Hindsight in 2020: Navigating a new normal with SARS-CoV2

Koshika Yadava, 8th July 2020

The spread of SARS-CoV2 across the world has revealed severe gaps in our preparedness for pandemics. Switzerland was amongst the few countries to respond rapidly, cancelling large events such as the Basel Fasnacht in February. In mid-March schools, restaurants, hairdressers, museums were all closed. By the 21st of March gatherings of more than five people were banned. Undoubtedly these lockdown measures were effective in limiting the virus spread, they do not provide a sustainable long-term solution. In the absence of an effective vaccine or treatment, we need to be continually vigilant about containing the virus. Here, we examine what we have learned about virus transmission, particularly the risk of airborne transmission and the measures to counter its spread. We also compare the effectiveness of commercially available respirators, surgical masks and different types of homemade masks in filtering aerosol particles and based on our observations make recommendations for their use in shared indoor spaces.

Introduction

There are many ways in which a virus can be transmitted. For respiratory viruses, common modes of transmissions are through contact with contaminated surfaces (fomites), infectious droplets and aerosols (also known as droplet nuclei) (Figure 1). The distinction between droplets and aerosols is at a somewhat arbitrary cut-off of 5uM diameter. In general, larger droplets(>5um) tend to settle down faster under the influence of gravity and aerosols (defined as <= 5uM) remain suspended for extended periods. Thus, size matters for short or long-range transmission, although a clear cut-off of 5uM is perhaps over-simplistic[1]. It is more likely a continuum where small droplets remain airborne, further shrink as they evaporate and behave as aerosols. Size of infectious droplet can also influence where the virus reaches when inhaled by a potential host. Large droplets do not follow the inhalation stream, whereas smaller droplets and aerosols may penetrate the lower respiratory tract down to the alveoli [2–4]. This difference in the deposition has implications for both the establishment of infection and the severity of the disease.

SARS-CoV2 belongs to the family of coronaviruses which includes the MERS virus and SARS-CoV1 (2003) strain, both of which are thought to have a potential for aerosol transmission[5,6]. SARS-CoV2 can infect sites other than respiratory tract causing a myriad of health complications[7] . For instance, the virus can infect the gut and is detected in the faeces, but so far, there is no evidence for feco-oral transmission[8,9]. Since the current outbreak, most recommendations, including guidelines from the WHO, focussed on counteracting the droplet and fomite modes of transmissions[10]. However, there is some indirect evidence for aerosol transmission of the virus which we discuss in the following sections. All these modes of transmission should to be considered for effective countermeasures as we establish a new normal after lifting the lockdown.

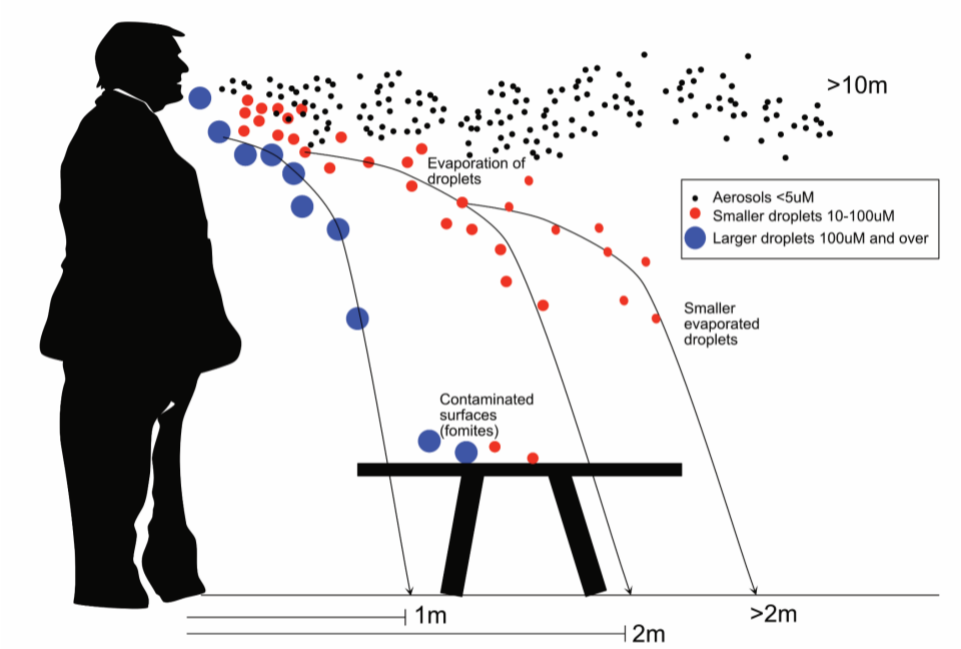

Figure 1: Estimates of the range of transmission of droplets and aerosols.

Evidence for SARS-CoV2 in aerosols

A study in April 2020, analysed the stability of SARS-CoV2 in laboratory-generated aerosols(<5uM)[11]. They found that the virus remained infectious within aerosols for up to 3 hours, providing a plausible indication for the risk of airborne transmission. Additional evidence came from a study measuring virus in air samples collected from patient environments at two hospital sites in Wuhan[12]. They found a higher viral concentration, particularly in poorly ventilated and crowded areas. Moreover, viral RNA was also deposited at 3m from the patient, ruling out the contribution of larger droplets.

A preprint from the Nebraska medical centre analysed viral loads in patients' environments as well as air samples from button samplers worn by personnel near patients[13]. In the patient environments, the highest levels of viral RNA were deposited at the air handling grates supporting the presence of the virus in the air. In line with the Wuhan study, a few air samples collected from over 2m from the patients contained viral RNA. All air samples collected from personnel were also positive, despite no coughing by patients when personnel were present. These studies determined viral load by measuring viral RNA using ddPCR or conventional qPCR. We have also validated the use of ddPCR in detecting low levels of virus and virus in air samples (Supplementary figure 1, Supplementary Table 1). Although these methods are sensitive and specific, an important caveat is that they do not distinguish between infectious and non-infectious virus. A preclinical study using Syrian hamsters provided direct evidence for infection by aerosol transmission of SARS-COV2[14]. To assess airborne transmission, they placed a naïve hamster and a SARS-CoV2 infected hamster in 2 adjacent stainless-steel wired cages at a distance of 1.8 cm. They also investigated fomite transmission by placing naïve hamsters in soiled cages from infected hamsters. They found that all three hamsters exposed to airborne transmission were infected while 1 of 3 through fomite transmission was infected. Similar results were found in another study using Ferrets as experimental animals. Here, to investigate airborne transmission, they placed an infected ferret and a naïve ferret in opposite cages separated by steel grids and 10 cm apart. They found viral RNA in 3 out of 4 animals exposed by this route for upto 13-17 days post exposure.

Notably the viral loads were similar to that obtained from naïve ferrets exposed though direct contact by cohousing with infected ferrets. Though these studies suggest that the virus transmission through air is comparable to fomites, it certainly requires validation in larger studies[15].

Aerosol and droplet generation by humans

At a biological source such as human being, it is not easy to make a clear distinction between droplets and aerosols purely by size. Not only do individuals produce a range of particles sizes, but smaller droplets can also further evaporate and shrink to the size of aerosols (<5uM). Coughing and sneezing generate particles of varied sizes, both large and small. Most guidelines recommend a physical distance of about 1m to 2m to mitigate the risk of contracting an infection from these droplets. This length is based on studies by Wells in 1934 who found that droplets < 100uM are likely to dry out before settling down within this range[16]. Nevertheless, this does not address the risk of infection from inhaled aerosols. Besides, the trajectories of droplets expelled on sneezing and coughing are far more complex. Beyond droplet size, extrinsic factors including temperature, humidity and airflow affect how fast these droplets settle and their rate of evaporation, potentially extending their range of transmission beyond 2m to 8m[17–19].

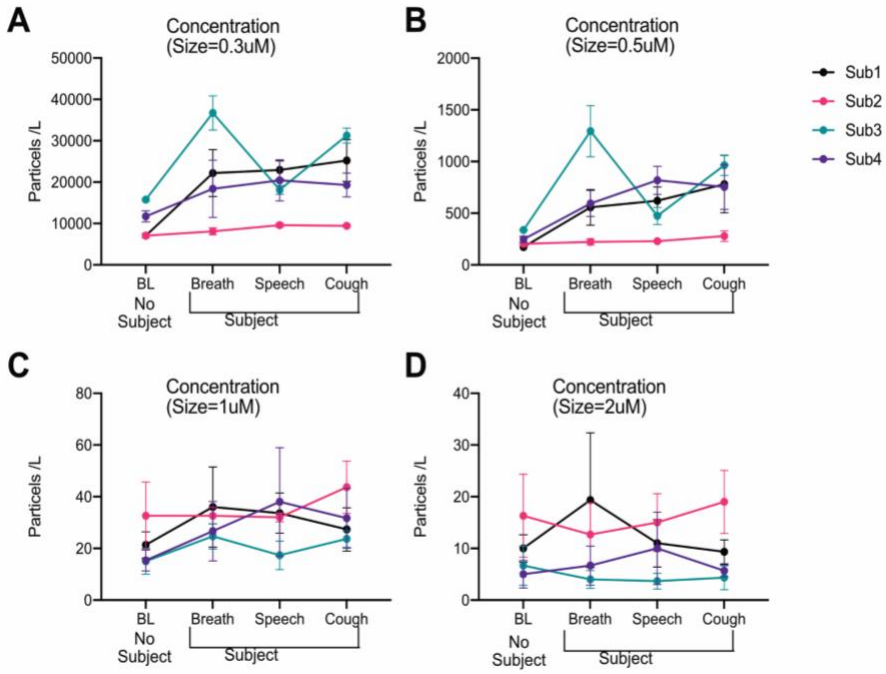

Figure 2 : Inter-Subject variability in particles generated during breathing, speech and cough. Four subjects breathed, spoke or coughed into a funnel connected to a particle counter (Fluka 985) for 10 seconds. Subjects spoke the identical words and coughed 6 times. The counts per liter is plotted for particles with a diameter of (A) 0.3uM, (B) 0.5uM, (C) 1uM and (D)2uM Mean of 3 replicates per condition per subject is plotted, error bars are standard deviation of the mean. BL=baseline, Sub=subject

In addition to sneezing and coughing, breathing and speech can generate small droplets and aerosols[20,21]. A recent study visualized droplets generated by speech which increased with loudness [22,23]. An earlier study which measured particle sizes more accurately found that small particles between 0.3-20uM were generated by mouth breathing, speech and coughing[24,25]. Notably voicing 'aah' generated more particles (1088 per L of exhaled air) than coughing (678 per L of exhaled air). If these particles can contain infectious virus, it could somewhat explain incidences of disease outbreak in indoor settings such as Church choirs and call centres where vocalization is widespread[26–30]. The inter-individual differences in expelled particles size and count during breathing and vocalization

(an observation we made using a simple experimental setup in our lab as well, Figure 2) may also partially explain the existence of some super spreaders.

How much virus present in air and is it infectious?

In patient environments, viral RNA has been detected in size segregated air samples containing particles between 0.25 and 1.0 µm and > 2.5 µm diameter. In Wuhan hospitals, the amount of viral RNA detected in air samples varies ranged from 19 copies/1000 L air sampled in toilets to 16 to 42 copies/1000 L in protective-apparel removal rooms (PPARs)[12]. In the Nebraska medical centres, the concentrations were much higher between 2-8 copies/L of air in air samples collected in hallways and 5-67 copies/L in personal air samples from personnel in patient environments[13]. Since the virus is found in nasal and salivary secretions and is about 100nm in size, it is plausible that it gets expelled in smaller droplets or aerosols produced by infected individuals and remains airborne. However, these studies do not provide conclusive evidence that the virus is indeed infectious within these airborne particles.

There is evidence for contagiousness of virus in exhaled air from studies with people infected with influenza[31]. Some studies have analysed both viral RNA and infectious particles using a controlled experimental setup by collecting fine particles (<5uM) from breaths of infected individuals [32,33]. They found that while RNA copy number and infectious viral particles correlated, RNA copy numbers were higher as it measures both viable and non-viable virus. They measured up to 10^3 infectious particles per 30 minutes of sampled breath without any episodes of coughing or vocalisation.

More recently two preprints detected SARS-CoV2 in exhaled breath of patients[34,35]. One of these studies estimated that patients could exhale 10^3-10^5 copies of viral RNA per minute but this is likely inaccurate as they didn't perform absolute quantification[34]. More importantly there is still no evidence of infectious virus in exhaled breath.

To spread infection, the presence of an infectious virus in the air on its own is insufficient. The quantity of infectious virus, how long it persists and how much of it is inhaled, are all crucial factors. The infectious dose 50 (ID50) of SARS-CoV1 is estimated to be 280 PFU, but we do not know the infectivity of SARS-CoV2 yet[36]. Assuming a breathing rate of 10L/min and comparable infectivity of SARS-CoV2 as SARS CoV1, a modelling preprint estimated a maximum exposure time of about 100 minutes to contaminated air within office spaces[37]. There is undoubtedly value in drawing parallels between SARS-CoV1 and SARS-CoV2. But SARS-CoV2 has spread far more extensively and infected many more individuals than SARS-CoV1. Infectious SARS-CoV2 decays at a similar rate to SARS-CoV1 within aerosols indicating that viral adaptations other than its physical stability contribute to its increased transmission[38]. Some of these adaptations pertain to mutations in the viral protein that might affect its tropism. For instance, the spike protein SARS-CoV2 binds with a much higher affinity to host cell receptor ACE2[39] and replicates more extensively in the bronchus than SARS-CoV1[40]. The shedding of higher viral loads and transmission by pre-symptomatic patients may also account for increased spread.

Viral shedding by presymptomatic infected individuals

A significant concern for virus spread is the shedding of infectious particles by presymptomatic people. A study from China estimated that infectiousness started 2-3 days before symptom onset[41]. Another study at a Nursing facility in Washington State, estimated a median of 4 days from detection of viral RNA in NP swabs to symptom onset[42]. In this facility,

76 residents tested positive for SARS -CoV2 after being in contact with an infected individual. Of these, 27 did not display any symptoms at the time of testing[42]. Additionally, the viral loads in nasopharyngeal swabs were comparable between symptomatic individuals and those with no symptoms. Studies in Italy and China have also found comparable viral loads in symptomatic and presymptomatic individuals[43,44]. The viral load peaks closer to the onset of symptoms[45]. It is important to bear in mind that most of these studies have assessed viral shedding by measuring viral RNA, which limits inference regarding infectiousness. Overall the evidence does support the transmission of infection by presymptomatic individuals and individuals with mild symptoms[46] . But there is no convincing evidence for the existence of truly asymptomatic carriers. Regardless, it may be best to err on the side of caution and enforce preventative community measures including masks, physical distancing, improved ventilation and limiting density of occupancy especially in shared indoor environments.

Mitigating virus spread

Most outbreaks have occurred in indoor settings globally whether it was a chalet in France, churches, offices or restaurants[26,27,30,47–49]. A preprint estimated the odds of transmission was 18.7 times greater indoors based on data from 11 clusters from Japan [50]. Another preprint from China found that all 318 outbreaks involving the three or more cases, occurred indoors[51]. Homes followed by transport were the most dominant venues for outbreaks. Additional community settings can also pose a risk as was found in Singapore, where 3 clusters mapped to a tour group, a conference and a local church[48]. Several outbreaks in Germany have also been linked to churches[29,52,53]. Some of these occurred in situations where strict hygiene and physical distancing should have been enforced. Most recently in Switzerland, a superspreader outbreak emanated from a club in Zurich, once again indoors[54]. An outbreak post lockdown also occurred in a slaughterhouse in Germany[55]. However, here the transmission was likely due to both poor ventilation and because the workers shared cramped living quarters where physical distancing was not possible.

As many people return to work, a pertinent question remains regarding the risk of contracting the virus in such as shared office space. Working in shared offices for long hours in a high-density environment can pose a significant risk as was seen in the outbreak in a call centre in South Korea[30]. To circumvent viral spread indoors, hand hygiene and physical distancing strategies have been adopted early on. Shops and offices, including FMI have placed hand sanitizers at points of entry. Occupancy is also limited in confined spaces such as elevators.

Modes of transmission are not mutually exclusive, and there is likely considerable overlap between droplet and aerosol transmission. Although incomplete, there is indirect evidence of airborne transmission of SARS-CoV2, that warrants consideration in practices within shared indoor spaces. Together with a lower density of occupation and physical distancing, adequate ventilation is vital to limit virus spread. An outbreak in a restaurant in China further emphasised the importance of air circulation[49]. Here viral transmission at distances greater than 1m was likely prompted by the airflow from an air-conditioner.

The use of masks is an effective mitigation strategy to prevent droplet and airborne transmission. Wearing masks can prevent the spread of airborne viruses such as influenza[56–58]. A recent study also showed that surgical masks were effective in reducing viral loads in aerosols and droplets expelled from infected individuals for several respiratory viruses, including coronaviruses[59]. However, some masks are more equal than others. Mask material

and mask fit, or sealing are essential considerations for the degree of effectiveness. Filtration efficiency of small particles in the aerosol size range vary with the kind of mask material and mask fit. Small particles which can slip between filter fibres can be trapped by electrostatic attraction by specific materials. Homemade masks vary widely in materials used, which impacts their effectiveness[60,61]. To date there has been no comprehensive study investigating the efficacy of homemade masks in preventing transmission, including designs recommended by the CDC in the current pandemic. Surgical masks are multi-layered comprising polymeric filter made of nanofibers placed between non-woven materials. Filtering facepiece masks (FFP) are respirators certified by the EU and classified as FFP1, FFP2, FFP3 based on their filtration efficiency, which is 80, 94 and 99% respectively. N95 is similarly a respirator which filters 95% of airborne particles and meets the standards set by the national institute for occupational safety and health in the USA. Although respirators are undoubtedly superior at filtering finer particles, their efficiency is mainly determined by their fit, which is affected by many factors, including facial hair. They are also less breathable and uncomfortable than cloth or surgical masks, thus likely to be less useful in a community setting.

An experimental demonstration of effectiveness of different masks for reducing aerosol exposure

We used a simple experimental set up to demonstrate the protection provided by different masks to the wearer from external environment (outside-in). Our setup consisted of a mannequin connected with a tubing to a particle counter (Fluka 985) as the subject and the ambient air in 14m^2 room as the external environment. We sampled between 0.47-0.5L of air for a duration of 10 seconds. We found that particle counts in ambient air varied a lot across days (Figure 3). Regardless the largest proportion of particles were <=0.3uM, followed by <=0.5uM.

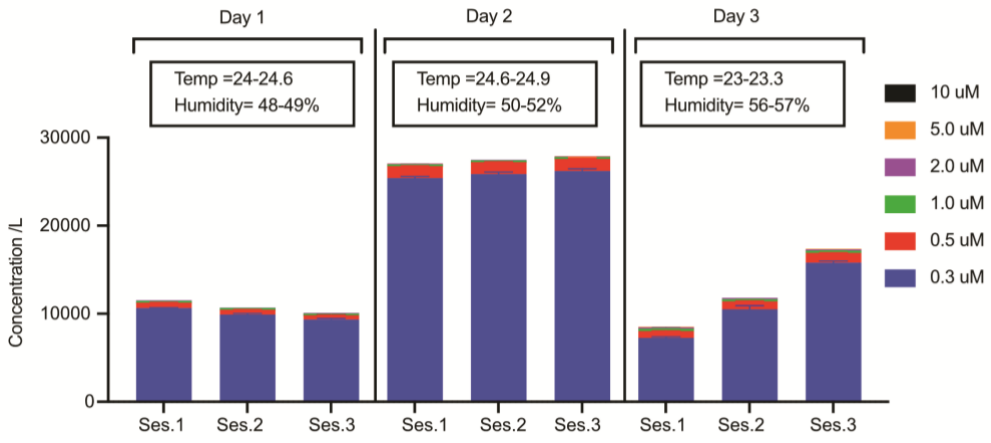

Figure 3: Profile of particles sizes in ambient room air. Ambient air was sampled in a 14m^2 room with closed windows and doors at three different sessions across 3 different days. Temperature and humidity were recorded for each day. Mean of 3 replicates per session is plotted, error bars represent standard deviation of the mean. Ses.=session

First, we evaluated commercially available surgical masks (one with ear loops and another with ribbons), FFP2 and FFP3 (Figure 4). We made three replicate measurements in three independent recording sessions for each condition. To improve sealing of the mask on the mannequin, we used a cut-out stocking worn over the mask. We calculated the effectiveness of the masks in reducing the particle counts as:

$$\text{Reduction}(\%)=(PC_{BL}-PC_{M})*100/PC_{BL}$$

Where PC_{BL} is the mean of the particle count of a given diameter at baseline for the recording session adjusted to volume of air sampled. Baseline in this setting is when the mannequin is exposed to ambient room air while wearing only the stocking seal. PC_M is the particle count of a given diameter when the mannequin is wearing a specific mask adjusted to volume of air sampled. We focussed on the effectiveness of masks at reducing particles from 0.3-2uM as these correspond to aerosol particles and were also most predominant in ambient room air. Surgical masks with ear loops didn't seal as well as the surgical masks with ribbons and both FFP2, FFP3 didn't seal well around the mannequin. With an effective seal, surgical masks performed at par with FFP2 and FFP3 in filtering small particles.

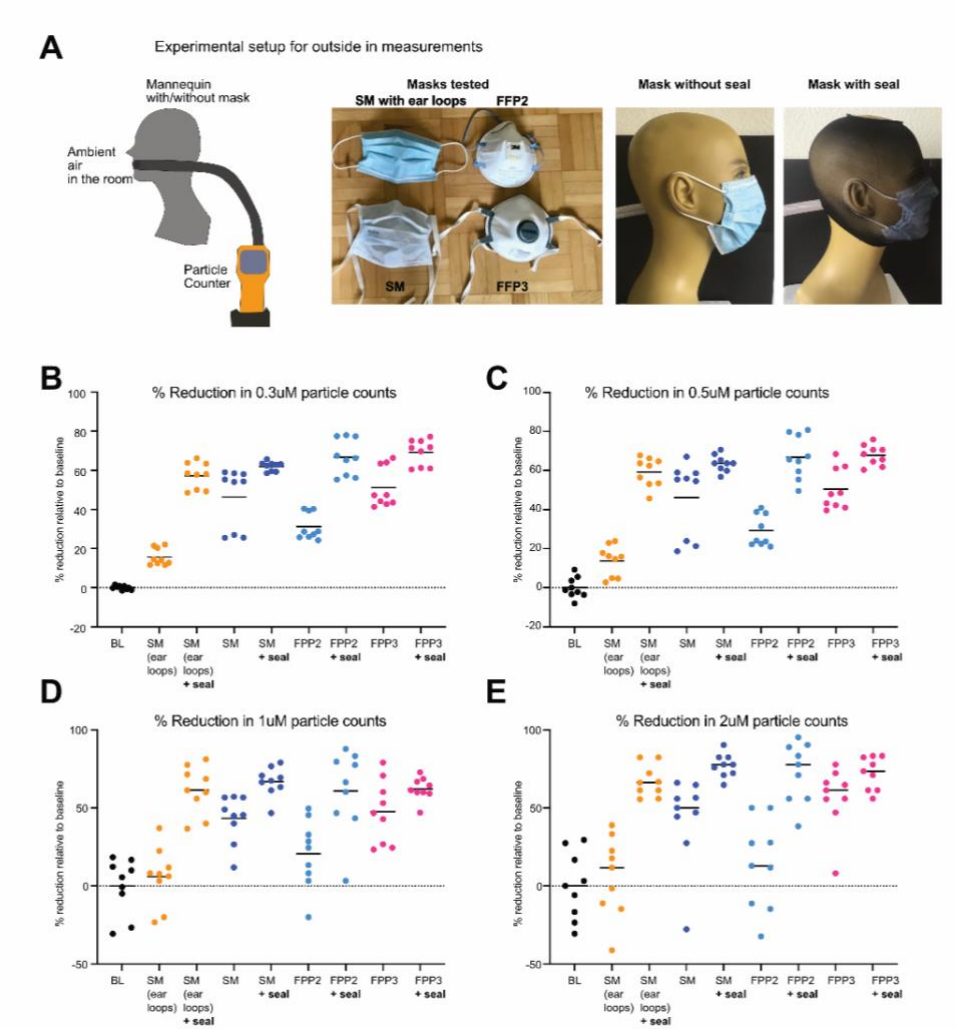

Figure 4: Comparison of commercial masks for outside-in protection. (A) Experimental set up showing masks evaluated and stocking used to seal masks around mannequin. Three independent sessions for all conditions were recorded and within each session three replicates for each condition were measured. All 9 points are shown. % reduction relative to baseline is plotted for particles with a diameter of (B) 0.3uM, (C) 0.5uM, (D) 1uM and (E)2uM. Line indicates mean. BL=baseline mannequin wearing only stocking seal. SM=surgical mask FFP=filtering facepiece mask.

In a community setting, people are more likely to wear homemade masks or surgical masks. We evaluated the protection to aerosol exposure conferred by a variety of homemade masks as compared to a surgical mask. We used the same set up for outside in measurements, this time using several different types of homemade masks with or without seal and compared it to a surgical mask with ribbons (Figure 5, Table 1). We found that homemade

masks varied in their effectiveness and none were as effective as the surgical mask. The best protection was provided by HM2, mask which was made of two internal layers of tightly knit cotton t-shirt fabric with an external polyester woven fabric. In the case of HM5 which was made of a multi-layered tea towel, lint from the fabric contributed to increased counts over baseline for particles 0.5-2uM.

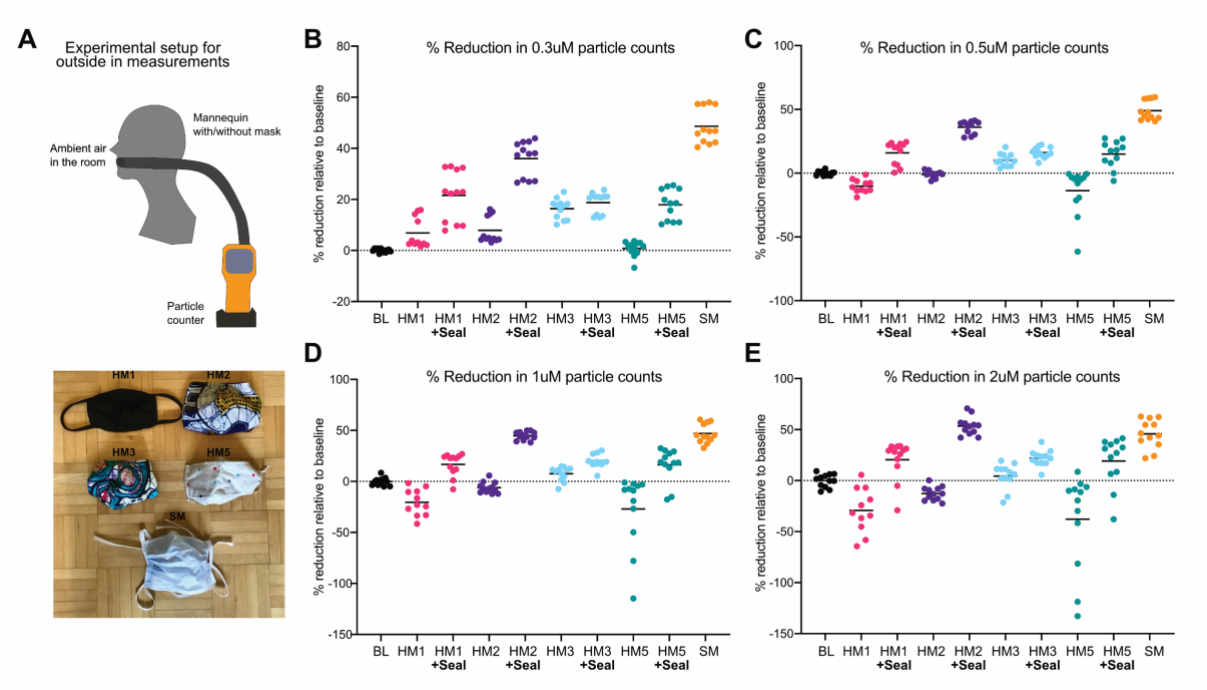

Figure 5: Comparison of different homemade masks for outside-in protection. (A) Experimental set up showing masks evaluated. Three independent sessions for all conditions were recorded and within each session four replicates for each condition were measured. All 12 points are shown. % reduction relative to baseline is plotted for particles with a diameter of (B) 0.3uM, (C) 0.5uM, (D) 1uM and (E)2uM. Line indicates mean. BL=baseline mannequin wearing only stocking seal. SM=surgical mask HM=homemade mask.

Mask ID	Mask Type	Description
HM1	Store brought	Single layer knit cotton with elastic ear loops
HM2	Three layer Homemade Olson mask without filter pocket	Machine Sewn mask with behind head and neck elastic loops and an adjustable nose bridge (twist tie). Material; Double layers of knit cotton t-shirt material with a polyester woven exterior.
HM3	Two layer Homemade Olson mask without filter pocket	machine sewn mask with one layer of woven polyester internal fabric and one external layer of woven cotton material.
HM5	Multilayered, 100% cotton, homemade	Multilayered mask made using a tea towel fitted with ear loops.

Table 1: Characteristics of homemade masks evaluated in our study

We next evaluated how well these homemade masks conferred protection to the environment from the wearer('inside-out')(Figure 6). For this we altered our experimental setup by connecting the mannequin to a nebuliser (Beurer IH 26) and nebulised water through the mouth of the mannequin with or without a mask. We placed the particle counter connected to a funnel at a distance of 12 inches from the mouth of the mannequin and to

make measurements. One caveat of this setup is that the particle counter could only measure the particles coming straight at it in the flow stream but not those that could leak from the sides or the top of the masks or go around the funnel. In our setup, most homemade masks worked well and at par with surgical mask for limiting inside-out spread.

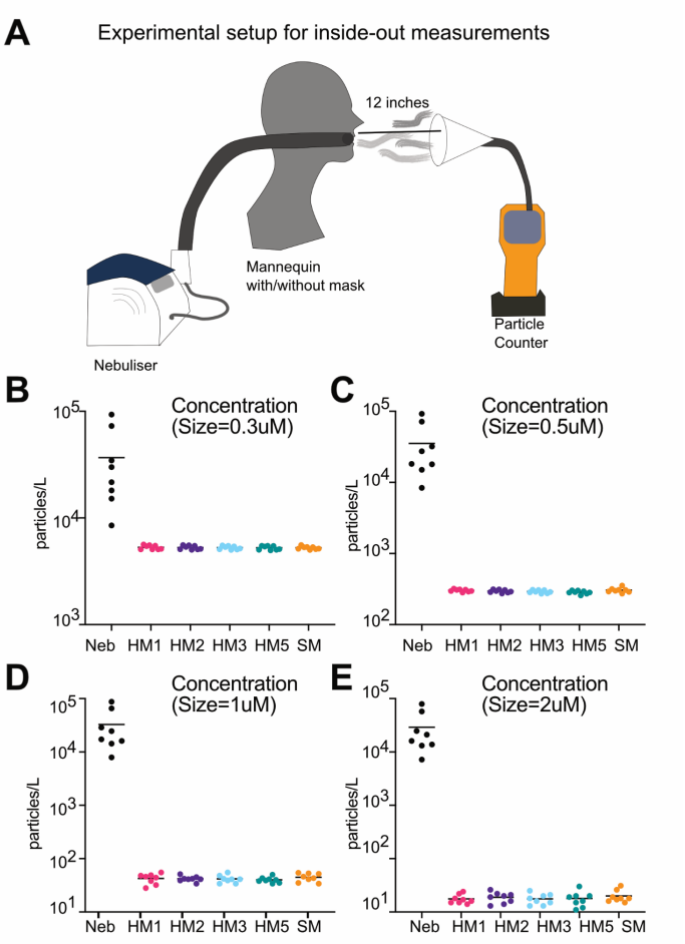

Figure 6: Comparison of different homemade masks for inside-out protection. (A) Experimental setup. Two independent sessions for all conditions were recorded and within each session four replicates for each condition were measured. All 8 points are shown. Particle counts adjusted to volume sampled is plotted for particles with a diameter of (B) 0.3uM, (C) 0.5uM, (D) 1uM and (E)2uM. Line indicates mean. neb=Nebuliser only. SM=surgical mask HM=home-made mask.

Overall, based on filtration efficiency alone, our results suggest homemade masks we tested work well for inside out protection while surgical masks are superior for outside-in. A disadvantage of surgical masks is that they are non-reusable, but there are studies ongoing to establish ways to decontaminate them without affecting their filtration capacity so that they can be reused[62]. Our results are in line with previous studies that found surgical masks were better than homemade masks[63,64]. These studies only demonstrate how well these masks work based on filtration of different particle size but do not provide real world evidence for the reduction in risk of disease transmission.

A recent meta-analysis of interventions showed that in a community setting wearing masks, including surgical or homemade multi-layered cotton masks reduced the risk of viral transmission[65]. This finding is important as in a community setting compliance with homemade mask and/or surgical masks is more likely, given they are more comfortable and easier to obtain. Since viral shedding and transmission can occur by presymptomatic individuals, masks would be useful in places such as public transport where physical distancing may be difficult.

Conclusion

The search for an effective vaccine and its adequate distribution to achieve herd immunity requires time. Nevertheless, there is reason to be optimistic as we start to gain knowledge of the virus and how it is spread. Recently Switzerland has launched a contact tracing app which has been downloaded by over a million people, and masks in public transport are now mandatory. These precautions also provide a useful reminder that although we are lifting the lockdown, the risk of infection remains. The strategy of extensive tracing, testing and quarantine combined with personal preventative measures such as hand

hygiene, distancing, and masks remain essential to establish a new normal. The importance of the use of masks is emphasized in the light of indirect evidence for airborne virus transmission. Given the incidences of recent outbreaks, the use of masks indoors in poorly ventilated areas with a high occupancy is a wise precaution. However, effectiveness of masks is determined by mask type, fit and comfort. For instance, although N95, FFP3 are superior in filtering small particles, they are only effective if they fit perfectly and they are extremely uncomfortable. Therefore, their use by health care professionals and in situations where there is increased risk of aerosol exposure to high viral loads, makes most sense. In our setup based on filtration efficiency, homemade masks worked well for inside out protection while surgical masks were superior for outside-in. The surgical masks with ribbons (KOLMI) which we evaluated were provided by the FMI. As FMI has provided a box of these masks to its staff and scientists, we recommend their use in shared indoor spaces where social distancing is not possible.

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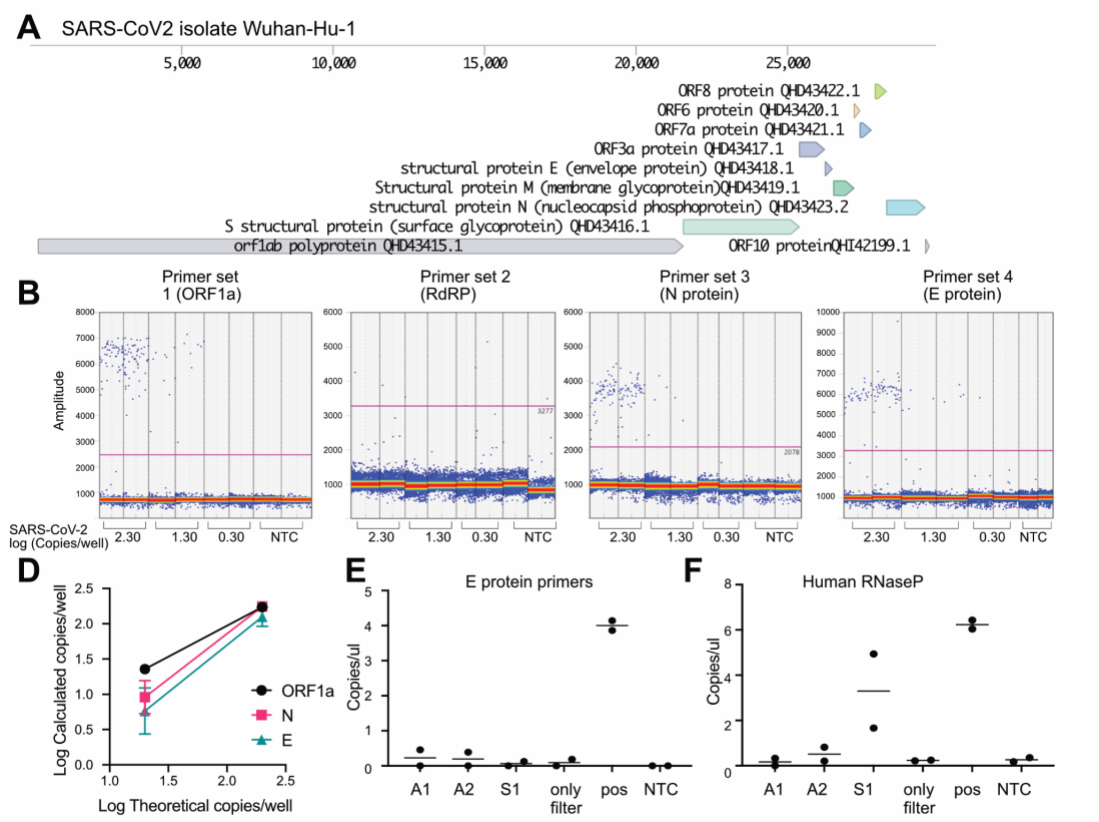

Supplementary figure 1: Detection of SARS cov2 using ddPCR A) use of different primers B) sensitivity c) measurements in air samples from FMI.

Supplementary Table 1: for Primer sequences used in ddPCR

Primer set	Sequences (5'-3')	Target	Reference
Primer set 1	ORF1ab-F CCCTGTGGGTTTTACACTTAA ORF1ab-R ACGATTGTGCATCAGCTGA ORF1ab-P FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ-1	ORF1a	Genescript
Primer set 2	RdRP_SARSr-F GTGARATGGTCATGTGTGGCGG RdRP_SARSr-R1 CARATGTTAAASACACTATTAGCATA RdRP_SARSr-P1 FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	RdRP (Pan Sarbeco)	WHO
Primer set 3	N gene-F GGGGAACTTCTCCTGCTAGAAT N gene-R CAGACATTTTGCTCTCAAGCTG N gene-P FAM-TTGCTGCTGCTTGACAGATT-TAMRA	N protein	Genescript
Primer set 4	E_Sarbeco_F1 ACAGGTACGTTAATAGTTAATAGCGT E_Sarbeco_R2 ATATTGCAGCAGTACGCACACA E_Sarbeco_P1 FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	E protein (Pan Sarbeco)	WHO