of fluorescent particles in the patient room and doffing room (Figure 7), (b) the net airflow in the patient room (~17 ± 5 m^3/h), and (c) the pressure differentials between the patient room and adjacent rooms (Figure 5). However, the time of appearance of fluorescent particles in the doffing room was different between the two exhaust failure conditions with and without the ductless cooling/heating unit on. By shutting off the ductless cooling/heating unit, the time required for particles to transport from the patient room to the doffing space was increased by approximately 3 min (Figure 7B). It is hypothesized that the airflow of the ductless cooling/heating unit helped push particles into the doffing room when the patient room lost negative pressure. The ductless cooling/heating unit in the patient room as depicted in Figure 6 faces the doffing room door. Therefore, assuming the ductless cooling/heating units are necessary for maintaining the comfort of the patient and HCWs upon failure of the BCU central HVAC system, future research should investigate different placement locations for the cooling/heating unit away from locations that will promote particle transport out of the patient room.

4 | CONCLUSIONS AND FUTURE WORK

In this study, we provide a systematic method for characterizing the isolation capabilities of a clinical BCU under two different HVAC

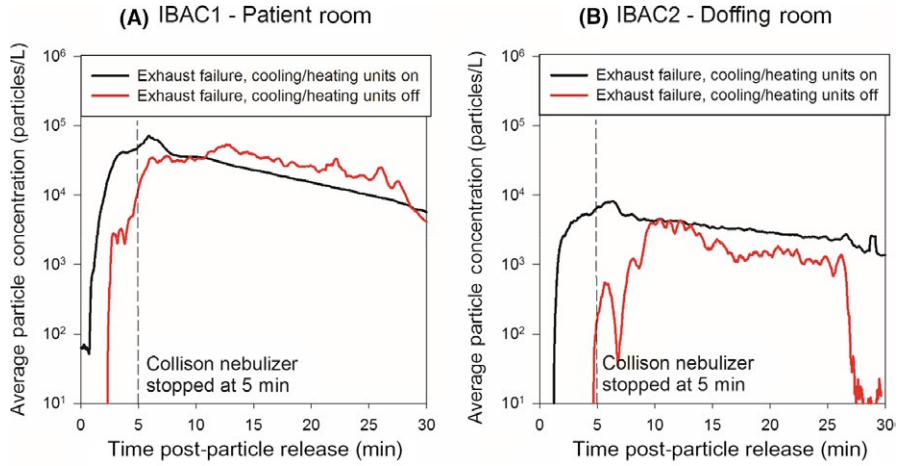

FIGURE 7 Time series plots for fluorescent tracer particle release as measured by the Instantaneous Biological Analyzers and Collectors (IBACs, FLIR Systems, Elkridge, MD) in the patient (IBAC1) and doffing rooms (IBAC2) during the exhaust failure condition during which the effect of turning off the ductless cooling/heating unit was investigated (n = 1 trial) vs exhaust failure with the cooling/heating units on (n = 3 repeat trials). During an exhaust failure of the HVAC (heating, ventilation, air-conditioning) system in the Johns Hopkins biocontainment unit (JH-BCU), the building supply air to the patient rooms is automatically turned off and a ductless cooling/heating unit in the patient rooms can be turned on to maintain air circulation and comfort. The dotted line in both figures represents the time when the Collison nebulizer was turned off to stop fluorescent particle nebulization

operating conditions: normal operation and HVAC exhaust failure. During normal HVAC operation, there were no fluorescent tracer particles detected outside of the patient room suggesting that the locations of and ventilation rates used by the patient room exhaust vents were sufficient to contain particles in the patient isolation room. For these tests, no HCWs were present and no patient care activities were performed. During HVAC exhaust failure, fluorescent tracer particles were detected in the areas adjacent to the patient room, including the doffing room and the hallway; ~6% of the fluorescent particles detected in the patient room were transported into the doffing space by airflow alone and not by HCW movement or opening/closing of doors. Thus, the HVAC automated response during exhaust failure to increase airflow to spaces adjacent to the patient rooms is not sufficient for maintaining negative pressure differentials and complete airborne isolation conditions. It is hypothesized that particles were transported from the patient room to adjacent areas of the BCU through cracks around the doors and door handles.

Therefore, future research will further characterize particle size-dependent penetration factors through patient room doors under different HVAC operating conditions with monodispersed aerosols. It should be further investigated how to leak protect the doors and door handles. Future research should also investigate different placement locations for the ductless cooling/heating units in the patient room away from locations that may promote particle transport out of the patient room. Additionally, updated protocols are needed during the emergency loss of negative pressure in the patient rooms, such as required use of respiratory PPE in all areas of the JH-BCU. This would also require identification of an emergency PPE doffing space.

Potential wider applications of this work include cross-comparison of the present JH-BCU airborne isolation capabilities to those of other BCUs throughout the country. There are 11 federally funded BCUs in the United States. However, there are no formal, mandated guidelines for BCU design, construction, and capabilities; therefore, specifications vary among the facilities.[8,22,23] The present study provides the first data examining the efficiency of a BCU environment to maintain isolation of released airborne particles. The methods used in the present study could be applied to evaluate the other BCUs with a focus on providing guidelines to standardize minimum BCU requirements for maintaining airborne isolation conditions. Additionally, the use of polydisperse, fluorescent tracer particles in combination with a networked array of real time, fluorescent aerosol sensors could be flexibly applied to other clinical spaces to investigate particle fate and transport throughout facilities. Because the fluorescent tracer particles used in this study (ie, optical brightener suspension) have been approved for release into public spaces,[32] this type of research could be conducted in both empty and occupied spaces to investigate the influence of patient and healthcare provider movement on pathogen simulant transport. This work could be coupled with and used for validation of numerical simulations to create a more complete picture of aerosol transport in any clinical space.

ACKNOWLEDGEMENTS

We would like to thank the staff of the Johns Hopkins Hospital and colleagues at other U.S. biocontainment facilities who helped inform and improve the JH-BCU design process and the creation of patient care protocols. The National Health Mission Area at the Johns Hopkins Applied Physics Laboratory provided funding for this study as part of an internal research and development project.

ORCID

Jennifer Therkorn http://orcid.org/0000-0001-7742-9938

REFERENCES

1. Sundell J. On the history of indoor air quality and health. *Indoor Air*. 2004;14:51-58.
2. Li Y, Leung GM, Tang JW, et al. Role of ventilation in airborne transmission of infectious agents in the built environment – a multidisciplinary systematic review. *Indoor Air*. 2007;17:2-18.
3. Neiderud C-J. How urbanization affects the epidemiology of emerging infectious diseases. *Infect Ecol Epidemiol*. 2015;5:27060.
4. Bogoch II, Creatore MI, Cetron MS, et al. Assessment of the potential for international dissemination of Ebola virus via commercial air travel during the 2014 West African outbreak. *Lancet*. 2015;385:29-35.
5. Semenza JC, Menne B. Climate change and infectious diseases in Europe. *Lancet Infect Dis*. 2009;9:365-375.
6. Oboho IK, Tomczyk SM, Al-Asmari AM, et al. 2014 MERS-CoV outbreak in Jeddah–a link to health care facilities. *N Engl J Med*. 2015;372:846-854.
7. Chan P, Chan M. Tracing the SARS-coronavirus. *J Thorac Dis*. 2013;5:S118-S121.
8. Schilling S, Fusco FM, De Iaco G, et al. Isolation facilities for highly infectious diseases in Europe – A cross-sectional analysis in 16 countries. *PLoS ONE*. 2014;9:e100401.
9. Walker JT, Hoffman P, Bennett AM, et al. Hospital and community acquired infection and the built environment – design and testing of infection control rooms. *J Hosp Infect*. 2007;65:43-49.
10. Mannheim JK. Isolation precautions. [MedlinePlus Website]. April 5, 2018. https://medlineplus.gov/ency/patientinstructions/000446.htm. Accessed December 12, 2017.
11. Siegel JD, Rhinehart E, Jackson M, et al. Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. 2007 [CDC Website]. December 2007. http://www.cdc.gov/hicpac/pdf/isolation/Isolation2007.pdf. Accessed December 20, 2017.
12. Beggs CB. The airborne transmission of infection in hospital buildings: fact or fiction? *Indoor Built Environ*. 2003;12:9-18.
13. Gralton J, Tovey E, McLaws M-L, Rawlinson WD. The role of particle size in aerosolised pathogen transmission: a review. *J Infect*. 2011;62:1-13.
14. World Health Organization (WHO). Infection prevention and control of epidemic- and pandemic-prone acute respiratory infections in health care. [WHO Guidelines]. April 2014. http://www.who.int/csr/bioriskreduction/infection_control/publication/en/. Accessed December 5, 2017.
15. Tang JW, Li Y, Eames I, et al. Factors involved in the aerosol transmission of infection and control of ventilation in healthcare premises. *J Hosp Infect*. 2006;64:100-114.
16. Xiao S, Li Y, Sung M, et al. A study of the probable transmission routes of MERS-CoV during the first hospital outbreak in the Republic of Korea. *Indoor Air*. 2018;28:51-63.
17. Li Y, Huang X, Yu ITS, et al. Role of air distribution in SARS transmission during the largest nosocomial outbreak in Hong Kong. *Indoor Air*. 2005;15:83-95.
18. Garibaldi BT, Kelen GD, Brower RG, et al. The creation of a biocontainment unit at a tertiary care hospital. The Johns Hopkins Medicine experience. *Ann Am Thorac Soc*. 2016;13:600-608.
19. The White House. Fact sheet: Progress in our Ebola response at home and abroad. [The White House Archives]. February 11, 2015. https://obamawhitehouse.archives.gov/the-press-office/2015/02/11/fact-sheet-progress-our-ebola-response-home-and-abroad. Accessed December 21, 2017.
20. Centers for Disease Control and Prevention (CDC). Hospital preparedness: A tiered approach. Interim guidance for preparing Ebola treatment centers. [CDC Website]. 2014. https://www.cdc.gov/vhf/ebola/healthcare-us/preparing/hospitals.html. Accessed December 21, 2017.
21. American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE). ASHRAE position document on airborne infectious diseases. February 2017. https://www.ashrae.org/about-ashrae/position-documents. Accessed December 20, 2017.
22. Smith PW, Anderson AO, Christopher GW, et al. Designing a biocontainment unit to care for patients with serious communicable diseases: a consensus statement. *Biosecur Bioterror*. 2006;4:351-365.
23. Herstein JJ, Biddinger PD, Kraft CS, et al. Current capabilities and capacity of Ebola treatment centers in the United States. *Infect Control Hosp Epidemiol*. 2016;37:313-318.
24. Drewry D, Sauer L, Shaw-Saliba K, et al. A novel approach to identify potential provider and environmental contamination on a clinical biocontainment unit using aerosolized pathogen simulants. *Health Secur*. 2017;16:83-91.
25. Centers for Disease Control and Prevention (CDC). Guidelines for environmental infection control in health-care facilities. [CDC Website]. 2005. https://www.cdc.gov/infectioncontrol/guidelines/environmental/background/air.html. Accessed December 21, 2017.
26. Foord N, Lidwell OM. The control by ventilation of airborne bacterial transfer between hospital patients, and its assessment by means of a particle tracer: I. An airborne-particle tracer for cross-infection studies. *J Hyg*. 1972;70(279-286):271.
27. Qian H, Li Y. Removal of exhaled particles by ventilation and deposition in a multibed airborne infection isolation room. *Indoor Air*. 2010;20:284-297.
28. Hambraeus A, Sanderson HF. The control by ventilation of airborne bacterial transfer between hospital patients and its assessment by means of a particle tracer: III. Studies with an airborne-particle tracer in an isolation ward for burned patients. *J Hyg*. 1972;70:299-312.
29. Pavelchak N, DePersis RP, London M, et al. Identification of factors that disrupt negative air pressurization of respiratory isolation rooms. *Infect Control Hosp Epidemiol*. 2015;21:191-195.
30. Nates JL. Combined external and internal hospital disaster: impact and response in a Houston trauma center intensive care unit. *Crit Care Med*. 2004;32:686-690.
31. Powell T, Hanfling D, Gostin LO. Emergency preparedness and public health: the lessons of hurricane sandy. *JAMA*. 2012;308:2569-2570.
32. United States Department of Homeland Security (USDHS). *Environmental assessment of proposed tracer particle and biological releases for the hazards of dynamic outdoor release (HODOR) project*. Washington, DC: Department of Homeland Security Science and Technology Directorate; 2017.
33. Ratnesar-Shumate S, Wagner ML, Kerechanin C, et al. Improved method for the evaluation of real-time biological aerosol detection technologies. *Aerosol Sci Technol*. 2011;45:635-644.
34. Xie X, Li Y, Chwang ATY, et al. How far droplets can move in indoor environments – revisiting the Wells evaporation–falling curve. *Indoor Air*. 2007;17:211-225.
35. DeFreez R. LIF bio-aerosol threat triggers: then and now. [SPIE Digital Library]. September 17, 2009. https://www.spiedigitallibrary.org/conference-proceedings-of-spie/7484/1/LIF-bio-aerosol-threat-triggers-then-and-now/10.1117/12.835088.full?SSO=1. Accessed December 22, 2017.
36. Santarpia JL, Ratnesar-Shumate S, Gilberry JU, Quizon JJ. Relationship between biologically fluorescent aerosol and local meteorological conditions. *Aerosol Sci Technol*. 2013;47:655-661.
37. Pereira ML, Knibbs LD, He C, et al. Sources and dynamics of fluorescent particles in hospitals. *Indoor Air*. 2017;27:988-1000.

38. Licina D, Tian Y, Nazaroff WW. Emission rates and the personal cloud effect associated with particle release from the perihuman environment. *Indoor Air*. 2017;27:791-802.
39. Bhangar S, Adams RI, Pasut W, et al. Chamber bioaerosol study: human emissions of size-resolved fluorescent biological aerosol particles. *Indoor Air*. 2016;26:193-206.
40. Yang S, Lee GW, Chen CM, et al. The size and concentration of droplets generated by coughing in human subjects. *J Aerosol Med*. 2007;20:484-494.
41. Huang J-M, Tsao S-M. The influence of air motion on bacteria removal in negative pressure isolation rooms. *HVAC&R Res*. 2005;11:563-585.
42. King MF, Noakes CJ, Sleigh PA. Modeling environmental contamination in hospital single- and four-bed rooms. *Indoor Air*. 2015;25:694-707.
43. Tang JW, Eames I, Li Y, et al. Door-opening motion can potentially lead to a transient breakdown in negative-pressure isolation conditions: the importance of vorticity and buoyancy airflows. *J Hosp Infect*. 2005;61:283-286.
44. Nicas M, Nazaroff WW, Hubbard A. Toward understanding the risk of secondary airborne infection: emission of respirable pathogens. *J Occup Environ Hyg*. 2005;2:143-154.
45. Nicas M, Hubbard AE, Jones RM, Reingold AL. The infectious dose of Variola (smallpox) virus. *Appl Biosaf*. 2004;9:118-127.
46. CDC. Bioterrorism. https://www.cdc.gov/smallpox/bioterrorism/public/index.html. Accessed July 3, 2018.
47. Ducharme J. The FDA approved an anti-bioterrorism drug meant to treat smallpox. [TIME Magazine]. July 14, 2018. http://time.com/5338775/fda-small-pox-approval/. Accessed July 17, 2018.
48. Tung Y-C, Shih Y-C, Hu S-C. Numerical study on the dispersion of airborne contaminants from an isolation room in the case of door opening. *Appl Therm Eng*. 2009;29:1544-1551.
49. Lidwell OM. The control by ventilation of airborne bacterial transfer between hospital patients, and its assessment by means of a particle tracer: II. Ventilation in subdivided isolation units. *J Hyg*. 1972;70:287-297.
50. Chen C, Zhao B, Zhou W, et al. A methodology for predicting particle penetration factor through cracks of windows and doors for actual engineering application. *Build Environ*. 2012;47:339-348.
51. Mosley RB, Greenwell DJ, Sparks LE, et al. Penetration of ambient fine particles into the indoor environment. *Aerosol Sci Technol*. 2001;34:127-136.

How to cite this article: Therkorn J, Drewry III D, Pilholski T, et al. Impact of air-handling system exhaust failure on dissemination pattern of simulant pathogen particles in a clinical biocontainment unit. *Indoor Air*. 2018;00:1–13. https://doi.org/10.1111/ina.12506