

Sanders Containment Filter

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- Culturability, and Effect of Surgical Masks

FROM THE DESK OF

SCOTT SANDERS, President

Sanders Inc.

March 8, 2020

Containment Filter

White Paper

Sanders Containment Filter's ability to capture and hold particles the size known pathogens is a true breakthrough in filtration. This new Containment filter is available to fill the gap between a viral outbreak's beginning and the time it takes to create a vaccine and it's distribution. This will greatly enhance our ability to contain the spread of respiratory viruses. The Containment Filter should be used immediately after an outbreak is recognized, during the period before a vaccine is developed and available to the general public.

Airborne contamination is a complex mode of transmission where many of the remaining communicable diseases are able to enter our body and cause harm. Airborne Transmission has the highest rate of transmission possible, indirect, personto-person. This process is explained in the ASHRAE Position Paper, "Airborne Infectious Disease," 2009 (see Appendix A). This document stipulates that Measles, Mumps, Influenza, and TB are all spread as Airborne Droplet Nuclei, submicron particles. A 2013 study conducted by the University of Maryland (see Appendix B), concluded that there are nine (9) times more culturable virus in the humidity exhaled from the lungs of a person infected with the flu, than in a cough or sneeze. We know that the coronavirus CoV-19 is spread as a respiratory disease similar to the flu. The new test kits for the corona virus recommends swabbing the inside the upper nose. If not airborne, or exhaled from the lungs, how would the virus be most prevalent in the inside of the upper nose?

One incident during the SARS Corona Virus outbreak in Hong Kong was found to infect over 100 people and killed 41 due to a sub-micron airborne cloud of virus in fecal particles that spread over very long distances as aerosols, created after a toilet flush from 1 infected person. The main method of Droplet Nuclei airborne transmission is by indirect contact in the same air space that was occupied by an infected person, earlier.

Identification of contagious individuals is not always possible during this outbreak, because people can go many days without symptoms. In order to combat this issue, we must protect ourselves from the undiagnosed or pre-diagnosed patient. These are truly the people who unknowingly spread the disease. During a respiratory outbreak, we must assume the air is contaminated, since we cannot be assured that it is not.

Sanders Containment Filter is a new, synthetic filter developed and now available, that has efficiencies *higher than HEPA*, 99.99980% @ 0.1 micron. Independently tested on virus VFE. *This* filters main advantage is that, unlike old, hard-sided, micro-fine glass HEPA's, it is a soft flexible pad with *a very low static pressure*, .17 W.C. @ 125 cfm/sq. ft. For the first time, this allows for HEPA or near HEPA filtration of the return air duct directly, in any room. This means that now all rooms may be filtered with HEPA air quality without any reconstruction costs of transforming the HVAC unit these works on all installed units. By attaching this pad over every return vent or at the main unit, you will capture most particles the size of known pathogens, as it is about to enter the HVAC system (*source capture*). The media is available in roll form and can be cut to fit in the field; this provides a user friendly, cost effective solution, never available before.

For large common areas like at airports, schools or convention centers, the filter would be cut into circles and placed on the air entering ports of a low or high velocity floor fan, up to 3000 cfm. This could easily be life saving technology when there is not an outbreak as well, preventing the airborne spread of more common disease, like Norovirus, Measles, Mumps, TB. This is not limited to hospitals, but also should be considered in schools, cruise lines, Dr, offices and any other common well-populated areas. This new method of prevention is no different than hand washing or vaccines; it removes particles the size of known pathogens thereby removing it from the air, prevent the illness from being inhaled and entering the body, therefore preventing catching the disease. Sanders Containment Filter is a new innovative breakthrough in air filtration that will totally change the way we think about clean air.



A new and unique method to help contain and remove particles, the size of known pathogens from facilities







CONTAINMENT FILTER

by Sanders, Inc.

For the first time, a filter allows for

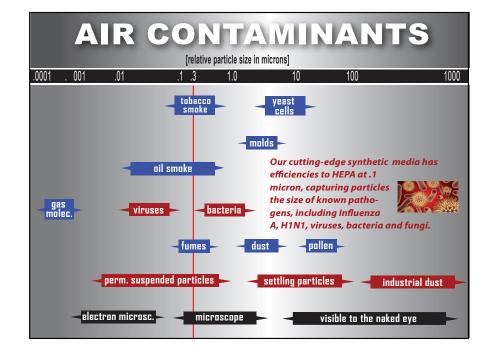


HEPA or near-HEPA filtration without the need for a retrofit of the HVAC system or holding frame.

Our filter is a soft, flexible pad with a very low static pressure,



unlike rigid micro-fine glass HEPA filters.



"Manufacturing the highest-quality synthetic air filters in the industry"

www.sandersfilters.com info@sandersfilters.com

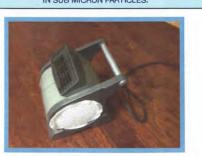
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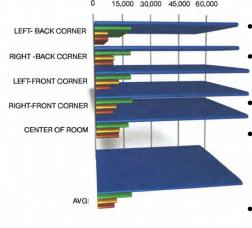


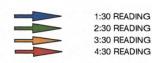
SUB MICRON PARTICLE REDUCTION

Nov 4, 2015 FLOOR DRYER TEST RESULTS 100 SERIES CONTAINMENT FILTER MEDIA, 8" **ROUND X 2, ON A 600 CFM FLOOR DRYER** TIME OF READING: (Numbers represent sub micron particles per liter of air) 1:30 PM 2:30 PM 3:30 PM 4:30 PM LEFT- BACK CORNER 51.500 4.600 4.600 12,600 RIGHT -BACK 12,300 6.600 6.300 52,000 LEFT-FRONT 56,100 12,300 8,100 RIGHT-FRONT CORNER 56,100 12,800 7,100 6,100 CENTER OF ROOM 56,000 11,300 8,100 7,800 AVG 54,300 12,260 6.900 5.980 **RESULTS: 89% REDUCTION** IN SUB MICRON PARTICLES.



IN A 3 HOUR PERIOD 15.000 30.000 45.000 60.000 LEFT- BACK CORNER





Sanders Containment Filter

- High efficiency submicron filtration has n ever been possible with a flat cut pad.
- The unpatrolled ease of use from a high efficiency submicron air filter, is now possible, by placing the Sanders
- Containment filter over the air entering ports of a floor dryer.
- This allow for immediate and convenient sub-micron filtration of any inhabited area. Scrubbing particles the size of known pathogens from any inhabited area.
- No retrofit to the existing HVAC system is required. Large common areas such as airports, schools, hospitals. Cruse ships, Dr. offices.
- Pre diagnosed people require AIR rooms that can be immediately created.
- Military barracks, or border retention holding areas.
- Literally anywhere people congregate or pass through should be filtered.

During a viral respiratory disease outbreak, "we cannot quarantee the air we breathe is clean, so we must assume it is not".

This current outbreak has brought to our immediate attention a problem we have never truly addressed.

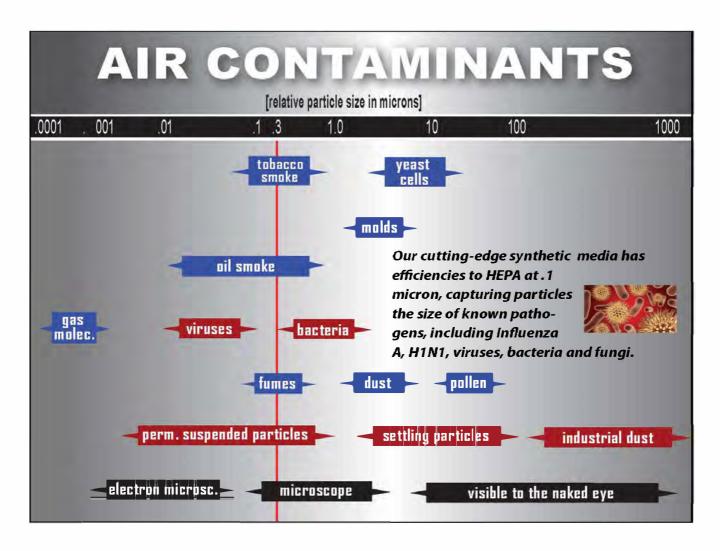
Airborne transmission has existed for many years, the outbreaks of Influenza, Measles, Mumps, Norovirus, and TB. And many others. The list is very large of the number of disease spread at least in part, as an Air-borne Droplet Nuclei sub-micron size pathogen.

These sub-micron size virus and bacteria are so small they act more a gas than a particle that allows them to stay suspended for hours or days. Viral shedding, humidity containing virus from an exhaled breath, of an infectious patient, a plume from a toilet flush and many other common activities can and do created aerosol that spread these disease.

Understanding this complex mode of transmission is crucial to slowing the spread of the disease, reducing deaths and human suffering.









Sanders Inc. 100 Series, Containment Filter

TECHNICAL DATA	WEIGHT	COLOR	MATERIAL COMPOSITION		
Medical Containment Media	100 g/m² 15 g/m²	WHITE WHITE	BLENDED SYNTHETIC FIBER SPUNBOND POLYPROPYLENE (other colors available)		
TOTAL MEDIA WEIGHT	105 g/m²				
AVAILABLE FORMS	SINGLE OR DOUBLE LAMINATED SCRIM / MELTBLOWN MEDIA ROLLS, SHEETS, COILS (SLIT TO WIDTH) & FABRICATED PARTS (INCLUDING HEAT SEALED OR WELDED)				

FILTRATION PERFORMANCE					
NaCl Penetration at 95 LPM	<20.00%	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
NaCl Efficiency at 95 LPM	> 80.00%	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
Pressure Drop at 95 LPM	< 2.1 mm H ₂ O	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
BFE Efficiency***	> 99.967%	Tested in accordance to Spec MIL-M-36954C By Nelson Labs			
VFE Efficiency***	> 99.950%	Tested in accordance to Spec MIL-M-36954C By Nelson Labs			
Air Permeability**	> 275 CFM	Tested in accordance to ASTM Spec ASTM D373			

TESTING APPARATUS / SAMPLE SIZE:

*RIG: TS18130 AUTOMATED LASER PARTICLE COUNTER

SAMPLE SIZE: 100 cm^2

**RIG: TEXTEST FX3300 AIR PEREABILITY TESTER

***VFE Efficiency = Viral Efficiency through a single pass of air ***BFE Efficiency = Bacterial Efficiency through a single pass of air



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TECHNICAL DATA SHEET

PRODUCT ID: Sanders 250 Series testing

TECHNICAL DATA	WEIGHT	COLOR	MATERIAL COMPOSITION		
Sander 250 SCRIM NETTING	250 g/m ² 15 g/m ² 180 g/m ²	WHITE WHITE CLEAR	BLENDED SYNTHETIC FIBER SPUNBOND POLYPROPYLENE (other colors available)		
TOTAL MEDIA WEIGHT	445 g/m²				
AVAILABLE FORMS	SINGLE OR DOUBLE LAMINATED SCRIM / MELTBLOWN MEDIA ROLLS, SHEETS, COILS (SLIT TO WIDTH) & FABRICATED PARTS (INCLUDING HEAT SEALED OR WELDED)				

FILTRATION PERFORMANCE					
NaCl Penetration at 32 LPM	< 0.50%	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
NaCl Efficiency at 32 LPM	> 99.50%	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
Pressure Drop at 32 LPM	< 1.6 mm H ₂ O	Tested in accordance to TSI8130 NaCl 0.1 micron particle size			
BFE Efficiency***	> 99.99995%	Tested in accordance to Spec MIL-M-36954C By Nelson Labs			
VFE Efficiency***	> 99.99980%	Tested in accordance to Spec MIL-M-36954C By Nelson Labs			
Air Permeability**	> 85 CFM	Tested in accordance to ASTM Spec ASTM D373			

TESTING APPARATUS / SAMPLE SIZE:

*RIG: TS18130 AUTOMATED LASER PARTICLE COUNTER

SAMPLE SIZE: 100 cm²

**RIG: TEXTEST FX3300 AIR PEREABILITY TESTER

***VFE Efficiency= Viral Efficiency through a single pass of air

***BFE Efficiency = Bacterial Efficiency through a single pass of air



info@sandersfilters.com www.sandersfilters.com



Nov 4, 2015 FLOOR DRYER TEST RESULTS 100 SERIES CONTAINMENT FILTER MEDIA, 8" ROUND X 2, ON A 600 CFM FLOOR DRYER

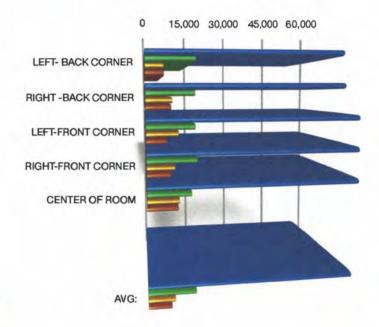
TIME OF READING:

	(Numbers represent sub micron particles per liter of air)					
	1:30 PM	2:30 PM	3:30 PM	4:30 PM		
LEFT- BACK CORNER	51,500	12,600	4,600	4,600		
RIGHT -BACK CORNER	52,000	12,300	6,600	6,300		
LEFT-FRONT CORNER	56,100	12,300	8,100	5,100		
RIGHT-FRONT CORNER	56,100	12,800	7,100	6,100		
CENTER OF ROOM	56,000	11,300	8,100	7,800		
AVG:	54,300	12,260	6,900	5,980		

RESULTS: 89% REDUCTION IN SUB MICRON PARTICLES.



SUB MICRON PARTICLE REDUCTION IN A 3 HOUR PERIOD





1:30 READING 2:30 READING 3:30 READING 4:30 READING





Airborne Influenza is the reason why there is a "flu season"

- Flu viruses are airborne and within that state are highly infectious. Airborne flu viruses penetrate into you lungs.
- 2. Breathing in only one to three airborne flu viruses will make you ill with severe flu.
- Humidity is the critical factor in how long flu viruses can live and far they can travel. Controlling indoor humidity (grains of moisture) is one key to preventing airborne flu transmission.
- 4. Schools are the "petri dish" for flu.
- 5. Washing your hands to prevent the flu is not very helpful.
- 6. There are plenty of solutions to prevent "flu season".

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Airborne Influenza Topics

- Current explanations for "flu" season
- · How do people eject flu viruses into the air?
- How does airborne flu infect people?
- · What different forms do airborne flu viruses take?
- How far can airborne flu viruses travel in a room, circulate within buildings and inside their HVAC units?
- · What conditions increase airborne flu virus survival?
- What technologies are available to sterilize, capture and/or kill (inactivate) airborne flu viruses?

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Two incorrect explanations for "flu season"



- 1. "Crowding" people spend more time indoors so they breathe & cough in closer crowded situations creating "flu season"
- 2. Cold weather makes people sicker in the wintertime which is around the time "flu season" occurs

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"Crowding"- people are in closer crowded situations because it's cold outdoors



This study looked at the correlation between cold weather "spells" when people would have to spend more time together in closer "crowded" situations, and they found no correlation to increased influenza illness.

"No consistent relations were found between various combinations of monthly mean temperatures and normalized excess deaths."

"Confidence intervals on the number of deaths attributed to cold weather are large, so we cannot conclude that influenza is a more important cause of winter mortality on an annual timescale than is cold weather."

NIH Scientists Jonathan Dushoff, Cecile Viboud, et al.
 Mortality due to Influenza in the United States 2006
 American Journal of Epidemiology v163 p181

This study is available @ GreenCleanAir.com

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Cold Weather makes people sick



It's assumed that cold weather can cause you to "catch a cold". There is no science linking being cold and being more likely to be infected with virus as a result.

"Researchers (the authors) have worked to identify and measure a seasonal component of influenza transmission with the goal of explaining large annual fluctuations in incidence. But, as we have seen here using simple models, these large fluctuations may be caused by exogenous seasonal changes in transmission that are too small to detect, amplified by the endogenous population dynamics of the host–pathogen system."

In non-scientific speak: The change in seasons is not the cause of increased influenza infections.

1. NIH Scientist Jonathan Dushoff, et al. Dynamical resonance can account for seasonality of influenza epidemics 2004 PNAS v101 p16,915

This study is available @ GreenCleanAir.com

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CDC's Top Influenza Scientist states that flu is Airborne



Dr. Nancy J. Cox Director of CDC Influenza Division

"It is generally accepted that influenza viruses are spread primarily by aerosols* of virus-laden respiratory secretions that are expelled into the air during coughing, sneezing, or talking by an infected person."

"School Absenteeism due to influenza often occurs early in the epidemic and children are believed to play an important role in disseminating the virus into the community during both epidemics and pandemics."2

1, Cox. N. GLOBAL EPIDEMIOLOGY OF INFLUENZA: Past and Present 2000 Annu, Rev. Med. v51 p407

*Droplet Nuclei are aerosols and are 5-10 microns which can stay airborne indefinitely. Even aerosols less than 20 microns can stay airborne for long periods of time. Aerosols are Not Large Droplets which are greater than 20 microns and are easily captured by the nose. Large droplets can travel@ 3-6 feet away but quickly fall to the ground preventing them from being breathed in

Expert Flu Virologist Professor Dr. John J. Treanor describes Airborne Flu Transmission



"Influenza virus infection is acquired by a mechanism involving the transfer of virus-containing respiratory secretions from an infected to a susceptible person. A number of lines of evidence indicate that **small particle** aerosols are the predominant factor in such person-toperson transmission.

The explosive nature and simultaneous onset in many persons suggest that a single infected person can transmit virus to a large number of susceptible persons."

Dr. John J. Treanor, Chapter 162, Influenza Virus This open source study is available @ GreenCleanAir.com

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CDC Flu Expert Dr. Jacqueline Katz- Author of: Influenza A virus transmission: contributing factors and clinical implications'



- "The infectivity of airborne virus in small respiratory dropletsapproximately <5 micrometers (1/1 millionth of a meter) in diameter can be very high: the infectious dose of influenza virus in humans following aerosol inhalation was reported to be as low as three (.6-3 viruses) 50% tissue culture infectious doses (TCID50)."
- "Smaller particles (<5 µm in diameter, or droplet nuclei) are capable of remaining suspended in air for longer durations of time and can be carried farther distances than large droplets, depending on the rate of particle desiccation and other environmental factors. Particles of this size are capable of penetrating deep into the respiratory tract following inhalation, which is generally not the case for inhaled large droplets."

Jacqueline Katz et al. Influenza A virus transmission: contributing factors and clinical implications 2010 Expert Reviews in Molecular Medicine

GreenCleanAir.com

How do people eject viruses into the air?

- 1. Coughing
- 2. Sneezing
- 3. Talking
- 4. Singing
- 5. Flatulence
- 6. Toilet flush aerosolization (indirectly)

Studies using DNA testing show that airborne flu viruses everywhere!



- · As an Indoor Air Quality (IAQ) testing consultant, I can attest to the difficultly of trying to capture and isolate airborne germs. Harvard's Don Milton said it best: "Infectious aerosols are usually extremely dilute, and it is hard to collect and culture fine particles."
- In 2006, virus sampling equipment was finally perfected to collect and enumerate airborne viruses. They used layers of sieves to filter out particles, bacteria and fungi to finally end up with viruses.
- The next breakthrough was DNA/RNA testing called Polymerase chain reaction (PCR). Now viruses can be precisely measured.
- Studies have found thousands of airborne flu viruses by this method.
- Approx. 90 flu "copies" found by PCR testing equals 1 "viable" particle.

How many viruses do people eject into the air?



Table 5. The calculated numbers of the respiratory droplets which are likely to contain pathogenic or commensal organisms

The calculations were based on the figures given in Tables 3 and 4.

Expiratory activity	30,000,000 commensals per ml.	1,000,000 pathogens per ml.	30,000 pathogens per ml.	1000 pathogens per ml.
One sneeze:				
Under 100μ	. 62,000	4,600	150	5
All sizes	73,000	14,000	3,100	430
One cough:				
Under 100μ	710	64	. 2	0
All sizes	910	230	47	6
Counting to '100'				
Under 100μ	36	. 3	. 0	0
All sizes	50	14	3	0

J.P Duguid The size and duration of air carriage of Respiratory Droplets and Droplet nuclei 1946 Journal of Hygiene (London) v44 p471

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^{1.} Milton, Don Roy, Chad Airborne Transmission of Communicable Infection-The Elusive Pathway New England Journal of Medicine April 2004 v350 p1710

Natural flu infection (airborne into lungs) is worse than intranasal (contact into nose)



(For nasal induced flu it takes 330 infectious flu viruses (TCID50) to get infected versus 1-3 infectious flu viruses for airborne infection in the lung.)

"To assess the relative effect of natural versus experimental (intranasal) influenza illness on pulmonary function, we compared 43 normal adults with documented non-pneumonic influenza A infection during three outbreaks, 1974 (A/Port Chalmers/74), 1975 (A/Port Chalmers/74), and 1976 (A/Victoria/75) to 24 normal volunteers following nasal inoculation with wild-type influenza A/England/42/72, A/Scotland/74 or A/Victoria/75."

In naturally acquired illness, abnormalities in small airway function and transiently increase airway reactivity were observed. In contrast, no such dysfunction was observed in experimentally induced illness. This group manifested milder illness and significantly shorter duration of cough."

Little, JW et al. Attenuated influenza produced by experimental intranasal inoculation. Journal of Medical Virology 1979;3(3):177-88.

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How many viruses are floating in a room to infect you?



In 2011 Dr. Linsey Marr of VA Tech published: "Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes"

"To determine the potential for influenza to spread via the aerosol route, we measured the size distribution of airborne influenza A viruses. Over 1 hour, the inhalation dose was estimated to be between 12 and 48 median tissue culture infectious dose (TCID50), adequate to induce infection. These results provide quantitative support for the idea that the aerosol route could be an important mode of influenza transmission."

Since it takes only 1-3 airborne viruses to infect you, at 1 virus per naïve person, fully 48 new people could be infected! Keep in mind that these were adults who are less infectious than children who can become "super-emitters" and spew out up to 200 viable flu viruses in a short time.

Linsey Marr et al. Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care centre and on aeroplanes J. R. Soc. Interface 2011 v8 p1176

This study is available @ GreenCleanAir.com

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Wake Forest Research discovers the "Super-Emitter" (New Typhoid Mary?)



Dr. Walter Bischoff discovered that flu infected persons at Wake Forest Hospital were spewing flu viruses in volume and distance. One 8yr old super-emitter spewed out 20,000 RNA copies= 200 infectious flu viruses.

This child could infect 66 (3 viruses) to 200 (1 virus) naïve classmates within 1 hour!

8 year old spewed out
20,000 RNA Flu copies

10 year old spewed out
RNA Flu copies

5 year old spewed out
6,000 RNA Flu copies

Exposure to Influenza Virus Aerosols During Routine Patient Care Walter Bischoff et al. Journal of Infectious Diseases Feb 2013

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How does Influenza A Virus infect people?



- 1. Fingers to nose
- 2. Fingers to eye
- 3. Fingers to mouth
- 4. Inhale Large droplets
- 5. Inhale Intermediate droplets
- 6. Inhale droplet nuclei
- 7. Toilet aerosolization
- 8. Sewer pipe aerosolization

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What's Influenza A Virus and how does it infect people?



- Influenza A causes disease primarily in the lungs as it loves to infect the lower respiratory tract (LRT).
- It is not a <u>rhinovirus</u> which primarily causes infection in the <u>nose</u> and upper respiratory system.
- Since your fingers can't reach into your lungs, washing your hands can't prevent flu viruses from entering deep into your lungs.
- No matter how sterile your hands are, you'll still be fully exposed to airborne Influenza viruses entering and depositing into your lungs and lower respiratory tract to cause disease.

How does Influenza A Virus kill people?



- Influenza A likes to multiply at 98.6F which is the temperature
 of the lower respiratory system. (The upper respiratory
 system- nasal cavity & pharynx- are approx. 94F which
 rhinoviruses favor for multiplication).
- · Influenza A infects and destroys its victim's lung tissue.
- Damaged lung tissue has compromised its protective layers which can lead to severe pneumonia or overwhelming bacterial infection.
- Victims can die from aggressive Staph infections like Methicillin Resistant Staphylococcus Aureus (MRSA).

Sanders Inc. Page 10

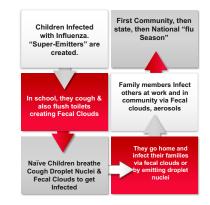
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Why are schools perfect petri dishes for Flu Transmission?

- Super-emitters Flu infected children can, with their immature immune systems become "super-emitters" and Wake Forest's Dr. Walter Bischoff discovered that an 8yr old super-emitter spewed out 20,000 RNA copies= 200 infectious flu viruses.
- <u>Dry environments</u> Many schools can have 15-25% relative humidity levels indoors! This is the PERFECT environment for airborne Viral transmission and contagion.
- Low MERV Filter Ratings Many schools have low MERV rated filters like MERV 4-6. You need a MERV 13 or higher to have any real effect on airborne viral capture.
- No Ultraviolet Lights Few schools in the US use ultraviolet lights. Schools with UV lights have enjoyed lower airborne viral transmission rates and higher indoor air
- $\underline{\textbf{Bathrooms with ceiling exhaust fans}} \ \ \textbf{Most bathroom designs do not incorporate}$ floor level exhaust vents.

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Children, Super-Emitters and National "Flu Season"



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Why Schools are the Vector Source For Flu Season





- In schools, the combination of low humidity, super-emitter children and toilet flush aerosolization are a toxic

Leading virologists Peter Wright, Gabrielle Neumann and Yoshihiro Kawaoka state that flu epidemics start in schools



Virologists Peter Wright¹, Gabrielle Neumann² and Yoshihiro Kawaoka³ state: "Increases in school absenteeism mark the beginning of a new epidemic, suggesting that school-age children play a critical role in disseminating influenza viruses. Increases in school absenteeism are typically followed by increases in work absenteeism.

These experts support my thesis that "Schools are the "petri dish" for flu." Since flu infected children can, with their immature immune systems, become "super-emitters", they easily infect their classmates as they all intermingle while traveling from classroom to bathroom to gym to lunchroom all the while super-emitters are spewing out infectious flu

Parents now have many good reasons to take a vested interest in advocating for clean and properly humidified air in the school especially in the dry wintertime.

- Professor Pediatrics, Pathology, Microbiology and Immunology Chief, Division of Pediatric Infectious Dise
 Associate Professor Department of Pathobiological Sciences School of Veterinary Medicine University of Micropain
- Associate Professor Cognition
 Toylor Misconsin
 Professor Department of Microbiology and Immunology University of Tokyo
 Field Virology 2007 Fifth Edition Page 1705

How far can Airborne Viruses Travel?



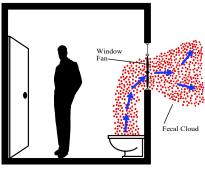
Large	Droplets/Aerosols	Droplet Nuc
1. Coughing	1-6 feet	200+ feet
2. Sneezing	8-15 feet	200+ feet
3. Singing, Talking	1-3 feet	200+ feet
4. Mouth Breathing	1-3 feet	200+ feet
5. Diarrhea*	1-5 feet+	200+ feet

^{*}As a Result of Toilet Water Aerosolization and Mechanical Fan Dispersion into outdoor air (2003 Hong Kong SARS Virus Epidemic)

Airborne SARS Transmission at Amoy Gardens Apartments 03.19-20.2003



Wang Kaixi was infected by airborne SARS viruses that he breathed in at the Prince of Wales Hospital. Since SARS produced diarrhea in the majority of patients, he flushed his toilet water likely heavily laced with SARS thereby aerosolizing his SARS viruses into the most toxic Fecal Cloud ever recorded. His window fan blew his SARS Fecal Cloud(s) outdoors where the wind and rising air currents spread them on to his unsuspecting Amoy Gardens neighbors



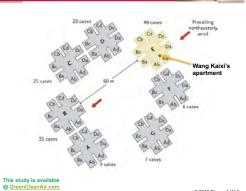
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The largest airborne infection event ever recorded-Amoy Gardens March 19-20, 2003

Retrospectively, Professor Yuguo Li documented the airborne toilet aerosolization SARS Plume created by Wang Kaixi. The plume first traveled upwards and infected nearly 100 neighbors in his building (Block E). It then traveled over 200 feet (70 meters) to infect more Amoy residents. Over 40 died.

Li, Yuguo et. al Evidence of Airborne Trans-mission of the Severe Acute Respiratory Syndrome Virus 2003 NEJM



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Over 330 people were infected downwind & 40+ were killed @ Amoy Gardens by 1 person!



Wang Kaixi's SARS toilet aerosolization plume is an amazing visual testament to the power of airborne viruses and their ability to travel long distances to infect new naïve victims.

Li, Yuguo et. al Evidence of Airborne Trans-mission of the Severe Acute Respiratory Syndrome Virus 2003 NEJM

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Stages of Infectious Droplets & Droplet Nuclei



Large droplets-20µ+

Mucus/water coa ted
 Viruses are aeros olized
 and they canit ev aporate
 fast enough and q uickly
 fall to the ground.

Small droplets/aerosols-10-20µ



 μ = micron or 1 millionth of a meter

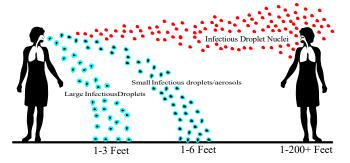
Droplet Nuclei- <10μ



Mucus/water coating has mostly evaporated leaving the virus with protein & salts. This is a Droplet Nuclei . Droplet Nuclei are so micro-scopic that they can float in the air indefinitely

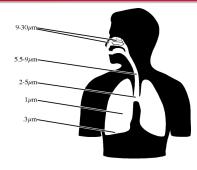
Infectious Droplets & Droplet Nuclei travel lengths





Droplet Nuclei Viruses are .3µ or Less & Penetrate Deeply into the Human Lungs

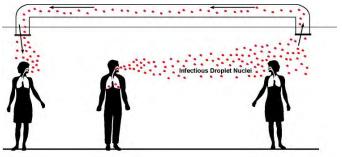




A µm is a micron or 1/1,000,000 of a meter. The smallest particle you can see is 30µm.

Droplet Nuclei Travel Within Buildings





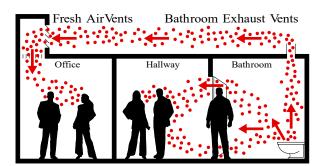
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How Toilets Aerosolize Flu Viruses Recirculation Vents suck them back in





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Toilet Water Viral Aerosolization





- Since 1955, many studies have documented how a toilet flush aerosolizes bacteria and viruses into the air above the bowl.
- Many scientists flushed toilet bowl water infected with a known quantity of viruses.
- British Scientist John Barker¹ in 2005, (post 2003 SARS Amoy Garden event) replicated the viral load and consistency of diarrhea. He added that to toilet water, flushed the toilet and took air samples to capture the aerosolized droplets. They were full thousands of viruses.
- 60 minutes afterwards every flush aerosolized additional viruses because porcelain is porous enough to harbor viruses (bacteria also).

Barker, John The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. 2005 Journal of Applied Microbiology v99 p339

This study is available GreenCleanAir.com

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Toilet Water Aerosolization Studies Reviewed by Dr. David Johnson for the CDC





"It may be concluded from the peer-reviewed studies discussed above that flush toilets of various designs spanning at least 50 years of production in Europe and the U.S. have been shown to produce substantial quantities of aerosol, that these aerosols are capable of entraining microorganisms at least as large as bacteria (includes viruses which are 10 timer smaller*), that such bioaerosols will be produced during multiple flushes after toilet contamination, that sufficiently small microbe-laden droplets will evaporate to form droplet nuclei bioaerosols the size of which can be consistent with that associated with respirable penetration, and that these droplet nuclei bioaerosols may remain viable in the air for extended periods and travel with air currents."

Toilet Plume Aerosol Occupational Hazards to Healthcare Facility Workers: A Review of the Literature with Suggestions for Future Research David L. Johnson, PhD, PE, ClH 2011 CDC funded this research so it is available @Greencleanizcom Dr. Johnson, Dr. Ken Mead et al. edited, peer reviewed and published online paper in the American Journal of Infectious Diseases, Oct. 2012 is titled: "Lifting the lid on toilet plume aerosol: A literature review with suggestions for future research".

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Toilet Water Viral Aerosolization



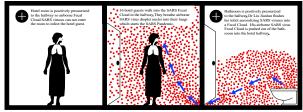
The 2003 SARS epidemic showcased the lethality of toilet water aerosolization which created Fecal Clouds in these published accounts:

- <u>Dr. Liu Jianlun</u> was the Chinese Doctor who initiated the worldwide SARS
 pandemic when he stayed in Hong Kong at The Metropole Hotel in February 2003.
 - Infected with SARS and having diarrhea, he infected 16 fellow hotel guests and
 - ${\bf 1}$ visitor through toilet water aerosolization. Those travelers flew around the world and one brought SARS to Toronto thereby devastating the city.
- Wang Kaixi was infected with SARS at the same hospital which was treating a SARS infected patient who visited a hotel guest's whose room was on the same hall as Liu Jianlun at the Metropole hotel.
 - ► Infected with SARS and having diarrhea, he infected over 300 Amoy Garden residents through toilet water aerosolization. Many lived over 200 feet away from his apartment.

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Airborne SARS Transmission at The Metropole Hotel 02.21-22 2003





The above scenario is different from the current belief that Dr. Jianlun spread his SARS viruses to his fellow Hotel guests by vomiting on the carpet outside his room. The currently accepted vomit theory is probably due to the World Health Organization's investigators speculating that Dr. Liu Jianlin may have vomited on the carpet outside his room. "It was speculated that he might have vomited, spit or heavily coughed near his room and, thus, contaminated this area of the corridor. In case of a vomit, the hotel staff might have been called for clean up. However, there is no record of such an incident." In addition, The Hong Kong Health authority called Mrs. Jianlun who said her husband Liu never vomited.

1. Page 8 The WHO Metropole Report 2003 available @ GreenCleanAir.com

The Metropole Hotel 02.21-22 2003





WHO investigators validated major parts of the above scenario confirming that Metropole guest rooms: "proved to be at positive pressure with respect to the corridor....(so) contaminated air could leave a room and transfer into the corridor with all doors closed." The rooms had wall air conditioning units (fan coil) which brought in outdoor air. When operating, the fan coil units created this situation: "The positive pressure slightly increased when the operating status of the fan coil was changed from stopped to low, medium and high fan speed. As expected, the higher fan speed produced higher room pressure and thereby higher room airflow from below the door" ² and out into the hallway corridor.

They also confirmed the Fecal Cloud creation scenario using a laser particle counter: "Particle counting was done at the rim of the WC and again approx. 300 mm (12 inches) above the WC during flushing. The tank flush produced approximately 0.2 mg/m³ in air." ³ These airborne droplet readings are the material evidence of the

1. Page 8, 2. Page 5, 3. Page 11 in: Final Report Metropole Hotel WHO 2003 available @ GreenCleanAir.com

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Sanders Inc. Page 13

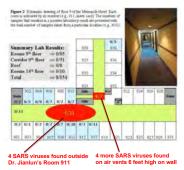
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The World Health Organization Metropole Hotel SARS report



The WHO investigators found SARS viruses on the carpet outside Dr. Jianlun's room and the two rooms on either side of him on April 27, 2003 (2 months later). Since the vast SARS debris field is at least 30 feet long, the vomit theory becomes less tenable. It's more logical that the WHO investigators found the settled droplet nuclei SARS es of Dr. Jianlun's Fecal Cloud on the carpet. In addition, they found SARS viruses on the air vent opening on the wall near the elevators which is over 6 feet above the floor and far from room 911.

That SARS viruses were found in spite of massive cleaning efforts: "It is interesting to note that genetic material (SARS) could be detected after almost two months and following an extensive decontamination and clean up in the hotel, particularly floor 9 and the associated guest rooms." 1



1. Page 5, Final Report Metropole Hotel WHO 2003 available @ GreenCleanAir.com

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2006 World Health Organization SARS report-Metropole Hotel Chapter 14



This illustration from a 2006 WHO report¹ shows how the airflows were moving on April 27^m, 2 months post-facto but needs clarification using the 2003 Final report. The air flowing out of the rooms is correct. But, "Corridor air also drifted towards the elevators. Corridor air movement in the vicinity of the rooms under study is very slow, with a drift towards the elevator lobby where an air extraction takes place... aerosols would slowly travel towards the elevator lobby".2 In addition, the report notes: "The air movement is so slow that a person walking into the corridor can cause a reversal of airflow".2

You may now appreciate how the Fecal Cloud moved up and down the corridors of the 9th floor, starting in the evening of the 21st and into the 22nd even after Dr. Jianlun finally went to Kwong

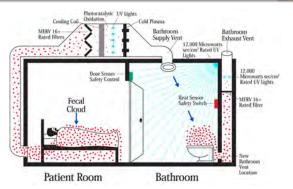
1. SARS: How a global epidemic was stopped 2006 WHO 2. Final Report Metropole Hotel WHO 2003

both are available @ GreenCleanAir.com

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Hospital Toilet Droplet Nuclei Infection Prevention





What conditions increase airborne flu virus survival, which increases infection probability?



- 1. Not being removed from indoor air by exhaust fans to outdoor air
- 2. Indoor Relative humidity below 40% at 70° (20% even better)
- 3. Not Captured by Gasket Sealed Nano-Rated HEPA Filters
- 4. No Exposure to Ultraviolet Light- "C" band, "germicidal" photons
- 5. No Exposure to Cold Plasma generated from needle-point units
- 6. No Exposure to Photocatalytic Oxidation Hydroxyl Radicals
- 7. No Exposure to Aerosolized Hydrogen-peroxide vapors

Does Humidity matters to airborne flu viruses? Yes.



- In 2011 Dr. Linsey Marr of VA Tech also published: "Dynamics of Airborne Influenza A Viruses Indoors and Dependence on Humidity"
- "Humidity is an important variable in aerosol transmission of Influenza A Viruses because it both induces droplet size transformation1 and affects Influenza A Viruses inactivation rates².....aerosol transmission route plays a significant role in the spread of influenza in temperate regions and that the efficiency of this route depends on humidity.'
- Her recommendation: "Maintaining a high indoor Relative Humidity and ventilation rate may help reduce chances of Influenza A Viruses infection.
- Since mucus is mostly water and surrounds the virus, low humidities evaporate mucus faster making the virus aerosol lighter and easier for human to suck down into their lungs. Droplet nuclei are the easiest to inhale deeply.
- 2. Since viruses aren't alive, you technically can't "kill them, you "inactivate" them making them non-viable/noninfectious.

Concentrations and size distributions of airborne influenza A viruses measured indoors at a health centre, a day-care cen and on aeroplanes J. R. Soc. Interface 2011 v8 p1176

centre This open source study is available @ GreenCleanAir.com

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VA Tech's Dr. Linsey Marr discovered why flu viruses love low humidity!



- In 2012 Dr. Linsey Marr of VA Tech published her experiments spraying human mucus with flu into the air with different humidities. She discovered that mucus's protein protects flu viruses from mucus salts in 50%rh or less air
- "Our findings in human mucus could help explain, at least in part, the transmission patterns of influenza. In temperate regions, wintertime heating reduces RH in the indoor environment to low levels, usually 40% (or less*).
- Low RHs not only help preserve the viability¹ of Influenza A Virus but also enable Influenza A Virus carrier² aerosols to persist longer in air because of their smaller size and lower settling velocities3 that result from more vigorous evaporation. Thus, transmission of influenza in temperate regions could be enhanced in winter primarily via the aerosol route.
- 1. Viruses are not "alive", so viable means being able to infect someone. Viruses hijack your cells, tricking them into making more viruses.
 2. Carrier is a person who may be sick and experiencing flu symptoms. Asymptomatic carriers have no symptoms but can infect people via aeroscis or total earosciscisco trotal earoscisciscisco.
 3. Settling velocities is how fast aeroscis fall to the ground. Microscopic droplet nuclei aeroscis are so light that they have a negligible settling velocity meaning that they can stay airborne for days or more!

 Added by Steven A Welty

 Added by Steven A Welty

 Added by Steven A Welty

Linsey Marr, et al. Relationship between Humidity and Influenza A Viability in Droplets and Implications for Influenza's Seasonality 2012 PLOS Journal v7, e-page 46789

@ GreenCleanAir.com

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Dr. G.J.Harper-1963 Experiment showed Influenza Survived in low humidity

The Influence of Environment on the Survival of Airborne Virus Particles in the Laboratory

G. J. Harper

"These results do show that relative humidity, temperature..are of great importance in determining the ability of viruses to survive in air long enough ... for transmission to the respiratory tracts of susceptible hosts"

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Dr. G.J.Harper-Experiments on Humidity's effect on Influenza Survival



Airborne micro-organisms: survival tests with four viruses

By G. J. HARPER

Microbiological Research Establishment. Porton Down. Salisburn. Wilts

Table 1. Viability of airborne virus 0-23 hr. after spraying

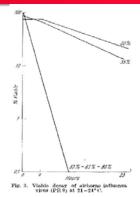
133		No.		Perce	ntage vial	ble at giv	en times (hr.)	
Temp. (°C.)	R.H. (%)	of tests	0*	1 2	1/2	1	4	6	23
			(b)	Influen	45.5				
7·0-8·0 43-46°	23 - 25	3	88	87	80	78	68	63	61
	51	3	66	49	75	61	39	42	61 19
20·5-24·0 69-75°	20-22	5	75	77	65	64	74	66	22
	34 - 36	3	86	93	58	59	66	53	22 14
	50 - 51	3	84	62	49	29	6-4	4.2	Trace
	64-65	3	77	45	29	15	6-6	3.2	N.D.
	81	4	67	55	22	13	6-4	5.0	Nil

@0040 Ottom A 187-16

Dr. G.J.Harper-Experiments on Humidity's effect on Airborne Influenza Survival



Viable decay of influenza (Fig. 3) Influenza virus showed a uniformly high viable decay rate at relative humidities above 50%. (Values at 50%, 65%, and 80% were so similar they are represented here by a single line.) After 4 hours, viabilities were around 6% at lower relative humidities, 20% and 35%, viable decay was slow, 14—22% viability being found in clouds 23 hours old.



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Leading virologists Peter Wright, Gabrielle Neumann and Yoshihiro Kawaoka state that low humidity is a critical factor to flu transmission



Virologists Peter Wright¹, Gabrielle Neumann² and Yoshihiro Kawaoka³ state: "The low relative indoor humidity during the winter months is believed to prolong the survival of influenza in aerosols and is believed to be responsible for the seasonal pattern in the northern hemisphere. The most effective spread among humans are aerosols. Most aerosol droplets formed during sneezing or coughing are less than 2 microns in diameter (droplet nuclei), and are preferentially deposited in the lower airways of the lung. Volunteers are readily infected by aerosol transmission. The often sudden onset of epidemics suggests that an infected individual can transmit the virus to a relatively large number of people.⁴

- Professor Pediatrics, Pathology, Microbiology and Immunology Chief Division of Pediatric Infectious Diseases Vanderbilt University School of Medicine
- 2. Associate Professor Department of Pathobiological Sciences School of Veterinary Medicine University of
- Wisconsin
- Professor Department of Microbiology and Immunology University of Tokyo
 Fields Virology 2007 Fifth Edition Page 1704

Droplet Nuclei Travel Within

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<u>Low</u> Indoor Humidity Increase Airborne Droplet Nuclei Levels



- Viruses Evaporate faster in Low Humidity levels (technically low grains¹) thus creating More Droplet Nuclei.
- Low humidity allows droplet nuclei to stay airborne longer as the droplets do not absorb extra water weight which would cause them to fall to the ground.
- Indoor Air currents both created by HVAC systems and people movement and their heat plumes assure that droplet nuclei will remain airborne nearly indefinitely indoors.
- This allows HVAC systems to redistribute droplet nuclei viruses throughout the building to infect more occupants.

Buildings



1. See my January 2010 article about this @ Greencleanair.com

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Correlation between low indoor humidity and increases in influenza morbidity and mortality



- Indoor wintertime humidity levels in the Northern Hemisphere especially in North America and Europe are between 15-35%.
- Since influenza loves low humidity air, the correlation between low indoor humidity and increases in influenza morbidity and mortality is logical given the correlation of airborne droplet nuclei creation and available contagion to infect humans.
- 3. What now establishes how one part of flu season is triggered is a new study¹ by the University of Virginia's Robert Davis linking the correlation between dry cold arctic air masses which descend upon New York City and subsequent flu deaths.

The Impact of Weather on Influenza and Pneumonia Mortality in New York City, 1975–2002: A Retrospective Study Davis, Robert PLoS 2012 v7 e-page 34091.

This open source study is available @ GreenCleanAir.com

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The Answer, My Friend, Is Blowing in the Wind* (Blame Canada!**)

Scientific Study: 17 days after **Dry & Cold** Canadian Air hits New York City: Influenza deaths increase

Cold & Dry Arctic Air Cold & Dry Arctic air masses descend upon NYC. Cold and dry Outdoor air is sucked indoors lowering indoor humidity.



Cold & Dry air allows efficient <u>Outdoor</u> flu transmission. More <u>outdoor</u> Flu Viruses can live longer to infect humans both outdoors and be sucked indoors.

Ideal Indoor Flu Survival & Transmission

Cold & Dry air is sucked into buildings increasing <u>Indoor</u> flu transmission. Heated air lowers Indoor Humidity even more. Indoor Flu Viruses can live longer to infect more naïve humans.

Davis, Robert The Impact of Weather on Influenza and Pneumonia Mortality in New York City, 1975–2002: A Retrospective Study 2012 PLOS v7 e-page 34091

*® Bob Dylan **® Southpark Creations

This open source study is available @ GreenCleanAir.com

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What technologies can sterilize, capture and/or kill (inactivate) airborne flu viruses?



- 1. Being removed from indoor air by exhaust fans to outdoor air
- 2. Indoor Relative humidity above 45% at 70° (50% even better)
- 3. Captured by Gasket Sealed Nano-Rated HEPA Filters
- 4. Exposure to Ultraviolet Light- "C" band, "germicidal" photons
- 5. Exposure to Cold Plasma/Bi-Polar from needle-point units
- 6. Exposure to Photocatalytic Oxidation Hydroxyl Radicals
- 7. Exposure to Aerosolized Hydrogen-Peroxide vapors

Ultraviolet Light can "Kill"/ Sterilize this % of Flu Viruses:



UVR Rating	%Viruses Killed/Sterilized
6- (75mw)	4.4%
7- (100mw)	5.8%
8- (150mw)	8.5%
10- (500mw)	25.7%
13- (2000mw)	69.5%
15- (4000mw)	90.7%
16- (5000mw)	94.9%

mw=Microwatt

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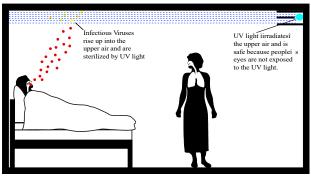
What is Ultraviolet Light and how does it work?



- Ultraviolet Germicidal (germ-killing) light is UV light in the "C" band (254 nanometers). It is invisible and is mostly filtered out our of sunlight before it reaches earth's surface. UV-C light <u>Sterilizes</u> germs by destroying the "T" bonds in their DNA. This prevents them from reproducing and they soon die.
- UV was artificially created in the 1890's and later commercially used to kill waterborne viruses & bacteria in France in 1909 for safe drinking water in Paris and other cities.
- By the 1930's Duke University surgeons were using in in operating rooms to reduce airborne bacterial and viral infections. In the 1930's and 1940's UV light was used in schools to successfully prevent airborne measles epidemics and in hospitals to prevent airborne disease transmission in the nurseries.

How Upper UV Room works to prevent airborne virus transmission



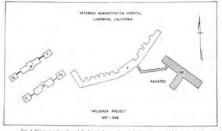


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Veteran's Hospital 1957 Flu Pandemic **Upper Room UV Study-100% Effective**







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Harvard Professor James McDevitt 2008 upper room UV virus Experiment

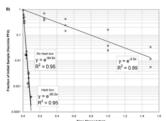


Professor McDevitt installed upper room UV lights to replicate the success of the 1957 Flu pandemic.

"Air disinfection using upper-room (UV) light can lower the airborne concentrations of infective organisms in the lower part of the room, and thereby control the spread of airborne infections among room occupants.

These data demonstrate that upper-room UVC has the potential to greatly reduce exposure to susceptible viral aerosols. These data may also be relevant to influenza, which also has improved aerosol survival at low RH."

Inactivation of Poxviruses by Upper-Room UVC Light in a Simulated Hospital Room Environment McDevitt, James 2008 PloS ONE v3 e-page 3186.



(inactivated) in just 6 minutes (.1 hour)

This open source study is available @ GreenCleanAir.com

Harvard Professor James McDevitt



room UV lights to replicate the success of the 1957 Flu pandemic and this time he used airborne influenza viruses.

"Using our experimental system, we measured influenza reductions as low as 98.2% by comparing samples with the UV light on to subsequent samples control samples with the UV light off.

This work provides an essential scientific basis for designing and utilizing effective upper-room UV-C light installations for the prevention of the airborne transmission of influenza."

Aerosol Susceptibility of Influenza Virus to UV-C Light McDevitt, James et al. Applied Enviror Microbiology 2012 v78 p1666.

This study is available @ GreenCleanAir.com

UCLA School of Medicine UV Experiment to kill Influenza-100% Effective



Inactivation of Airborne Viruses by Ultraviolet Irradiation

MARCUS M. JENSEN

tment of Medical Microbiology and Immunology, School of Medicine, University of California, Los Angeles, California Received for publication 11 May 1964

TABLE 1. Inactivation of viral aerosols during passage

1,4000 11	through a h				,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		
Virus	Concn of virus suspension†	Amt of viral suspen- sion dis- pensed UV celi		No. of virus PFU collected per ft ² of air with		Percentage of virus inactiv- ated by UV light	
		per min		UV off	UV on	UV light	
		mi mi	ft!/min				
Adenovirus	3.4 × 10*	0.144	100	29,235			
Coxsackie B-1.	4.0 × 10 ⁷	0.143	200 100	28,016 10,755			
			200	9,000			
Influenza A	1.0 × 10	0.145	100 200	920 690	0	>99.86	
Sindbis	7.5 × 10*	0.150	100	5,644	26	99.53	
Vaccinia	1.0 × 10*	0.128	200 100	3,793			
	2.0 × 107	0.142	200	2 265		>99.96	

0 Infectious Flu Viruses at 100 & 200 cubic feet per min (cfm)

This study is available @ GreenCleanAir.com

Mechanical Air Filters can trap this % of Swine Flu Viruses:



•	
1-5	1-5%
6	6.2%
7	7%
8	11%
10	12%
13	46%
15	71%
16	76%
17 (HEPA)	99.9%

Viruses can be captured & sterilized with a combination of MERV Filter & **URV** rated **UV-C** Light



- · Adding filters and UV together in successive layers can provide a lethal force to prevent distribution of airborne viruses into occupied spaces.
- A MERV 10 filter alone captures only 10% or flu viruses whereas adding a Ultraviolet rating of URV 10 triples that total single pass capture/sterilize to 35%.
- A MERV 13 alone has an 84% capture/sterilize rate with the addition of UV light. That is a very achievable goal for any
- Adding additional UV lamps can achieve a total single pass capture/sterilize of 99.9%.

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MERV rated filters & UV lights prevent airborne influenza



		Filter Model and Removal Rates, Fraction						
Pathogen	Mean size, μm	MERV 6	MERV 7	MERV 8	MERV 10	MERV 13	MERV 15	MERV 10
Influenza	0.098	0.062	0.07	0.11	0.12	0.46	0.71	0.76
Table 2. Ultray	riolet Germicidal Irradia	tion Kill Rates	of Design Ba	sis Biological	Weapon Agents			

Pathogen	(cm ² /μW-s)	$75~\mu W/cm^2$	$100~\mu W/cm^2$	$150~\mu W/cm^2$	$500~\mu W/cm^2$	$2000~\mu W/cm^2$	$4000~\mu W/cm^2$	5000 μW/cm
Influenza	0.001187	0.044	0.058	0.085	0.257	0.695	0.907	0.949
Table 3, Com	bined Removal Rat	es for Biologic	al Weapon Age	ents				
		FILTRATION	AND LITRAVIO	DLET GERMICI	DAL IRRADIAT	TON REMOVAL	RATES FRACT	ON

URV 8

0.19

Wladyslaw Modeling Immune Building Systems for Bioterrorism lournal of Architectural Engineering, 2003 Vol. 9, page 86

0.10

URV 6

0.12

MERV 13 URV 13

0.84

URV 10

MERV 10 URV 10

0.35

URV 13

URV 15

0.97

0.988

Combined UV Light & Filtration Capture Kill/Sterilize this % of Flu Viruses:



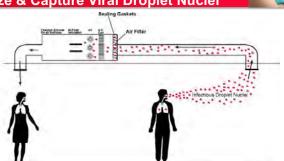
MERV & UVR Combined	%Viruses Killed/Sterilized
6	10%
7	12%
8	19%
10	35%
13	84%
15	97%
16	98.8%

Photocatalytic Oxidation (PCO), Cold Plasma/Bi-Polar Ionization



- Photocatalytic Oxidation is created when Ultraviolet light photons strike Titanium Di-Oxide to create Hydroxyl radicals. These newly liberated airborne Hydroxyl radicals can rupture and destroy the cellular material of viruses & germs they encounter.
- · Cold Plasma/Bi-Polar Ionization creates positively and negatively charged oxygen and hydrogen molecules which act like hydroxyl radicals and destroy germ's cell wall and inner cellular material.

Air Filters, UV Lights, P.C.O. and Cold Plasma/Bi-Polar Ionization Can Kill, Sterilize & Capture Viral Droplet Nuclei



The % of influenza captured, sterilized or killed will depend upon the Air Filter's MERV rating, intensity of Ultraviolet Output, the total surface area coated with Titanium Dioxide and the Bi-Polar ionization Output

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Cases of Ultraviolet Lights Preventing Indoor Virus transmission and infection



- 1. Germantown Friend's School 1942. Am J Public Health Nations Health. 1943 (Measles)
- 2. Livermore Veterans Hospital-1957. American Review of Respiratory Diseases. 1961 (1957 Flu Pandemic)

Japanese Hospital **Humidity Guidelines**



		summer		winter		
section	location	dry-bulb temperature (°C)	RH (%)	dry-bulb temperature ("C)	RH (%)	
hospital ward	patient bedroom* nurse station day room	24 - 26 - 27 24 - 26 - 27 26 - 27	50-60 50-60 50-60	22-23-24 20-22 21-22	40-50 40-50 40-50	
outpatient department	consulting room ^b waiting room dispensary ER	26 - 27 26 - 27 25 - 26 23 - 24 - 26	50-60 50-60 50-55 50-60	22 - 24 22 - 24 20 - 22 22 - 26	40 - 50 40 - 50 40 - 50 45 - 55 - 60	
central medical care areas	operation room recovery room ICU birthing room* newborn baby room general survey room X-ray studies room* kydrotherapy treatment room*	23 24 26 24 26 24 26 24 25 26 26 27 25 26 27 25 26 27 25 26 27 25 26 27	50 60 50 60 50 60 50 60 50 60 50 60 50 60 50 60 50 60	22 26 23 25 23 25 23 25 23 25 25 27 20 22 24 25 20 22 26 28	45 55 60 45 50 55 45 55 55 45 55 55 45 56 60 40 50 40 50 50 65	

Julian Tang , MD, PhD. J. Royal Soc. Interface (2009) v6, pS737 This study is available @ GreenCleanAir.com @ 2013 Steven A Welty

Now Liquid Desiccation systems can produce Clean Humidity



- New Patented Liquid Desiccant systems can add humidity to the air through micro-pores.
- This solves the problems of bacterial and fungal contamination that current steam and water spray humidification systems. These systems can cause downstream contamination in the ductwork when droplets fall out and wet the surfaces.
- See my May 2010 article in Engineered Systems for more information @GreenCleanAir.com.

1. Wash your hands.

2. Cover your cough.

on preventing the Flu

3. If you're sick, stay home.

Public Health Officials advice

4. Get a Flu vaccination

This advice doesn't address the problem of studies showing that up to 40% of infected influenza carriers have no symptoms.

It also doesn't address both human airway aerosolization and toilet water flush aerosolization of viruses. These both are critical modes of airborne infectious disease transmission within indoor spaces.

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Can Hand washing prevent flu transmission? CNN's Elizabeth Cohen challenged the CDC



In my June 2009 EPA Flu presentation, I said: "Since your fingers can't touch your lungs, washing your hands won't likely prevent flu viruses from entering deep into your lungs." I did this to indirectly challenge the CDC's recommendation, widely heralded by the media that, aside from a flu shot, the best advice to prevent you from getting the flu was to "wash your hands". I knew that there was no published scientific study anywhere which showed that someone with flu viruses on their fingers could infect themselves.

In September 2009, CNN Medical reporter Elizabeth Cohen was the first correspondent that pressed the CDC to produce the scientific documentation backing up their hand washing/sanitizing recommendation.

Her actions forced CDC to admit that hand washing to prevent influenza flu transmission was **not supported by any peer-reviewed, published papers anywhere**: "We don't have solid data on the effect that hand washing has on the transmission of H1N1 (flu virus)," CDC spokesman Tom Skinner wrote in an e-mail to Ms. Cohen. That "lack of solid data" really means there's no published data or paper or successful experiment showing someone getting the flu by hand inoculating themselves by touching their nose, lips, eye or mouth.

"Some doubt hand washing stops H1N1" CNN Elizabeth Cohen September 24, 2009

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More expert dismiss hand washing to prevent flu transmission



- In Ms. Cohen's article "Some doubt hand washing stops H1N1" (link below) she posits: "Hand washing: A false sense of security from H1N1? Some infectious disease experts said they're concerned messages from the CDC to wash hands to prevent H1N1 have given people too much faith in hand washing.
- 'Washing hands really is wonderful for preventing many diseases, such as the common cold, but it's not very helpful to prevent influenza,' said Arthur Reingold, professor of epidemiology at the University of California-Berkeley.' 'Everyone's eager to promote hand washing, and certainly it won't do any harm, but to rely on a hand washing as a way to prevent influenza is a serious mistake,' said Reingold.
- Dr. Monto is a world renown influenza expert with over 60 peer reviewed & published articles on influenza: 'Don't kid yourself that you're going to protect yourself from the flu completely by washing your hands, 'said Arnold Monto, a professor of epidemiology at the University of Michigan School of Public Health.'
- She also reported: "Dr. Peter Palese, a professor of medicine and infectious diseases at Mount Sinai School of Medicine in New York City, said 'hand washing isn't all that helpful against the flu because the flu isn't like other respiratory diseases. 'The flu virus isn't very stable on the hand,' he said. 'The virus has a lipid membrane that flattens out when it's on your hand, and it gets inactivated."

"Some doubt hand washing stops H1N1" Elizabeth Cohen September 24, 2009

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Recommendations to prevent and mitigate airborne flu transmission



- 1. Seal your filter rack & HVAC system.
- 2. Get the highest MERV rated filter that your air handling fan can tolerate.
- Put as much UV light within your coil plenum to achieve a 99.9% single pass kill rate along with Upper Room UV.
- Add Cold Plasma/Bi-Polar Ionization, Photocatalytic Oxidation and Nano-rated HEPA Filtration for viral capture and inactivation.
- Install bathroom exhausts 1-12" above the floor behind the toilet to capture aerosolized toilet water. Supply in ceiling.
- 6. Coughing/sneezing occupants wear a mask or stay at home.

How to Solve Flu Season



Raise
Humidity
to 45%+

Increase
air
changes
to 12 per
hour

Nom
Lowered
Lower

Flu Season Eliminated!

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Flu season is not a necessary evil, so: "Live Long and Prosper" by implementing my scientific based recommendations herein!



Old Chinese Proverb
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Toilet Aerosolization Studies



1959. Infective hazards of Water Closets. Darlow, HM, Bale WR. Lancet v6:17084) p1196: "Any process involving the splashing or frothing produces droplets, which remain suspended in the air for a variable period depending upon the mass and evaporation-rate of the droplets, and the velocity and direction of the local air currents. Apart from explosive exhalations such as coughs and sneezes, the commonest process predisposing to the formation of infective aerosols must surely be the flushing of a water-closet." More information about this article is available @ GreenCleanAir.com.

1975. Microbial Hazards of Household toilets: Droplet Production and the Fate of Residual Organisms. Gerba, Charles Applied Microbiology 1975 v2 p229 "It appeared that significant numbers of bacteria and viruses were being absorbed to the toilet proceialin and then eluted during the flushing action... viruses from experiments performed several days earlier were still present in the room. Click here for copy @ National Library of Medicine.

1985. Method of detecting Viruses in Aerosols. Appl Environ Microbiol. Wallis, C. v50 p1181 Recovered an average of 1500 airborne viruses due to a toilet flush. Click here for copy @ National Library of Medicine

2000 . Survival of Salmonella in bathrooms and toilets in domestic homes following salmonellosis. Barker John, Journal Applied Microbiology 2000 Jul v89 p137 Click here for copy @ Journal of Applied Microbiology.

2005. The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. Barker, John Journal of Applied Microbiology v99 p339. "Aims: to determine the level of aerosol formation and fallout within a toilet cubicle after flushing a toilet contaminated with indicator organisms (viruses) at levels required to mimic pathogen shedding during infectious diarrhea." Airborne viruses were still aerosolized 30 minutes and 60 minutes after the first flush. Click here for copy @ Journal of Applied Microbiology.

Airborne Droplet Nuclei Infection Studies



1966. Human Influenza from Aerosol inhalation Alford, RH Proceeding of the Society Environmental Microbiological Medicine v22 p800 Found that it took only. 6 to 3 viruses to infect "volunteers" with aerosolized influenza. Contrast that with studies showing it took 330 viruses to infect someone nasopharyngeally. More information about this article is available @ GreenCleanAir.com

1970. An Airborne Outbreak of Smallpox in a German Hospital and its Significance with Respect to other Recent Outbreaks in Europe. Bulletin of the World Health Organization. The a recent outbreak not epidemiological studies have clearly indicated that 17 of the cases were infected by virus particles disseminated by air over a considerable distance within a single hospital building ... several features ... were common similar to a similar outbreak in the Federal Republic of Germany in 1961 in which airborne transmission also occurred. This open source study is available @ GreenCleanAir.com.

Nosocomial Influenza Infection as a cause of Intercurrent Fevers in Infants. Hall, Caroline Breese Pediatrics. V55 p673 "Six of seven infants shed the virus for 7 to 21 days." More information about this article is available

1979. Indoor Spread of Respiratory Infection by Recirculation of Air. Riley, Richard Bulletin of European Physiopathology Respiratory 175 p699 One measles-infected student went on to infect 28 others in classrooms throughout the school. "The wide distribution of the 28 cases among children who had never occupied the same room as the index patient and the fact that about 70 per cent of the air was recirculated and hence shared by all the children served by the ventilating system, led to the conclusion that measles reached the different classrooms by way of the ventilating system. 93% of the first generation infections could have been prevented by disinfecting recirculated air. This would have aborted the entire outbreak. More information about this article is available \(\text{Oregan Green Clean Air com} \)

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Airborne Droplet Nuclei Infection Studies (cont.)



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An outbreak of Influenza aboard a commercial airliner. Moser, MR 1979. American Journal of Epidemiology v110 p1. Of the 53 passengers on the plane, 38 (72%) became infected with the same strain of influenza as a passenger with the flu. "Spread of Influenza is via droplets or droplet nuclei and the period of infectivity of these particles is prolonged by low humidity." More information about this article is available & Green Cleanakir come.

Airborne transmission of Chickenpox in a Hospital. Leclair, JM New England Journal of Medicine v302 p450 Chickenpox patient infected 13 other patients not only through indoor air but through her open window which, like Wang Kaixi, allowed air currents to blow her viruses downwind to infect others. "Her room was at positive pressure with respect to the hall and the outside of the building, these conditions promoted the escape of virus contaminated air. Once in the hall, air, presumably bearing droplet nuclei, was blown into the other rooms of the ward." More information about this article is available @ GreenCleanAir.com.

Measles Outbreak in a Pediatric Practice. Bloch, Alan 1985 Pediatrics. V75 p676 "Airflow studies demonstrated the droplet nuclei generated in the examining room used by the index patient were dispersed throughout the entire office suite. (Large) droplet spread is utilikely because three of the patients with secondary cases were never in the same room as the source patient. More information about this article is available @ GreenCleanAir com

A Measles Outbreak at University Medical Settings Involving Health Care Providers Sienko, DG American Journal of Public Health 1987 Y77 p1222 "In 1985, a measles outbreak involved 14 students and non-student contacts in Michigan. Eight transmissions occurred at university medical facilities; five of these were likely airborne transmissions. Medical students and a medical resident were involved in the outbreak's propagation." More information about this article is available @ CeasonCleak die.

Selected Viruses of Nosocomial Importance. 1998 Hospital Infection, 4th Edition. "Influenza A and B viral infections are among the moist communicable diseases of humans. Person to person transmission is believed to take place primarily by droplet nuclei. These aerosols help account for the explosive nature of influenzy outbreaks, since, in a closed environment, one interfed person can potentially infect large numbers of susceptible persons." More information about this article is available @ GreenCleanAir.com.

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Airborne Droplet Nuclei Infection Studies (cont.)



2004. Airborne Transmission of Communicable Infection-the Elusive pathway. Roy, CJ; Milton, DK. New England Journal of Medicine v350 p1710. "The current paradigm, as initially described by Charles Chapin in 1910, supports the belief the most communicable respiratory infections are transmitted by means of large droplets over short distances or through the contact with contaminated surfaces. What underlies the low repute of airborne transmission? First, the vod diseases whose aerosol transmission is most widely acknowledged, measles and tuberculosis, have been largely controlled with vaccination or drug therapy. As a result, the impetus to understand the aerobiology of infectious diseases has faded. Second. contamination of water, surfaces and large droplet sprays can be easily detected. It is difficult, however, to detect the contaminated air, because infectious aerosols are usually extremely dilute, and it is hard to collect and culture fine particles. But the reduction of airborne transmission of influenza by means of air sanitation in school could prove important with the emergence of the next pandemic influenza virus.

2005. Viral Load Distribution in SARS Outbreak. Chu, CM. Emerging Infectious Diseases 2005 Dec;11(12):1882-86. Showed how Amoy Garden victims of Wang Kaixi's SARS virus had higher levels of viruses in their nasal passages depending on how close they were to his apartment.

2006. Review of Aerosol transmission of Influenza A Virus. Tellier, Raymond. Emerging Infectious Diseases v12 p1657-82. *Larger dorplet transmission as the predominant mode by which influenza viruses is acquired. As a consequence of this opinion, protection against infectious aerosols is often ignored for influenza. This position contradicts the knowledge on influenza viruses transmission accumulated in the past several decades. Indeed, there relevant chapters of many reference books, written by recognized authorities, refer to aerosols (droplet nuclei) as an important mode of transmission for influenza... human cases of avian influenza were acquired by exposure to an aerosol (droplet nuclei) since large droplets would not have delivered the virus to the lower respiratory tract." See also "Review: Aerosol transmission of influenza" by Raymond Tellier. Journal of the Royal Society 2009.

2006. Disease Mitigation Measures in the Control of Pandemic Influenza. Ingesby, TV. Biosecurity and Bioterrorism v4 p366-75. "There are no data to demonstrate that hand-washing deters the

Studies (cont.)

Airborne Droplet Nuclei Infection



and Bioterorism v4 p366-75. "There are no data to demonstrate that hand-washing deters the spread of influenza within a community. General respiratory hygiene, such as covering one's mouth when coughing and using disposable paper tissues, is widely believed to be of some value in diminishing spread, even though there is no hard evidence that this is so. It has been recommended that individuals maintain a distance of 3 feet or more during a pandemic so as to diminish the number of contacts with people who are infected. The efficacy of this measure is unknown."

2006. Factors involved in the Aerosol transmission of infection and control of ventilation in healthcare facilities. Tang, JW. Journal of Hospital Infection v64 p100. Journal of Hospital Infection Control.

"Recent guidelines from the UK review the evidence for influenza transmission more comprehensively ... influenza can become truly airborne. Droplets generated by talking, laughing and sneezing potentially lead to the generation of infectious aerosol (droplet nuclei). The survival of such aerosolized pathogens depends on environmental conditions such as temperature and relative humidity. Long range transmission occurs between distant location and is primarily governed by air flows driven by pressure differences generated by ventilation systems."

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Studies on "Flu Season" due to Low Indoor Humidity



 $\textbf{1960} \ \text{Viruses survival as a seasonal factor in influenza and poliomyelitis. Hemmes, JH. \ Nature \ \ v218\ p430$

1964. Survival of Measles in Air. DeJong, JG. Nature v201 p1054 "Relative humidity indoors might be an important factor in the seasonal variation of measles (virus)."

1976. Survival of airborne influenza virus: effects of propagating host, relative humidity and composition of spray fluids. Schaffer, FL. Archives of Virology 1976 v51 p263-73.

1979. An outbreak of Influenza aboard a commercial airliner. Moser MR. American Journal of Epidemiology Jul;110 p1-6. Of the 53 passengers on the plane 38 (72%) became infected with the same strain of influenza as the sick passenger. "Spread of Influenza is via droplets or droplet nuclei and the period of infectivity of these particles is prolonged by low humidity."

2006. Factors involved in the Aerosol transmission of infection and control of ventilation in healthcare facilities. Tang, JW. Journal of Hospital Infection Control V44 p100-14. "The survival of such aerosolized pathogens depends on environmental conditions such as temperature and relative humidity."

2007. Influenza Virus Transmission is Dependent on relative Humidity and temperature. Lowen AC. PLoS Pathology. Oct 19 v3 epage1470-6. "Long term exposure to dry air is likely to affect influenza viruses growth in the upper respiratory tract, and may indeed play a role in influenza seasonality. (Influenza) transmission was highly efficient at low relative humidity levels-20% or 35%."

 $\underline{\text{More information about these articles is available } \underline{\text{@ GreenCleanAir.com}}$

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ASHRAE Position Document on

Airborne Infectious Diseases

Approved by ASHRAE Board of Directors June 24, 2009



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Executive Summary

This position document has been written to provide the membership of the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) and other interested persons with information on the health consequences of exposure to airborne infectious disease and on the implications of this knowledge for the design, installation and operation of heating, ventilating, and air-conditioning (HVAC) systems. In this paper three methods of transmission of Airborne Infectious Diseases are discussed, namely through direct contact, large droplet contact, and inhalation of droplet nuclei. The practice of the HVAC&R professional is likely limited to reduction of disease transmission to those diseases transmitted by droplet nuclei. The conclusions regarding needed research and advice for the practitioner are listed in Table 1.

ASHRAE's sole objective is to advance the arts and sciences of HVAC&R to serve humanity and promote a sustainable world through research, standards writing, publishing and continuing education. Therefore, the health effects of airborne infectious disease transmission are relevant to ASHRAE.

ASHRAE's position at the present is:

- Many infectious diseases are transmitted through inhalation of airborne infectious particles termed droplet nuclei,
- Airborne infectious particles can be disseminated through buildings including ventilation systems,
- Airborne infectious disease transmission can be reduced using dilution ventilation, specific in-room flow regimes, room pressure differentials, personalized and source capture ventilation, filtration, and UVGI.

ASHRAE should commit to improving the health of individuals who occupy buildings and should support further research on engineering controls to reduce infectious disease transmission.

1.0 Issue

The potential for airborne transmission of disease is widely recognized although it generates much controversy and discussion for example which diseases are spread via the airborne route or via other mechanisms of dissemination. Three issues are pertinent for engineers:

- the impact of ventilation on disease transmission,
- the disease for which ventilation is important for either transmission or control,
- the control strategies are available for implementation in the buildings of interest.

This position paper addresses each of these.

2.0 Background

2.1 Introduction to Infectious Disease Transmission

Infectious diseases are typically transmitted based on certain characteristics and spread through populations in predictable ways. Diseases can be spread from a single source, i.e., a "point source" such as an individual with active tuberculosis in a restaurant, or in an ongoing way, in a person-to-person pattern. The relationship of the incubation period (the time period between acquisition of the infection and its clinical appearance) to the pattern of onset of illness ("epidemic curve") identifies whether something arises from a single source or represents ongoing transmission (Sartwell 1995). As the shape of the epidemic curve deviates from a normal or log-normal distribution, ongoing person-to-person or ongoing point source transmission becomes more likely than transmission from a single source at a given point in time. Successive waves of epidemic transmission are usually assumed to represent person-to-person transmission.

Infectious diseases are transmitted through three primary routes: (1) direct contact and fomites, which are <u>inanimate</u> objects that transport <u>infectious organisms</u> from one individual to another; (2) large droplets (generally with a mass median aerodynamic diameter (MMAD) of >10 micrometers - μm); and (3) particles with MMAD <10 mm sometimes termed droplet nuclei. Recent work by Xie and colleagues (2007) indicate that large droplets are those larger than 5-100 mm at the original time of release. Nicas and colleagues (2005) show by modeling that emitted large droplets will evaporate to 50% of their initial value and that if the initial diameter is < 20 mm this process will happen instantaneously. These three primary routes each require different control strategies, evolved over many years of infectious disease practice. They have generated standards of practice for infectious disease and hospital epidemiology (APIC 2008). This classification represents a fundamental belief among infectious disease physicians and infection control professionals (Mandell 1999). Additional transmission routes, such as blood transfusions, intravenous injections, or injuries are not of concern here.

Direct contact implies the passage of the infectious agent through surface contact. For example, the infectious agent resides on the skin or in secretions on hands is left on doorknobs, bed rails and surfaces; and is picked up by the next victim. This form of transmission requires the implementation of barrier precautions, such as gloving, handwashing, and cleaning of contaminated surfaces. Prototypical diseases transmitted in this way are rhino virus-induced upper respiratory tract illness, the common cold, and hepatitis C, a cause of viral liver disease.

Some infectious agents are secreted in large droplets, such as may be coughed or sneezed. These droplets usually fall to the ground within three feet, and transmission via the airborne route to persons greater than three feet distant is considered unlikely so that a six-foot protection ring is considered needed. Many diseases transmitted from person-to-person follow this pathway. Infectious pneumonias like pneumococcal disease (Hogue et al. 1994), or plague

(CDC 2001) are thought to be transmitted in this way. Humidity affects survival of the infectious agent although not always in predictable ways.

Finally, some diseases are transmitted through the airborne route in particles with a MMAD of <10 mm. These particles are typically generated by coughing and sneezing, and to a lesser extent, singing and talking. Such particles remain airborne for hours at a time and can be transported far distances. There is thought to be a large range in the rate of production of these airborne infectious particles, depending on differences in patients and diseases (Riley and Nardell 1989). Tuberculosis represents the prototypical airborne transmission disease although a few outbreaks of small pox have been documented (Wehrle et al. 1970) and even recently of SARS (Chu et al. 2005) appear to have followed this pathway. As these particles remain airborne for some period of time, HVAC system operation affects the concentration in several ways.

2.2 Mathematical Model of Airborne Droplet Nuclei Infection

Riley and Nardell (1989) present a standard model of airborne infection usually referred to as the Wells-Riley equation. This equation is useful for understanding the relationship between the number of new infections, C, and the number of susceptibles (S) and infectors (I), the number of doses of airborne infection (q) added to the air per unit time by a case in the infectious stage, the pulmonary ventilation per susceptible (p) in volume per unit time, the exposure time (t), and the volume of fresh or disinfected air into which the quanta are distributed (Q):

$$C = S(1 - e^{-Iqpt/Q})$$
 (1)

In this equation, the exponent represents the degree of exposure to infection and $(1-e^{-lqpt/Q})$ is the probability of a single susceptible being infected. The parameter q is derived from the term quantum, which Wells used to indicate an infectious dose, whether it contains a single organism or several organisms (Wells 1955). The ability to estimate q is difficult at best and has been reported in the literature to be 1.25 to 249 quanta per hour (qph) in tuberculosis patients (Catanzaro 1982; Riley et al. 1962), and 5480 qph for measles (Riley et al. 1978). Fennelly and colleagues (2004) measured cough aerosol directly from tuberculosis patients. The patients generated infectious aerosol that contained 3-4 colony-forming units (cfu) to a maximum of 633 colony-forming units (cfu is a direct measure of infectiousness using culturing techniques). Also the size distributions that were measured in this study suggest that most of the viable particles in the cough-generated aerosols were immediately respirable.

Equation 1 is useful for understanding the impact of increasing the volume of fresh or disinfected air on airborne infection. Increasing Q decreases exposure by diluting infectious particles with more infectious droplet-nuclei free air. Q can also be impacted through the use of other engineering control technologies including filtration and ultraviolet germicidal irradiation, as discussed below.

2.3 For which Diseases is the Airborne Transmission Route Important?

Standard textbooks of infectious diseases and of infection control classify agents by primary transmission route. Tables 1 and 2 present the standard beliefs in the field on how each of these diseases is transmitted. Table 1 presents those infections widely considered likely to be transmitted through the air; those for which airborne, i.e., droplet nuclei, transmission has been documented are identified with an *. Recent controversy, primarily focused on small pox and influenza transmission, is worth acknowledging. The theoretical basis and the implications are important for engineers as they have major consequences for air handling. Table 2 presents those infections that are not transmitted through the air, but through other routes. This is included in order to clarify those infections that *cannot* be influenced by ventilation.

Only in the 1950s did the relationship of particle size, airborne suspension and transmission implications begin to become clear. Particle size distributions of coughed materials are thought to encompass a broad range of diameters, from very small to large airborne droplets and macroscopic elements. There is not, however, enough data to describe the particle size distributions of cough-generated aerosols or to predict these distributions based on the infected person's viscosity of secretions, anatomical structures in the oropharynx (roughly meaning throat) and airways, and disease characteristics. Research is needed to better characterize cough-generated aerosols.

Although small pox was assumed to result from fomite and large droplet transmission, two outbreaks documented that airborne transmission could occur (Gelfand and Posch 1971; Richter 1971). It remains unclear whether this resulted from peculiarities of the source patients, who might have had anatomic abnormalities that generated smaller particles; some characteristics of the infection with wetter secretions and therefore small particles; or whether the local clinical staff was more observant about true transmission and this route had simply been missed elsewhere.

Similarly the Severe Acute Respiratory Syndrome (SARS), a corona virus, like the common cold, was assumed to result from large droplet transmission although many health-care workers preferred to rely on respirators effective against droplet nuclei. In general, no major difference was seen in protection capabilities between devices with protection against very small particles (N95 respirators, according to current standard classifications) and those without such protection (surgical masks). Still in one dramatic outbreak, in the Amoy Gardens high-rise apartment, airborne transmission through droplet nuclei most likely represented the primary mode of disease spread, likely due to the dried-out floor drain, through airborne dissemination by the toilet exhaust fan and winds (Yu et al. 2004, Li et al., 2005).

Work by Dick and colleagues suggest that the common cold may in fact be transmitted through the same airborne droplet nuclei route (Dick et al. 1967, 1987). Experimental studies (Dick et al. 1987) document the possibility of transmission beyond three feet under controlled conditions in experimental chambers and strongly suggest airborne transmission as at least one

component even of rhinoviral infection. A recent field study (Myatt 2004) supports that result and documents its likely importance in a field investigation.

Care of patients with seasonal influenza has for decades relied upon (large) droplet precautions. The sparse older epidemiologic literature suggests this as adequate. Nevertheless there is some evidence suggesting a far greater importance for airborne transmission by droplet nuclei. A 1959 study of influenza prevention in a Veterans Administration nursing home (ARRD) identified an 80% reduction in influenza in staff and patients through the use of upperroom ultraviolet germicidal irradiation (UVGI) (McLean 1961). This suggests that air currents to the higher room areas where the UVGI was present carried the airborne infectious particles, and they were inactivated. The less infective particles were therefore unable to infect staff and patients in control areas with UVGI as compared to areas without UVGI. Influenza transmission occurred from one index case to 72% of the 54 passengers aboard an airliner, on the ground in Alaska, while the ventilation system was turned off (Moser 1979). This outbreak is widely thought to represent a second piece of evidence for airborne transmission and it is also thought that the high attack rate was due in part to the ventilation system not being in operation (Moser 1979). A recent review (Tellier 2006) acknowledges the importance of these papers and suggests including consideration of airborne transmission in pandemic influenza planning. Older literature, too, acknowledges the potential importance though it suggests that droplet transmission is far more important than airborne droplet nuclei transmission, at least for other common viral diseases such as the common cold (Gwaltney and Hendley 1978). At present, planning for pandemic influenza in the U.S. relies on "social distancing," i.e., maintaining at least three feet of distance between individuals to reduce the likelihood of transmission, on minimizing public contact (work at home, closure of schools, etc), and on both respiratory hygiene (coughing into tissue or towels) and hand cleanser use (USDHHS 2008).

2.4 Implications for Engineers

ASHRAE has a long tradition of relying on United States public health agencies as the cognizant authorities on public health, more recently including international health agencies and following those recommendations. It dos not generally rely on its own interpretations of the health literature. ASHRAE's role and the purpose of this Position Document is to use the health science, combined with engineering principles and practices to identify how ASHRAE programs, publications and research can better address the proper design and operation of HVAC system to prevent the spread of disease through airborne transmission.

Considering the three main transmission routes (direct contact, large droplets > 10 μm and droplet nuclei < 10 μm) it is clear that ventilation has no influence on direct contact transmission. Control strategies for large droplet transmission include respiratory hygiene, i.e., coughing into handkerchiefs or putting masks on ill individuals to prevent dissemination of particles (CDC 2001). Because such particles are quite heavy and drop quickly, general dilution and even enclosures and exhaust ventilation will not significantly influence airborne particle concentrations and the potential for transmission. Although some of the moisture content may evaporate, this does not happen quickly enough to change large droplets into droplet nuclei,

especially as 95% of the content must evaporate for the MMAD to decrease by 50%. Droplet nuclei particles may be transported through ventilation systems, as has been documented for tuberculosis, Q-fever, and measles (Li et al., 2007). If influenza transmission occurs not only through direct contact or large droplets, as is the long-standing public health tradition, but also through the airborne route, as newer data suggest, HVAC systems may contribute far more both to transmission of disease and, potentially, to reduction of transmission risk. In the absence of controlled intervention trials, this remains of great interest but of undetermined value.

Some biological agents potentially used in terrorist attacks may be purposefully transmitted through HVAC systems, such as small pox, plague pneumonia, and hemorrhagic viruses.

The following technical solutions are of interest: dilution ventilation, laminar and other in-room flow regimes, differential room pressurization, personalized ventilation, source capture ventilation, filtration (central or unitary), and ultraviolet germicidal irradiation (upper room, in-room and in the air stream).

Ventilation represents a primary infectious disease control strategy through dilution of room air around a source (CDC 2005). Directed supply and/or exhaust ventilation such as laminar flow and displacement is important in several settings including operating rooms (AIA 2006).

Room pressure differentials are important for controlling airflow between areas in a building (Garner et al. 1996). For example, TB isolation rooms are kept at negative pressure with respect to the surrounding areas to keep potential infectious agents within the rooms; hospital rooms with immuno-compromised individuals are kept at positive pressure to keep potential infectious agents out of the rooms.

Another strategy from an exposure control perspective could be the use of personalized ventilation systems that supply 100% outdoor air, highly-filtered, or UV disinfected air, (i.e., the ventilation provision per person) directly to the occupant-breathing zone (Cermak et al. 2006; Sekhar et al. 2005). Additionally, providing supplemental (either general dilution or exhaust/capture in a specific location) ventilation in locations in which infectious sources are located will reduce exposure potential, such as what is done in TB isolation rooms (CDC 2005). The value of these strategies is unproven and individual case study may be required to justify their application.

The addition of highly efficient particle filtration to central ventilating systems is likely to reduce the airborne load of infectious particles. ¹ This control strategy may prevent the transport of infectious agents from one area, such as patient rooms in hospitals or lobbies in public access

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¹ Filter efficiency varies with particle size, so the type of filtration required in order to be effective will vary with the type of organism and the aerosol that carries it. ASHRAE Standard 52.2 describes a minimum efficiency reporting value (MERV) for filter efficiency at various particle sizes and HEPA filtration may not be necessary. Specific personnel safety procedures may be required when changing filters, depending on the types of organisms and other contaminants that have been collected on the used media.

buildings, to other occupied spaces, when these areas share the same central ventilation system. Such systems are common in buildings in the U.S. Additionally, local efficient filtration units (either ceiling mounted or portable) reduce local airborne loads and may serve purposes in specific areas such as healthcare facilities or high-traffic public occupancies (Miller-Leiden et al 1996; Kujundzic et al. 2006).

There are three general UVGI strategies: installation into ventilating ducts, irradiation of the upper zones of occupied spaces, and in-room irradiation after one occupant and before the next. All depend upon inactivation of viable agents carried in droplet nuclei. In both the duct and in-room UVGI, the amount of radiation applied can be much higher compared to what can be used for upper-zone UVGI, resulting in higher exposures and quicker inactivation. When effectively applied, duct-mounted UVGI functions similarly to filtration. Upper-zone UVGI, when effectively applied, inactivates infectious agents locally and can be considered in public access and high-traffic areas such as cafeterias, waiting rooms, and other public spaces. Inroom UVGI can be considered as a kind of disinfection between successive occupants of a room. There is research that shows UVGI in both the upper-room and in-duct configuration can inactivate some disease transmitting organisms (Riley et al. 1962; Ko et al. 2002; CDC 2005; Kujundzic et al. 2007; VanOsdell and Foarde 2002; Xu et al. 2003, 2005) and that it can affect disease transmission rates (McLean 1961). Additional research is needed showing clinical efficacy specifically in occupancies with high-risk sources (such as jails, homeless shelters, and health-care facilities) and facilities where high-risk susceptible individuals congregate, such as nursing homes and healthcare facilities. Such research may lead to other recommended changes in HVAC system design. More research is also needed to document intrinsic (specific to microorganism) airborne virus and bacteria inactivation rates. See Table 3 for a summary of occupancy categories in which various strategies may be considered and priorities of research needs.

The 2006 Guidelines for Design and Construction of Health Care Facilities (AIA 2006) describe criteria including ventilation rates, filtration and pressure relationships among rooms that can guide HVAC designers of these facilities. ASHRAE's ANSI/ASHRAE Standard 170-2008, Ventilation of Health Care Facilities, covers similar requirements (ASHRAE 2008).

When outbreaks occur in the workplace, transmission through HVAC systems must be considered. Although there is currently inadequate information to suggest the need for or benefits from the control strategies discussed above, engineers should consider their possible application. As other routes are blocked by more efficient prevention strategies, the airborne route is likely to become relatively more important. It is unclear by how much infectious particle loads must be reduced to achieve a measurable reduction in disease transmissions and whether the cost-benefit implications or efficiencies warrant use of these controls. Societal disruption from epidemics and the unexpected transmission of disease in workplaces, public access facilities, and transportation warrants both modeling and field research of engineering controls.

3.0 Recommendations

ASHRAE holds a strong position that engineers play a key role in reducing disease transmission that occurs in buildings.

ASHRAE recommends that

- a strategic research agenda be developed to address the role of HVAC systems in the spread of infectious disease;
- this topic be included in ASHRAES future strategic plans;
- further research be conducted to understand how reducing the energy footprint of buildings will impact infectious disease transmission;
- further research be conducted on engineering controls to reduce infectious disease transmission. Table 3 summarizes the control strategies available and the occupancy categories in which these controls can be used. The research priority for each control is provided. Filtration and UVGI controls research are given top priority because less is known about how these controls can be applied in buildings and HVAC systems to decrease disease events.

ASHRAE should commit to improving the health of individuals that occupy buildings and to reduce the risk of airborne infectious disease transmission.

Table 1. Diseases Spread by Droplet or Airborne Transmission (*diseases are those where airborne transmission is reasonably certain even if it is not the primary mode)

Disease	Organism	Clinical Manifestations	Healthcare/personal care workers at risk
Adenovirus	Adenovirus	Rhinitis, pharyngitis, malaise, rash, cough	All, especially those in intensive care units, long-term pediatric care facilities and ophthalmology clinics
Influenza*	Influenza virus	Fever, chills, malaise, headache cough, coryza, myalgias	All, especially physicians and nurses
Measles (Rubeola)*	Rubeola virus	Fever, rash, malaise, coryza, conjunctivitis, Koplik's spots, adenopathy, CNS complications	All
Meningococcal disease	Neisseria meningitides	Fever, headache, vomiting, confusion, convulsions, petechial rash, neck stiffness	Emergency medical personnel, emergency department staff
Mumps*	Mumps virus	Painful/swollen salivary glands orchitis, meningoencephalitis	All, especially pediatricians, dentists, daycare workers
<u>Pertussis</u>	Bordetella pertussis	Malaise, cough, coryza, lymphocytosis, "whooping" cough	All
Parvovirus B19	Parvovirus B19	Rash, aplastic anemia, arthritis, myalgias	All, especially nurses
Respiratory Syncitial Virus	RSV	Often asymptomatic; respiratory symptoms	All
<u>Rubella</u>	Rubella virus	Fever, malaise, coryza, rash	All
Tuberculosis*	Mycobacterium species	Fever, weight loss, fatigue, night sweats, pulmonary disease, extra pulmonary involvement including lymphatic, genitourinary, bone, meningeal, peritoneal, miliary	All, especially nurses, pathologists, laboratory workers, housekeeping staff
<u>Varicella</u>	Human Herpesvirus 3	Chickenpox or zoster presentation	All

^{*}Infections for which airborne, i.e., droplet nuclei, transmission has been documented

Table 2. Diseases (Organism) Spread by Routes other than Droplet or Airborne Transmission

Contact with Blood or Body Fluids or via Percutaneous Exposure	Fecal –Oral Route	Skin Contact
Hepatitis B (Hepatitis B Virus)	Helicobacter pylori (Helicobacter	<u>Herpetic Whitlow</u> (Herpes
	pylori)	simplex <u>)</u>
Hepatitis C (Hepatitis C virus)	Hepatitis A (Hepatitis A virus)	<u>Tinea</u> corporis ringworm
		(Microsporum, trichophyton species)
AIDS/HIV Infection (Human	Norovirus (Norovirus)	Warts (Papilloma virus)
Immunodeficiency Virus)		
Viral hemorrhagic fevers-including	Polio (Poliomyelitis virus)	
Lassa fever, Marburg virus, Crimean		
hemorrhagic fever, Ebola virus		
(Various viruses)		
Other diseases that have been	Salmonellosis (Salmonella species)	
transmitted via percutaneous		
injuries (laboratory, research		
facilities): <u>Blastomycosis</u> ,		
<u>Brucellosis</u> , <u>Crypotococcosis</u> ,		
<u>Diphtheria</u> , <u>Gonorrhea</u> , <u>Herpes</u>		
Simplex, Leptospirosis, Malaria,		
Mycoplasmosis, Rocky Mountain		
<u>Spotted Fever</u> , <u>Scrub Typhus</u> ,		
<u>Herpes B Virus</u> , <u>Sporotrichosis</u> ,		
Staphylococcal Disease,		
<u>Streptococcal</u> <u>Disease</u> , <u>Syphilis</u> ,		
<u>Toxoplasmosis</u> , <u>Tuberculosis</u> , <u>Yellow</u>		
<u>Fever</u> , Creutzfeldt-Jacob disease,		
Leishmaniasis?		
	Shigellosis (Shigella species)	
	Enterotoxigenic e. coli (?)	
	Campylobacter (?)	

Table 3. Airborne Infectious Disease Engineering Control Strategies: Occupancy Categories Applicable for Consideration and Research Priorities*

Strategy	Occupancy Categories Applicable for Consideration**	Research Priority
Dilution Ventilation	All	9
Personalized ventilation	1, 4, 6, 9, 10	8
Source capture	1, 2, 8, 14	10
Central system filtration	All	4
Local air filtration	1, 4, 6, 7, 8 10	5
Upper room UVGI	1, 2, 5, 6, 8, 9, 14	1
In-room UVGI	1, 2, 7, 8, 14	3
Duct UVGI	1, 2, 3, 4, 5, 6, 8, 9, 14	2
In-room flow regimes	1, 6, 8, 9, 10, 14	7
Differential pressurization	1, 2, 7, 8 11, 14	6

**Occupancy Categories

- 1. Health Care (Residential and Outpatient)
- 2. Correctional Facilities
- 3. Educational < age 8
- 4. Educational > age 8
- 5. Food and Beverage
- 6. Internet Café / Game Rooms
- 7. Hotel, Motel, Dormitory
- 8. Residential Shelters
- 9. Public Assembly & Waiting
- 10. Transportation Conveyances
- 11. Residential Multi-Family
- 12. Retail
- 13. Sports
- 14. Laboratories where infectious diseases vectors are handled.

*Note: In considering going beyond requirements that include codes and standards, planners may use guidance from published sources such as CDC 2005, AIA 2006, APIC 2008, Table 3 above, and discuss risk with the facility user. HVAC system designers can assist closely allied disciplines such as architects and plumbing engineers to understand how unplanned airflow can impact airborne infectious disease transmission. Examples include wastewater drains, especially if improperly trapped; and wall and door leakage, including the pumping action of swinging doors.

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Influenza Virus Aerosols in Human Exhaled Breath: Particle Size, Culturability, and Effect of Surgical Masks

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Abstract

The CDC recommends that healthcare settings provide influenza patients with facemasks as a means of reducing transmission to staff and other patients, and a recent report suggested that surgical masks can capture influenza virus in large droplet spray. However, there is minimal data on influenza virus aerosol shedding, the infectiousness of exhaled aerosols, and none on the impact of facemasks on viral aerosol shedding from patients with seasonal influenza. We collected samples of exhaled particles (one with and one without a facemask) in two size fractions ("coarse">5 μ m, "fine"=5 μ m) from 37 volunteers within 5 days of seasonal influenza onset, measured viral copy number using quantitative RT-PCR, and tested the fine-particle fraction for culturable virus. Fine particles contained 8.8 (95% CI 4.1 to 19) fold more viral copies than did coarse particles. Surgical masks reduced viral copy numbers in the fine fraction by 2.8 fold (95% CI 1.5 to 5.2) and in the coarse fraction by 25 fold (95% CI 3.5 to 180). Overall, masks produced a 3.4 fold (95% CI 1.8 to 6.3) reduction in viral aerosol shedding. Correlations between nasopharyngeal swab and the aerosol fraction copy numbers were weak (r=0.17, coarse; r=0.29, fine fraction). Copy numbers in exhaled breath declined rapidly with day after onset of illness. Two subjects with the highest copy numbers gave culture positive fine particle samples. Surgical masks worn by patients reduce aerosols shedding of virus. The abundance of viral copies in fine particle aerosols and evidence for their infectiousness suggests an important role in seasonal influenza transmission. Monitoring exhaled virus aerosols will be important for validation of experimental transmission studies in humans.

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Introduction

Transmission of influenza virus between humans may occur by three routes: (1) direct or indirect contact between an infected and a susceptible person, usually resulting in contamination of a susceptible person's hands followed by hand to respiratory mucosa contact; (2) large droplet spray wherein droplets of respiratory fluid greater than approximately 100 µm in diameter are expelled with sufficient momentum to deliver a direct hit on the respiratory mucosa; and (3) aerosols generated by release of smaller, virus-containing droplets, as may occur during tidal breathing and coughing [1,2], that rapidly evaporate into residual particles (droplet nuclei), which are inhaled and deposited in the respiratory tract [3–6]. There is significant evidence for each of these routes [7,8], but their relative importance is not known [3]. As a result, the Institute of Medicine recommended that healthcare workers in contact with 2009-H1N1 patients use protection against all of the

possible routes of infection, including use of fit-tested N95 respirators [3]. A year after the 2009 pandemic, there was no greater clarity on the importance of the various modes of transmission [9].

The U.S. Centers for Disease Control and Prevention recently funded an experimental study of person-to-person transmission to address this important knowledge gap [10]. However, an experimental study using intranasal inoculation to infect experimental donors [11] will need to show that the donors and naturally infected persons shed similar virus aerosols with regard to quantity, particle size distribution, and infectiousness, given that earlier experiments suggested that intranasal inoculation requires quantitatively larger doses and produces qualitatively milder illness than does inoculation via aerosol [12].

In an occupational hygiene context, personal protection is usually the last resort, after source mitigation and environmental controls are exhausted [13]. Thus, it is worthwhile considering

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Author Summary

The relative importance of direct and indirect contact, large droplet spray, and aerosols as modes of influenza transmission is not known but is important in devising effective interventions. Surgical facemasks worn by patients are recommended by the CDC as a means of reducing the spread of influenza in healthcare facilities. We sought to determine the total number of viral RNA copies present in exhaled breath and cough aerosols, whether the RNA copies in fine particle aerosols represent infectious virus, and whether surgical facemasks reduce the amount of virus shed into aerosols by people infected with seasonal influenza viruses. We found that total viral copies detected by molecular methods were 8.8 times more numerous in fine (\leq 5 μ m) than in coarse (>5 μ m) aerosol particles and that the fine particles from cases with the highest total number of viral RNA copies contained infectious virus. Surgical masks reduced the overall number of RNA copies by 3.4 fold. These results suggest an important role for aerosols in transmission of influenza virus and that surgical facemasks worn by infected persons are potentially an effective means of limiting the spread of influenza.

whether surgical facemasks could be effective as a means of source control. The CDC recommends that persons with influenza wear surgical masks when in contact with susceptible individuals [14,15]. However, there is only one report studying mask impact on containment of infectious large droplet spray during influenza infection [16], and no data on surgical mask impact on release of infectious viral aerosols.

In the current study of patients infected with seasonal influenza, we describe the number of copies of viral RNA in two aerosol size fractions, report the culturability of virus in the fine-particle fraction, and the effect of surgical masks.

Results

We screened 89 volunteers: 33 (37%) tested positive for influenza using the rapid test (20 influenza A and 13 influenza B) and were asked to provide exhaled breath samples. Eight additional volunteers with negative rapid tests who reported a cough and who had a temperature of ≥37.8°C were also invited to participate. In total, 38 volunteers were confirmed to have influenza virus infection by PCR of nasopharyngeal specimens. Exhaled breath data with and without a surgical mask are complete for 37 of the 38 volunteers (21 influenza A, 16 influenza B); data for one volunteer has been excluded due to laboratory error in sample processing. One of the infected subjects reported receiving influenza vaccine for the current year. None of the subjects sneezed during the sample collection. Table 1 shows the sex, symptom and fever prevalence, and influenza virus type and Table 2 shows descriptive statistics for age and viral RNA copy number in swabs and exhaled aerosol fractions of the 37 volunteers with confirmed influenza infection. The viral copy numbers in each of the five specimens for all 37 cases are shown in Table S1.

We detected influenza virus RNA in the coarse fraction (particles greater than 5 μ m) collected from 11% (4 of 37 volunteers) while wearing surgical masks and from 43% (16 of 37) while not wearing a mask (relative risk for virus detection with mask = 0.25, 95% confidence interval (CI) 0.09 to 0.67; McNemar's test p = 0.003). The median number of coarse fraction viral

Table 1. Participant's sex, symptoms, temperature, and influenza virus type.

	N	Percent
Number with complete data	37	100
Male	30	81
On antiviral medicine ^a	0	0
Asthmatic ^a	5	14
Flu shot this season ^a	1	3
Flu shot previous seasons ^a	12	32
Current smoker ^a	9	24
Tachypnea ^a	13	35
Breathing difficulty ^a	16	43
Lymphadenopathy ^a	18	49
Feverish ^a	19	51
Temperature ^b ≥37.8°C	10	27
Type A	21	57

^aSelf-reported.

^bAt time of exhaled breath measurement.

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copies (Figure 1) was below the limit of detection with and without facemasks; the 75^{th} percentile dropped from 37 to below the limit of detection with use of surgical masks. Using Tobit analysis, we estimated that the geometric mean coarse fraction copy number without a facemask was 12 (95% confidence interval (CI), 4 to 37) and that the effect of facemasks was to produce a statistically significant 25 fold reduction in the copy number (95% CI 3.5 to 180, p = 0.002) to <0.5 copies per 30 min sample.

We detected viral RNA in 78% (29 of 37) of fine particle samples collected from volunteers when they were wearing a mask and in 92% (34 of 37) of samples collected when they were not wearing a mask. Thus, the relative risk for any virus detection with mask versus without a mask was 0.85 and borderline statistically significant (CI 0.72 to 1.01; McNemar's test p = 0.06). However, the reduction in copy number was statistically significant: The median number of viral copies in the fine particle fraction was 250 with masks and 560 without masks. The geometric mean copy number in the fine particle fraction without a facemask was 110 (95% CI 45 to 260) and the facemasks produced a 2.8 fold reduction in copy number (95% CI 1.5 to 5.2, p = 0.001).

Combining the coarse and fine fractions, we detected viral RNA in 29 (78%) subjects when wearing facemasks and 35 (95%) when not wearing facemasks (McNemar's test p = 0.01). Surgical masks produced a 3.4 (95% CI 1.8 to 6.3) fold reduction in viral copies in exhaled breath.

Fine fraction copy numbers were on average 8.8 (95% CI 4.1 to 19) times larger than coarse fraction copy numbers. The coarse and fine fraction copy numbers were correlated (r=0.60, p<0.0001). The viral load in the nasopharyngeal swab specimen, however, was not correlated with that in the coarse fraction (r=0.17, p=0.31) and only weakly with that in the fine fraction (r=0.29, p=0.08). There was no significant difference in copy number between influenza A and B virus infection in either the coarse (p=0.28) or fine (p=0.26) fraction. Reported asthma (p=0.029) and feverishness (p=0.014) were associated with significantly lower fine fraction copy numbers. However, coarse fraction copy numbers were not significantly impacted and temperature measured at the time of testing was not associated with exhaled copy numbers. Vaccination in any prior year was

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Table 2. Descriptive statistics.

	Percentiles					
	Min	25 th	Median	75 th	Max	
Age	18	18	19	20	54	
Days since onset ^a	0	1	2	3	5	
Nasopharyngeal swab copy number	1.7×10 ³	8.3×10 ⁴	4.2×10 ⁵	1.8×10 ⁶	3.4×10 ⁷	
Coarse particle copy number with mask	0	0	0	0	7.7×10 ¹	
Coarse particle copy number no mask	0	0	0	3.7×10^{1}	2.9×10 ⁴	
Fine particle copy number with mask	0	5	2.2×10 ¹	2.5×10^{2}	2.4×10 ⁴	
Fine particle copy number no mask	0	1.1×10^{1}	1.1×10^{2}	5.6×10 ²	1.3×10 ⁵	

^aAt time of exhaled breath measurement.

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associated with a non-significant trend toward lower copy numbers in coarse (p = 0.11) and fine fractions (p = 0.15); there were too few having received the current season's vaccine to analyze. Self-reported tachypnea, breathing difficulty, smoking, and lymphadenopathy were not associated with significant shifts in exhaled copy numbers.

We recovered infectious virus from fine particle samples (with and without mask) produced by the two subjects with the highest numbers of viral RNA copies in the fine particle fraction after blind passage on MDCK cells. Sequence analysis showed that the two isolates were seasonal H1N1, with sequence differences from each other and unrelated to any viruses present in the Veterinary Medicine laboratories at the time these samples were cultured.

Virus copy number (Table 3) declined with time since onset of symptoms. In the coarse fraction, each additional day after onset was associated with a 6.0 fold drop in the number of virus copies detected (95% CI 1.7 to 21 fold). Fine particles also declined with time, each additional day after onset was associated with a 2.4 fold drop in the number of copies detected (95% CI 1.1 to 5.1 fold).

Discussion

We measured exhaled influenza viral particle copy number by quantitative RT- PCR in two particle size fractions, ${\geq}5~\mu m$ (coarse) and ${<}5~\mu m$ (fine), and assayed the fine fraction for culturable virus. We observed that viral copy numbers were greater in the fine than in the coarse fraction, and recovered infectious virus from the fine particle fraction collected from the two samples with the highest RNA copy numbers. These results, combined with older data suggesting that the infectious dose via aerosol is about two orders of magnitude lower than via large droplets [12], suggest an important role for aerosols in seasonal influenza transmission.

Surgical masks nearly eliminated viral RNA detection in the coarse aerosol fraction with a 25 fold reduction in the number of viral copies, a statistically significant 2.8 fold reduction in copies detected in the fine aerosol fraction, and an overall statistically significant 3.4 fold reduction of viral copy number in the exhaled aerosols. This finding supports current Centers for Disease Control and Prevention recommendations that healthcare facilities encourage patients with influenza-like illness to don surgical facemasks as one component of an influenza infection control program [17].

When volunteers were not wearing surgical masks, we detected virus RNA in coarse particles exhaled by 43% and in fine particles exhaled by 92% of influenza patients. This is in contrast to the

report by Johnson et al [16], who detected influenza virus RNA in cough generated large droplet spray from 100% of influenza patients over two brief sampling trials, and from 78% on each trial. These discrepant findings are likely due to the very different collection techniques and particle sizes collected in these two studies. We used a specially designed aerosol sampler to collect particles from 0.05 to 50 μm in diameter. Johnson et al, by contrast, used simple deposition on petri dishes, and based on particle settling rates and collection times, that method would have been unlikely to collect particles with diameters of less than approximately 50 μm because smaller particles would have remained suspended in air and flowed around the petri dishes.

We view results from Johnson et al and the present study as complementary. Together the studies show that surgical masks can limit the emission of large droplet spray and aerosol droplets larger than 5 µm [16]. However, surgical masks are not as efficient at preventing release of very small particles. It is well known that surgical masks are not effective for preventing exposure to fine particles when worn as personal protection [18]. We had hypothesized that when used as source control, exhaled droplets might be large enough prior to evaporation to be effectively captured, primarily through impaction. This appears to be true for virus carried in coarse particles. But the majority of virus in the exhaled aerosol appear to be in the fine fraction that is not well contained. Nevertheless, the overall 3.4 fold reduction in aerosol copy numbers we observed combined with a nearly complete elimination of large droplet spray demonstrated by Johnson et al. suggests that surgical masks worn by infected persons could have a clinically significant impact on transmission. For example if one hypothesized that all transmission were due to aerosol particles <50 μm, and estimated a reproductive number of 1.5 for influenza (i.e. each infection generates 1.5 new infections on average at the start of the epidemic) [19], then the use of surgical masks by every infected case could reduce the reproductive number below 1 [20]. Compliance, however, would be a major limitation resulting in lower efficacy in real-world practice [21,22].

While it is generally assumed that large droplets shed from the respiratory tract contain infectious virus, there are limited data that indicate that fine particle aerosols released from the human respiratory tract contain infectious virus. In one previous study by Lindsley et al, infectious virus was detected in 2 of 21 cough aerosol samples, once with a sampler that did not discriminate between coarse and fine particles and once in the coarse particle fraction of a second instrument [23]. This observation, along with our observation that it was possible to recover culturable virus

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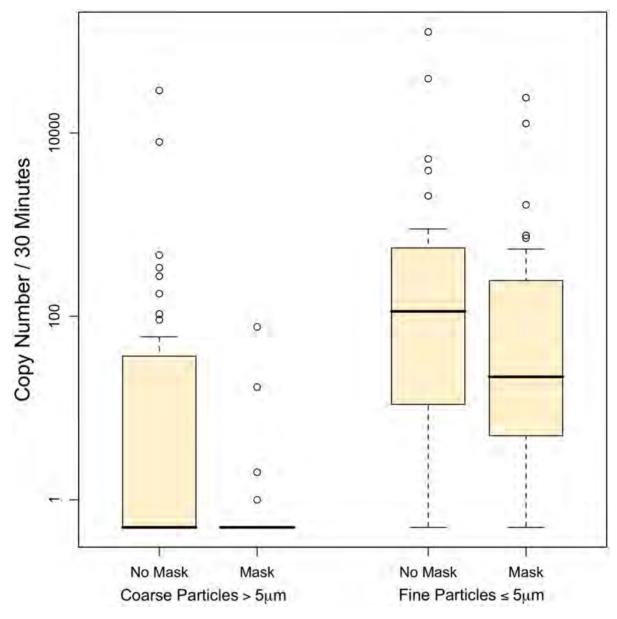


Figure 1. Influenza virus copy number in aerosol particles exhaled by patients with and without wearing of an ear-loop surgical mask. Counts below the limit of detection are represented as 0.5 on the log scale. doi:10.1371/journal.ppat.1003205.g001

from the fine-particle fraction using our device demonstrates that humans generate infectious influenza aerosols in both coarse and fine particle fractions. This lends support to the hypothesis that aerosols may be a common pathway for influenza transmission among humans [8,24]. However, a clear test of the hypothesis requires intervention studies that can interrupt only one mode of transmission without interfering with others [25].

We only detected infectious virus in exhaled breath samples with high $(10^4 \text{ to } 10^5)$ copy numbers by quantitative RT-PCR. This implies that the ratio of total viral particles to infectious virus was about 10^3 to 10^4 , compared with 10^2 to 10^3 for laboratory stocks and experimental aerosols [26]. It is not yet known whether the low recovery of infectious virus (despite high copy numbers of

viral RNA) represents technical difficulties in sampling and culturing exhaled breath samples or whether the vast majority of the virus exhaled by influenza A patients is actually non-infectious. These findings are consistent with those by Lindsley et al. [23] We designed the sampler specifically to overcome problems with existing bioaerosol samplers, including efficiently collecting submicron particles into a liquid and use of appropriate buffer to preserve infectiousness [27]. We have previously shown that collection on solid, dry collection media resulted in large losses of culturability [26]. Therefore, we did not attempt to culture the coarse fraction collected on a Teflon substrate. Subsequent studies in our laboratory indicated that about 50% of the infectious virus is lost during the concentration step of our procedure (data not

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Table 3. Copy number coarse and fine exhaled particles without surgical mask by day since onset of influenza symptoms.

			Number of Virus Particles			
Days Since Onset ^a	Particle Size	Number of Cases	Min	Median	Maximum	
1	Swab	10	2.1×10 ⁴	1.1×10 ⁶	3.4×10 ⁷	
	Coarse		<ld< td=""><td>2.3×10¹</td><td>2.9×10⁴</td></ld<>	2.3×10 ¹	2.9×10 ⁴	
	Fine		4	6.1×10 ²	1.3×10 ⁵	
2	Swab	15	1.7×10 ⁴	1.0×10 ⁵	3.4×10 ⁶	
	Coarse		<ld< td=""><td><ld< td=""><td>4.7×10²</td></ld<></td></ld<>	<ld< td=""><td>4.7×10²</td></ld<>	4.7×10 ²	
	Fine		<ld< td=""><td>2.1×10¹</td><td>3.9×10⁴</td></ld<>	2.1×10 ¹	3.9×10 ⁴	
3	Swab	7	2.3×10 ⁴	1.4×10 ⁶	1.0×10 ⁷	
	Coarse		<ld< td=""><td><ld< td=""><td>1.1×10²</td></ld<></td></ld<>	<ld< td=""><td>1.1×10²</td></ld<>	1.1×10 ²	
	Fine		2	3.7×10 ¹	5.3×10 ²	
4	Swab	3	8.1×10 ⁴	4.2×10 ⁵	1.5×10 ⁶	
	Coarse		<ld< td=""><td><ld< td=""><td><ld< td=""></ld<></td></ld<></td></ld<>	<ld< td=""><td><ld< td=""></ld<></td></ld<>	<ld< td=""></ld<>	
	Fine		3.2×10 ¹	7.5×10 ¹	4.4×10 ²	

^aBecause there were only single cases studied on day 0 (day of onset) and on day 5 since onset of symptoms, only data for cases studied on days 1 through 4 after onset of symptoms are shown.

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shown), suggesting that this is one contributing factor in the low rate of recovery of infectious virus in this study.

The lack of strong correlation between the viral load in the nasopharyngeal and aerosol samples is possibly of interest. This may merely be a result of nasopharyngeal sample variability; in future studies, control for sample quality by PCR of a cellular gene may be helpful. Our sampler, as is the case with all samplers for fine and ultrafine particles, has an upper limit to the size droplet that can be pulled into its inlet airstream. Thus, a second possible explanation for the lack of correlation is that the nasopharynx is primarily a source for very large droplets (>50 µm) that we would not have detected. Furthermore, none of our subjects sneezed; an efficient method of generating droplets from the upper respiratory tract. This may imply that the smaller droplets we detected were generated in the lower respiratory tract and that the viral load at that location is not strongly correlated with the nasopharyngeal load. Alternatively, shedding into aerosol droplets may be driven by other host factors (e.g. asthma, symptom severity, and immune response), co-infection with other agents, virus factors affecting release from the epithelium, or the nature of the resident microbiome. If shedding into aerosol is determined in large part by the location of infection in the respiratory tract, this may have implications for experimental studies of transmission [11,28]. Such studies will need to monitor aerosol shedding to determine whether nasal inoculation of donors results in aerosol shedding that mimics naturally acquired infection to validate the experimental design and aid the interpretation of results.

Most of the viral aerosol generation we observed occurred during the first days of symptomatic illness (Table 3), consistent with studies of shedding monitored by nasal washes [29]. We studied each individual on only one occasion and, by design, have little data beyond day 3. Further longitudinal studies of viral aerosol generation are needed to confirm these findings. New study designs will be needed to examine aerosol generation before and on the day of symptom onset in community acquired infection. A limitation of our study is that we recruited patients with certain signs and symptoms or who were positive on a rapid test or had fever, and therefore our data could be biased towards patients with higher viral loads [21]. However, we still observed

significant inter-individual variation and modeling suggests that cases with higher viral loads are disproportionately important in the spread of influenza [30,31]. Additional studies are also needed to determine how aerosol generation correlates with symptoms (including milder disease), presence of other health conditions, age (we studied a narrow age distribution), and co-infection with other respiratory viruses so that recommendations for infection control can be critically evaluated.

Methods

Patient population

We recruited volunteers with influenza-like illness from the Lowell, MA community, primarily among students and staff of the University of Massachusetts, beginning January 29 and ending March 12, 2009. The study protocol was approved by the Institutional Review Boards of the University of Massachusetts Lowell, Lowell General Hospital, and Saints Memorial Hospital, Lowell, MA. Oral informed consent was obtained by providing each subject with a detailed consent information form. Collection of a signed copy of the form was waived because it would have been the only personally identifiable information retained by this minimal risk study.

Volunteers learned of the study through flyers and notices posted on campus and by referral from health care providers. We screened self-referred volunteers by telephone for influenza-like illness (ILI). Persons who reported onset of fever and cough within the preceding 72 hours or were referred by a health care provider were invited to the laboratory for testing. We collected a nasopharyngeal specimen using a flocked swab (501CS01, Copan Diagnostics, Murrieta, CA) and temperature was taken with a digital ear thermometer (Model 18-200-000, Mabis Healthcare, Waukegan, IL). All volunteers with a temperature ≥37.8°C and a cough and volunteers without fever who provided a nasopharyngeal specimen positive for influenza by point of care testing (QuikVue Influenza A/B, Quidel Corp., San Diego, CA) were invited to provide exhaled breath samples, answer a questionnaire, and provide a second nasopharyngeal specimen for analysis by PCR. Only subjects with influenza infection confirmed by PCR were included in the data analysis.

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Exhaled breath collection

We collected exhaled breath with the subject seated in front of the inlet for a sampler designed for human exhaled breath collection, Figure 2, (G-II) described in detail by McDevitt et al. [27] Briefly, the G-II inlet was cone shaped so that the subject's face was situated inside the large end of an open cone with air drawn continuously around the subject and into the sampler. The cone allows the subject to breathe normally and unlike use of a mouthpiece, the subject could also wear a mask. The cone served as a capture type ventilation hood allowing collection of exhaled breath with minimal fugitive emissions even when the subject was wearing a mask with resultant redirection of flow. Intake air (130 L/min) flowed through a conventional slit impactor that collected particles larger than 5 µm on a Teflon surface ("coarse" particle fraction). To collect a "fine" particle fraction, water vapor was condensed on the remaining particles, which created droplets large enough to be captured by a 1.0-µm slit impactor. The 1.0-um impactor was composed of a slit and a steel impaction surface sealed inside a large reservoir. Impacted droplets drained from the impaction surface into a buffercontaining liquid in the bottom of the reservoir. Concentrated buffer was pumped into the reservoir during collection to match the accumulation of water from collected droplets and maintain phosphate buffered saline with 0.1% bovine serum albumin throughout collection. The sampler was shown to be 85% efficient for particles greater than 50 nm in diameter and was comparable to the SKC BioSampler for detection and recovery of influenza A/PR/8/34 H1N1 by PCR and culture. Between subjects, the apparatus was disassembled and cleaned with a 0.5% hypochlorite solution.

Exhaled particles were collected for 30 minutes while the subject wore an ear-loop surgical mask (Kimberly-Clark, Roswell, GA) and then for 30 minutes without a mask. Subjects were asked to cough 10 times at approximately 10-minute intervals for a total of 30 coughs during each 30 minute sample. One subject coughed frequently such that forced coughs were not required. No subjects were observed to sneeze.

Sample analysis

Immediately after collection, the Teflon impaction surface was removed and temporarily stored at $-20^{\circ}\mathrm{C}.$ The impactors were scraped with a flocked swab wetted with Dulbecco's phosphate buffered saline with calcium and magnesium (Hyclone, Thermo Scientific, Waltham, MA) with 0.1% bovine serum albumin (DPBS++BSA). The swab was eluted in 600 μ l of DPBS++BSA for 1 minute with vortexing. The resulting sample was stored at $-80^{\circ}\mathrm{C}.$

The fine particle fraction collected in DPBS++BSA buffer (100 to 150 ml volume) was maintained at $4^{\circ}C$ and concentrated by ultrafiltration using Amicon Ultra 15 filter units with a molecular weight cut off of 100 kD (Millipore, Bedford, MA) to a volume of approximately 400 μl . Following ultrafiltration, the filter was washed with 200 μl of DPBS++BSA, and the wash solution was combined with the retentate. Samples were stored at $-80^{\circ}C$.

RNA extraction in Trizol-chloroform, reverse transcription, and quantitative PCR were performed as previously described [1,32]. Quantitative PCR was performed using an Applied Biosystems Prism 7300 detection system (Foster City, CA) for coarse fraction samples or a LightCycler 480 (Roche, Indianapolis, IN) for the fine particle fraction. Duplicate samples were analyzed using influenza A and B primers described by van Elden et al. [33] A standard curve was constructed in each assay with cDNA extracted from a stock of influenza A (A/Puerto Rico/8/1934, Advanced Biotechnologies Incorporated, Columbia, MD) with a concentration of



Figure 2. Exhaled breath collection system. Each volunteer sat as shown with face inside the inlet cone of the human exhaled breath air sampler inside a booth supplied with HEPA filtered, humidified air for 30 min while wearing an ear-loop surgical mask. Three times during the 30 min each subject was asked to cough 10 times. After investigators changed the collection media, the volunteer sat in the cone again, without wearing a surgical mask, for another 30 min with coughing as before

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3.0×10¹¹ virus particles per mL or a stock of influenza B (B/Lee/ 1940, Advanced Biotechnologies Incorporated, Columbia, MD) with a concentration of 8.6×10^{10} virus particles per mL as determined by electron microscopy. Results are expressed as the total number of virus particles by reference to the standard curve, rounded to the closest integer value. The limits of detection were 6 and 11 viral RNA copies per qPCR well for influenza A and B respectively. Fine particle samples from all subjects were cultured for infectious virus on MDCK cells. Confluent cells in 24-well plates (Corning, NY, USA)were inoculated with 0.1 ml of the concentrated sample diluted 1:1 in OptiMEM® I medium (Invitrogen, Carlsbad, California). The plates were incubated at 37°C for 1 h with rocking every 15 min, and 0.8 ml of OptiMEM® I media with 1 µg/ml of TPCK-trypsin was added to each well and incubated for 72-96 h. The cells were checked daily for cytopathic effect (CPE) and if none was detected, two blind passages were performed using cell supernatant. At each passage, supernatants were tested for influenza virus by hemagglutination (HA) assay using 0.5% chicken red blood cells. Positive samples were confirmed by Flu DETECT (Synbiotics, CA, USA) strip test and by amplification of the hemagglutination (HA) gene by RT-PCR followed by sequencing.

Statistical analysis

We analyzed the effect of surgical masks as a) log relative risk for production of any virus aerosols assuming a binomial distribution using generalized estimating equations with exchangeable withinsubject correlation to account for repeated measures, and b) the geometric mean counts of virus particles detected in exhaled breath by qPCR and fractional reduction in copy number using Tobit regression analysis on log copy number with a random effect to account for variability between individuals. Tobit analysis was also used to compare coarse and fine particle fractions. Tobit regression avoids bias that would arise from assigning samples below the limit of detection a specific value such as zero or the limit divided by the square root of 2. Surgical mask use was the dependent variable. We also computed McNemar's test for paired samples to examine mask effect and Spearman's correlation coefficient to examine the relationship between the load in the nasopharyngeal swab and aerosol fractions. Statistical analyses were performed using SAS (Procs GenMod, NLMixed, Lifereg, Freq, Corr, and Means, version 9.2, Cary, NC).

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Supporting Information

Table S1 Copy number and influenza type in five assayed samples per subject. (DOCX)

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Author Contributions

Conceived and designed the experiments: DKM MPF JJM. Performed the experiments: DKM MPF JJM. Analyzed the data: DKM. Wrote the paper: DKM MPF BJC MLG JJM. Performed confirmatory experiments: MLG. Provided statistical consultation: BIC.

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