

At “half mask” or “nose commando:” A note demonstrating nasal breaths can spread microorganisms when improperly wearing a mask during COVID-19

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Summary

Many individuals are wearing face masks improperly at ‘half mask’ and potentially breathing out microbes that can contaminate the air as well as anything below the nose. This note provides the first report that bacteria and fungi breathed out during nasal air exhalation are able to be cultured after landing on blood agar plates. The CFU’s are higher after both 10 breaths and extremely significant for 20 breaths compared to the control plates exposed to the air. Implications of this finding are that going ‘nose commando’ may be able to continue the spread of respiratory diseases such as COVID-19. Minute bioaerosols carrying bacteria may be designated as microsnots.

Introduction

As COVID-19 gripped the United States, mandates for wearing face masks in indoor venues were issued to attempt to limit the spread of the SARS-CoV-2 virus. After observing an increasing wearing of masks, I was shocked when stopping at a truck stop in the mountains of Virginia and observed most of the employees at

the various restaurant counters wearing their masks below their nose. One lady at a sandwich counter was struggling during food preparation, breathing heavily just above where her hands were preparing food for a customer (Figure 1).

Subsequent personal observations have resulted in noting an increasing number of customers and employees in businesses open during the pandemic wearing their PPE face coverings at “half mask” or “nose commando (1)!” Concern over this un-hygienic behavior, and probable contamination of air and objects, led to this small study of microorganisms in exhaled nasal breaths.

There is currently strong agreement that both airborne droplets and bioaerosols carry infectious organisms and are able to lead to the transmission of disease (2, 3). The value of facemasks for reducing infections was reviewed (4) and with metanalysis found that the average risk of infection was less than 20% compared to those without masks. The wearing of face masks is currently highly recommended (5, 6) as breathing may be enough to spread the virus (7).

Oral exhalation carries bioaerosols (8, 9) and the amount is very high in some individuals, even in tidal breathing through the mouth. The amount of bioaerosols was greatly reduced with use of a saline spray, and yet this simple type of protective measure has not yet been adopted to reduce Coronavirus transmission.

The presence of mycobacterial bacilli was reported (10) from nasal breaths, but not cultured. Numbers of the bacteria were high, including a mean of 3.8×10^4 per breath in untreated patients. Viral RNA was detected via PCR from nasal breathing (11) using a special mask and one sample cultured in HeLa cells grew viruses.

An earlier study (12) swabbed the nares and into the nasal cavity with probable *Serratia marcescens* (listed as *Bacillus prodigiosus*) and did detect some cultural growth after “strong exhalations,” and after large blood agar plates were exposed for five minutes of “normal” nasal breathing did detect an average of two colonies (range 0 to 6) on the plates. Though a confusing discussion appears to indicate that these could not be distinguished from the colonies growing on open ‘control’ plates.

No studies have been found clearly demonstrating the presence of culturable microbes in exhaled nasal breath, which were not inoculated into the nasal passages.

The hypothesis for this simple study is that nasal breaths, if they carry minute bioaerosols containing microbes, will result in a significantly greater number of bacterial colonies than open controls on blood agar media. This is the first paper demonstrating growth of bacteria and fungi, that were not inoculated, from breaths exhaled through the nose.

Materials and Methods

The goal of this experiment was to determine if microbes were present in breaths of air nasally exhaled, and able to be cultured.

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The experimenter had their nose 0.5m above the agar plates surface which were uncovered. The nose was exposed and without covering of the mask, which had been pulled down to ‘half-mask’ position.

Ten breaths were breathed downward toward the exposed agar surface. Breaths included a deliberate inspiration and then exhalation, while a comfortable breath without forcing, each breath was double to triple that of the tidal volume and similar in volume to a deep sigh.

The plates were then incubated at 35°C and checked daily at 24, 48 and 72 hours and the colonies (CFU’s) counted. The plates were incubated agar side up.

Two more experiments were conducted similarly except that 20 breath exhalations were used. The time plates were open for both were less than 1.5 minutes, and controls were opened for 3 minutes of exposure to the room air.

Blood agar plates (5% defibrinated sheep’s blood, 1.5%

tryptone, 0.5% Soytone, 0.05% NaCl, 1.5% agar) were used to culture the microbes landing on the plates.

Results

The data are shown graphically in Figure 2 for the number of colonies, or Colony Forming Units (CFU’s) of bacteria and fungi, growing on the blood agar plates. The descriptive statistics for the controls, the 10 breath plates, and the 20 breath plates are detailed in Table 1. These demonstrate clear increases in colony numbers on the plates breathed on from a mean for the control of 2.27, to 9.8 for 10 breaths, and 16.9 for those exposed to 20 exhaled breaths.

Figure 3 shows examples of the blood agar plates and the colonies (CFU’s) that were counted. Table 1 contains the raw data

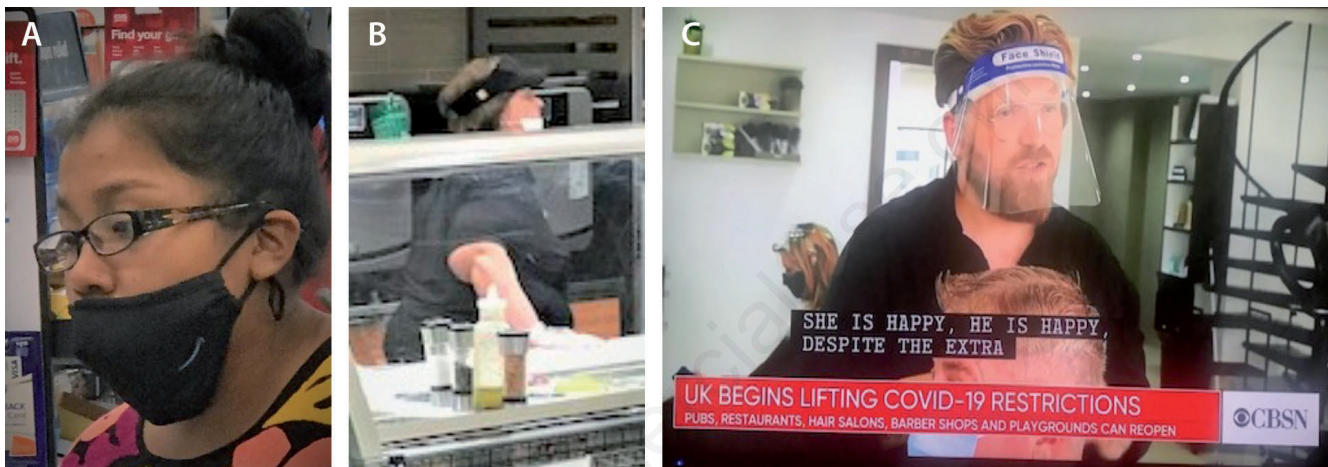


Figure 1. A) is a shopper wearing the mask below the nose. B) is a food preparer who is at “half mask” and was breathing hard through their nose while making sandwiches. C) shows a person wearing a face shield and breathing directly onto the customer below.

COLONY COUNTS (CFU'S) CONTROLS & 20 BREATHS

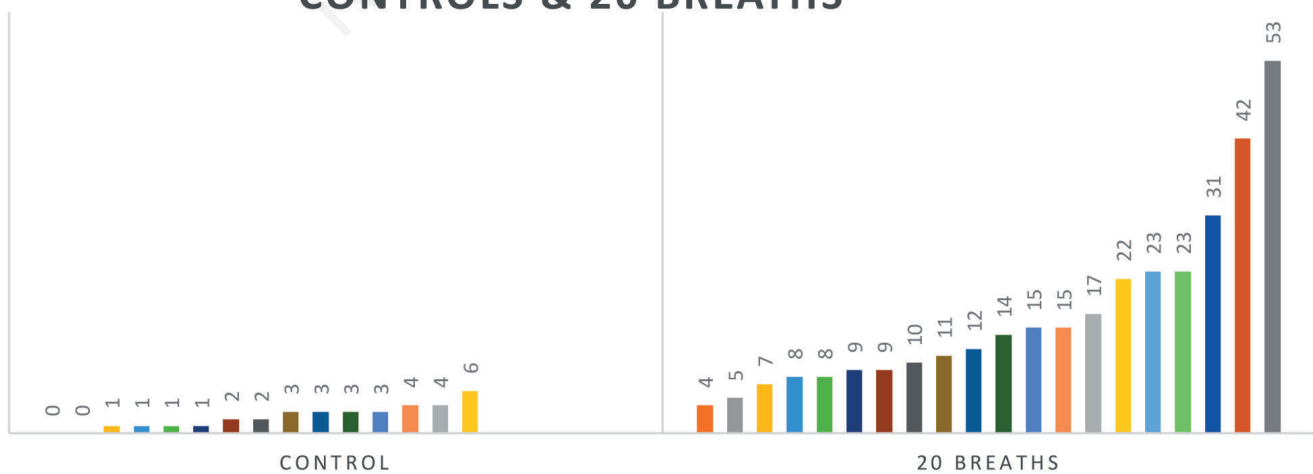


Figure 2. Graphic representation of the colony number (CFU’s) counts from the blood agar control plates exposed to the air for 3 minutes and plates exposed to 20 exhaled nasal breaths.

and descriptive statistics for each experimental group. The means of the groups were found to be significantly different.

The calculated difference of means from the 20-breath group and Control group showed a difference of 14.233, a standard error of 3.301, a 95% Confidence Interval of 7.5169 to 20.9499, a t-statistic of 4.311, with a DF of 33. The difference was

extremely significant with a Significance level of $P=0.0001$.

The difference in 10-breath group and early control had a difference of 6.4, a Standard error of 1.924, a 95% CI of 1.9643 to 10.8357, a t-statistic of 3.327, DF of 8, with a Significance level of $P=0.0104$. The null hypothesis of no difference between the means is rejected for both exhalation groups.

Table 1. Descriptive statistics of the raw data from the experiments of 10 exhaled breaths, 20 exhaled breaths, and the Control plates exposed to the air for 3 minutes.

10 Breaths		20 Breaths		Control	
Mean	9.8	Mean	16.9	Mean	2.26666667
Standard Error	1.655295	Standard Error	2.83019434	Standard Error	0.430577316
Median	9	Median	13	Median	2
Standard Deviation	3.701351	Standard Deviation	12.65701387	Standard Deviation	1.667618776
Sample Variance	13.7	Sample Variance	160.2	Sample Variance	2.780952381
Kurtosis	-1.17374	Kurtosis	2.70989508	Kurtosis	0.160659382
Skewness	0.607393	Skewness	1.683193469	Skewness	0.578805281
Range	9	Range	49	Range	6
Minimum	6	Minimum	4	Minimum	0
Maximum	15	Maximum	53	Maximum	6
Sum	49	Sum	338	Sum	34
Count	5	Count	20	Count	15

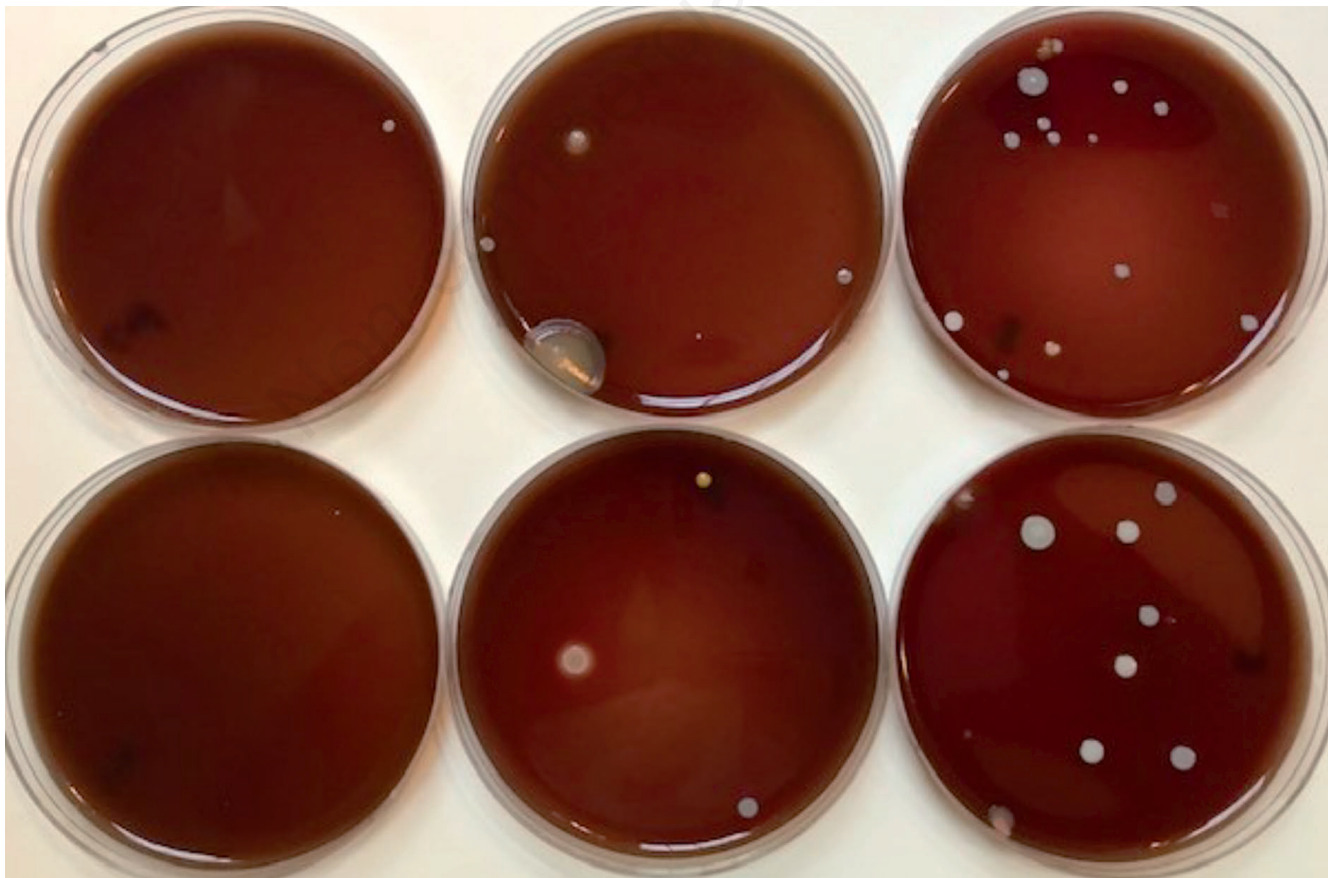


Figure 3. Blood Agar Plates showing examples of results of colony growth from Control (left), low colony (CFU's) growth (center) and higher CFU's (right), both for 20 breaths.

Discussion and Conclusions

The first issue raised by this study is that people wearing a mask as PPE at 'half mask' are breathing out microorganisms, including any potential pathogens that are present in their lungs, bronchi(-oles), pharyngeal and nasal mucosa as they exhale. This occurs even without any noticeable droplets or aerosols, and contaminates all nearby surfaces and remain in the air for others to breathe.

The exposure and dosage may be low from nasal breathing. Most infectious disease researchers have only expressed concern about coughs, sneezes and talking (more recently including singing), but even low doses of an infectious agent may cause disease. This can occur via continual buildup from constant breathing, the ability of very small bioaerosols to penetrate deeply into the lungs, the chance of infection of very sensitive tissues, and with SARS-CoV-2 also having the ability to infect the GI tract via aerosols breathed onto food (including the risk of reinfection or adding to the infection from feces, and toilet aerosols).

Plastic face shields, while protecting the wearer from facial spray of any kind, can allow for breath exhalations and their aerosols to contaminate surfaces below. Wearing a mask with the shield should be considered a requirement (13).

A second issue that needs to be raised is that individuals wearing their masks below their noses are breathing in the droplets, aerosols, and dust as much as not having a mask on. It has been assumed that the mask protects the wearer more than others (14). Since inhalation is primarily nasal going 'half-mask' completely negates protection and olfactory nasal cells appear easily infected, by 200-700 fold (15). In addition, it has been found that warmer and moister nasal conditions reduce the risk of infection, and these would be increased while using a face mask properly.

Breathing onto the mask itself from the nose contaminates the outside, and later breathing out or coughing through the mask may dislodge the organisms present on the surface and propel them into the air. This could increase the risk of spread of potential pathogens.

Many wear masks below the nose because they think they can breathe better, in response to questions (Wolff, Pers. Obs.). Some believe that wearing the mask below the nose meets the requirements of the 'law' for wearing a mask. The WHO provides guidelines for mask use (5), but fails to include the need to cover the nose and mouth.

Thirdly, while many wear the mask improperly because they feel they will not get infected, or it is their 'right' to resist authorities, it needs to be clear that the highest risk is not to themselves but to others that they pass the infection to. This includes family members, store customers, coworkers, and certainly those on the front lines taking care of the sick and doing other needed business.

A recent report (16) documented high levels of SARS-CoV-2 viral Nucleic Acids in the nasal pharynx of children, especially those 5 and younger. This rate of 10X to 100X more RNA makes it clear that masks must be worn fully covering the nose to prevent spread to others and critical for the resumption of in-person schooling. Young children not properly masked have a high risk of breathing in the bioaerosols, becoming infected, and then passing these viruses on to other children and adults. Children essentially become biological vectors of the pathogen, which means that control of this public health threat is necessary.

While colony growth on open agar plates was low, the significantly higher CFU's from breathing represent only those actually hitting and adhering to the agar surface, only those culturable on the blood agar, and does not include viruses which are not culturable on this media.

Because breathing out of the nose can deposit microbes on surfaces below, it should be noted that this must also be considered in laboratories when cultures (and other work) is being conducted, and masks should be used. It is even possible that some reports of

organisms on plates that have been attributed to other sources might actually be due to contamination from nasal (and oral) breathing, or this could be a reason for some experimental results that are anomalous or misleading.

Lastly, this note is the first report of culturable bacteria and fungi occurring naturally in exhaled nasal breaths. The minute bioaerosols exhaled should be referred to as microsnot.

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