Are aerosols generated during lung function testing in patients and healthy volunteers? Results from the AERATOR study

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ABSTRACT
Pulmonary function tests are fundamental to the diagnosis and monitoring of respiratory diseases. There is uncertainty around whether potentially infectious aerosols are produced during testing and there are limited data on mitigation strategies to reduce risk to staff. Healthy volunteers and patients with lung disease underwent standardised spirometry, peak flow and FE\textsubscript{NO}\textsubscript{0} assessments. Aerosol number concentration was sampled using an aerodynamic particle sizer and an optical particle sizer. Measured aerosol concentrations were compared with breathing, speaking and voluntary coughing. Mitigation strategies included a standard viral filter and a full-face mask normally used for exercise testing (to mitigate induced coughing). 147 measures were collected from 33 healthy volunteers and 10 patients with lung disease. The aerosol number concentration was highest in coughs (1.45–1.61 particles/cm\textsuperscript{3}), followed by unfiltered peak flow (0.37–0.76 particles/cm\textsuperscript{3}). Addition of a viral filter to peak flow reduced aerosol emission by a factor of 10 without affecting the results. On average, coughs produced 22 times more aerosols than standard spirometry (with filter) in patients and 56 times more aerosols in healthy volunteers. FE\textsubscript{NO}\textsubscript{0} measurement produced negligible aerosols. Cardiopulmonary exercise test (CPET) masks reduced aerosol emission when breathing, speaking and coughing significantly. Lung function testing produces less aerosols than voluntary coughing. CPET masks may be used to reduce aerosol emission from induced coughing. Standard viral filters are sufficiently effective to allow guidelines to remove lung function testing from the list of aerosol-generating procedures.

INTRODUCTION
Due to the pandemic, concerns about viral transmission by aerosols and droplets mean that many respiratory diagnostic services are being cancelled or delayed.1 There is an urgent need to restart these vital diagnostic services and mitigate risks to patients and staff from viral transmission. However, there remains uncertainty around the role of lung function testing in the generation of aerosols and onward transmission risk.

A rapid systematic review identified seven sources that classified the aerosol-generating procedure (AGP) status of lung function tests.2 Four of these defined lung function tests as an AGP, two defined them as a possible AGP and one source

stated that these were not an AGP. All cited sources were guidelines, none of which referred to primary experimental data that could support these recommendations. Current advice from expert groups and guidance bodies is contradictory, leading to variation in clinical practice.3-5 Previous experimental work has been performed outside an ultra-clean environment, which does not allow accurate attribution of aerosol and can potentially misclassify it, does not conform to clinical standards, and does not include patients with lung disease.

In this study, we aimed to generate primary data on aerosol generation during lung function tests and assess mitigation strategies using highly sensitive technology in an ultra-clean, laminar flow theatre.

METHODS
This study was performed as part of the AERosolisation And Transmission Of SARS-CoV-2 in Healthcare Settings (AERATOR) study to assess the risk of aerosolised transmission of SARS-CoV-2 in healthcare settings.

Environmental set-up, recruitment and procedures
Full technical methods and images of the set-up are found in online supplemental appendix S1 and have been reported elsewhere.6 In brief, healthy volunteers and patients with lung disease were recruited to allow collection of aerosol emission in ultra-clean, laminar flow operating theatres. Participants underwent protocolised testing (breathing, speaking and coughing) followed by lung function tests designed to represent standard clinical practice. Time stamps were used to indicate timing and duration of tidal breathing and forced manoeuvres, including formal spirometry (as per American Thoracic Society/European Respiratory Society (ATS/ERS) guideline), peak flow measurement and FE\textsubscript{NO}\textsubscript{0} measurement.

All testing was performed by an accredited lung function technician using a Vantus spirometer (Vyaire), with FE\textsubscript{NO}\textsubscript{0} testing using a NIOX device (Aerocrine). The spirometer systems used single-use bacterial viral filters (BVF); peak flow meters were tested both with and without BVF (see online supplemental appendix S1 for images).

A proof-of-concept test of mitigation to reduce the risk of procedure-induced cough aerosolisation was undertaken using a reusable full-face
cardiopulmonary exercise test (CPET) face mask with BVF in five subjects. The sampling funnel was positioned over the point of greatest exhalation flow.

Measurements of aerosol were taken simultaneously by two separate devices, an aerodynamic particle sizer (APS; measured size range 0.3–20 μm) and an optical particle sizer (OPS; measured size range 0.3–10 μm); details are found in online supplemental methods. We report geometric mean and SD, with comparisons made by unpaired t-tests on the log-transformed data from the APS, unless stated. Both devices rely on fundamentally different technologies (aerodynamic size vs optical size) and therefore both were included to provide robustness in all reported measurements and reduce the chance of technical bias affecting the results.

RESULTS

Full results and patient demographics are found in online supplemental appendix S2. Correlation between the APS and OPS was extremely high (r>0.95), and therefore only APS results are reported in the text. In brief, the highest aerosol emission was generated during a voluntary cough, with the next highest in unfiltered peak flow (table 1 and figure 1). Patients with lung disease generated higher aerosol concentrations than volunteers when breathing (0.29 vs 0.04 particles/cm³, p<0.01) and speaking (0.20 vs 0.10 particles/cm³, p=0.04), but not when coughing (1.43 vs 1.61 particles/cm³, p>0.2).

Unfiltered peak flow produced approximately half the aerosol of voluntary coughing, but the addition of a filter reduced this by an order of magnitude in volunteers (0.76 vs 0.9 particles/cm³, p<0.01) and patients (0.37 vs 0.01 particles/cm³, p<0.01), with no clinically significant change in measured peak flow value (online supplemental figure S3).

Filtered spirometry also produced little aerosol (0.11 particles/cm³ in volunteers and 0.10 particles/cm³ in patients), with voluntary cough producing on average 56 times more aerosol in volunteers (and 22 times more aerosol in patients). We could not elicit any aerosol emission from the FEV₁ device.

A potential mitigation strategy for induced coughing, the CPET mask, was tested in five volunteers, with formal results reported in online supplemental appendix S2. Briefly, large reductions in aerosol emission during breathing (0.02 vs <0.0001 particles/cm³, p=0.08, paired t-test on log-transformed data for all comparisons), speaking (0.1 vs <0.001 particles/cm³, p=0.06) and coughing (1.12 vs 0.06 particles/cm³, p<0.01) were observed, although this did not meet a significance threshold due to the low number of participants.

Finally, we tested whether receiving nebulised salbutamol altered subsequent aerosol emission in patients and we identified no significant change (online supplemental results and figures S4–S6).

DISCUSSION

This study provides much-needed high-quality experimental data characterising aerosols generated during standard clinical lung function tests in volunteers and patients. The finding that spirometry, peak expiratory flow (with standard BVF) and exhaled nitric oxide testing do not generate significant aerosols (in comparison with cough) suggests there is likely limited additional risk associated with lung function testing outside of the potential to generate aerosols via coughing.

Peak flow testing without a filter does produce aerosols (although less than coughing). However, the addition of a filter reduces this aerosol concentration to negligible levels and renders the procedure non-aerosol-generating. Together this points to the effectiveness of standard CE-marked viral filters in reducing aerosol emission.

The study also identifies a potential effective mitigation measure (reusable filtered CPET mask) for aerosols generated by subjects who cough as a result of the procedure, with promising data showing large reductions in aerosol emission, although we caveat this is due to the small number of participants. Finally we provide reassurance that salbutamol nebulisation used as part of bronchodilator reversibility testing does not induce higher aerosol emission during subsequent testing. Taken together, we believe that this may allow for a significant increased diagnostic capacity through reduced need for air room changes between subjects.

This study has a number of strengths. First, we performed spirometry as following standard UK practice in an ultra-clean, laminar flow theatre with extremely low background aerosol concentration. This allows us to make confident conclusions...
about the source and level of aerosols produced. Second, we measured aerosol production from healthy volunteers and patients with a mixture of chronic lung conditions that were suspected to affect aerosol emissions.

There are some limitations to our study. We included patients with a mixture of small airways and suppurative lung conditions in our study, but the patient group was too small and heterogeneous to draw any definitive conclusions about the comparison between individual lung diseases. Common to all prior studies and for safety reasons, none of the subjects was known to be suffering from viral infections which have been shown to increase aerosol production. Finally, the link between recorded aerosol concentration and risk of onward infection remains unknown, with the capture and quantification of infectious SARS-CoV-2 or other pathogens in exhaled air methodology. They identified cough as the manoeuvres outside of an ultra-clean environment. Two patients with a mixture of chronic lung conditions that were suspected to affect aerosol emissions.

There are three comparable studies that have examined this question. First, Helgeson et al’s small-scale study identified aerosol generation during spirometry performed by five healthy volunteers. It detected aerosol production despite inclusion of viral filters, but excluded participants who coughed and only measured aerosols at a distance from the source, being unable to definitively identify the source. Greening et al included 33 healthy volunteers who performed different respiratory manoeuvres outside of an ultra-clean environment using particles in exhaled air methodology. They identified cough as the major source of aerosols and found varied results with other lung function testing. However, the authors acknowledge that their pulmonary function tests (PFTs) did not meet the ERS/ATS spirometry criteria and 22 of the volunteers performed only tidal breathing. Finally, Li et al performed lung function testing on 28 patients. Only one measure of aerosol (OPS) was performed during PFTs and for 30–60 min following the procedure after the patients and researchers had left the room. The paper concludes that PFTs should be considered an AGP; however, on examination of the presented data, measured aerosol levels remained steady or fell during the procedures. The peak aerosol production occurs when the patients moved away from the apparatus and breathed, spoke and coughed without a mask on. In our view, this data more likely reflect aerosol emission from normal respiratory activity such as coughing, rather than relating to the lung function testing, and highlight the need for measurement at the source of aerosol emission and an ultra-clean background.

Bringing all the data together, including our and others’ evidence that aerosol is generated from normal respiratory activity, this paper suggests that the major risk in lung function testing remains the potential for infectious aerosols to be generated by coughing rather than in performing spirometry. Risk assessments should focus on the risk of infection to the patient, ventilation of the room and whether induced coughing can be mitigated, rather than on the type of lung function testing performed.

CONCLUSIONS

Spirometry (performed with a standard filter) and FE_{NO} do not generate significant aerosol concentrations compared with coughs in healthy volunteers and patients with lung disease. Peak flow does generate aerosols, although a viral filter reduces this >10-fold. Reusable CPET masks with filters applied to subjects prior to testing may be a potential solution to mitigate aerosol emission from induced coughing before and after the procedure.

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Contributors JW, DAM, FWH, SS, FKAG, DTA, GWN and JB designed the experiments. CR performed the lung physiology testing. SS and FKAG analysed the data, with BRB and JPR providing supervisory support and analysis. GWN performed supplementary experiments.

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Investigation of respiratory disease: risk of aerosolisation from spirometry, peak flow, and other associated tests.

Supplement 1 - Methods

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This study was performed as part of the wider AERATOR study to assess the risk of aerosolised transmission of SARS-CoV-2 in healthcare settings. Ethical approval was given by the North West Research Ethics Committee (Ref: 20/NW/0393, HRA Approved 18/9/20).

Aerosol measurement

Aerosol measurements were recorded using two devices simultaneously: an Optical Particle Sizer (OPS) and Aerodynamic Particle Sizer (APS). Technical specifications were detailed in a previous publication from our group but are replicated here.[1] Images of the set up are shown at the bottom of this document.

The APS (TSI Incorporated, model 3321, Shoreview, NM, USA) measures aerosol at a sampling flow rate of 1 L min\(^{-1}\) with accompanying sheath flow of 4 L min\(^{-1}\). The APS reports the aerodynamic size of particles in an aerosol plume, size-resolving aerosol number concentration into 52 size bins ranging from 0.5 µm to 20 µm in diameter with a time integration of 1 s. The size bins are equally spaced in log(diameter) space, apart from the smallest size bin (0.5 - 0.523 µm).

The OPS (TSI Incorporated, model 3330, Shoreview, NM, USA) samples air at 1 L min\(^{-1}\) and detects particles by laser optical scattering. The OPS reports the particle number concentration and optical size distribution within the diameter range 300 nm to 10 µm with a time resolution of 1 second. The OPS is widely used for aerosol studies from laboratories / clean rooms to more demanding outdoor environments. It is calibrated by the manufacturer using polystyrene latex spheres and its performance conforms to the ISO standard 21501-4:2018. The reported optical size of the particles is based on an assumed refractive index of pure water at 600 nm wavelength (1.333).

Both the APS and OPS were connected to the same sampling funnel, which was 3D printed (RAISE3D Pro2 Printer, 3DGBIRE, Chorley, UK) from PLA with a maximum diameter of 150 mm, cone height of 90 mm with a 10-mm exit port. Two conductive silicone sampling tubes of 0.3 m length and internal diameter 4.8 mm (3001788, TSI) were connected to the neck of the sampling funnel, with one connected to the APS and the other to the OPS.

Environmental set up and patient recruitment

Participants were recruited in an ultra-clean laminar flow operating theatre (EXFLOW 32, Howarth Air Technology, Farnworth, UK) with high efficiency particulate air (HEPA) filtration and an air supply rate of 1200 m\(^3\)/s. This ventilation system has a canopy ‘clean zone’ where surgical procedures are
performed; the air circulation velocity is 0.2 m.s$^{-1}$ at 1 m above the floor below the canopy and produces 500–650 air changes per hour. All aerosol recordings were performed under the canopy, and the background aerosol concentration was sampled prior to each measurement for a mean sampling duration of 43 s.

**Statistical analysis**

Aerosol generation differs greatly among people, with an approximate log-normal distribution in number concentration.[2,3] As such, our analysis focused on comparing the relative aerosol number concentrations from different procedures performed by each individual. We report the number concentration, an intensive property that does not depend on scale (i.e. is independent of the time or volume sampled) as reported by the instruments measured over a sample period, selected to be 1 s.

We have reported one of two parameters for each activity: either the peak particle number concentration reported across the full number of samples of the measurement for single, forced exhalations such as coughing (cm$^{-3}$); or, the mean particle number concentration reported as the average across all samples for continuous activities such as breathing or speaking (cm$^{-3}$). We then visualised size distributions of aerosol emission across the volunteers and compared aerosol emission across activities. We report geometric mean and geometric standard deviations, and all comparisons are by t-tests on the log-transformed data, unless stated.

Data analysis was performed by collating raw data of sampled aerosol concentration output by the APS and OPS instruments using Aerosol Instrument Manager 9.0 (TSI Incorporated, Shoreview, NM, USA) and Microsoft Excel. A custom-written software in LabVIEW (National Instruments, Texas, USA) was used to automate the analysis process for increased efficiency.

**Images**
Example images: Peak flow device without filter
Example images: Peak flow device with filter

Example image: FENO device, with arrow commenting on where aerosol was measured from.
Example images: CPET mask with standard bacterial / viral filter
Experimental set up:

This figure shows one of the study researchers sitting in the laminar flow theatre and demonstrating the funnel. The two devices connected to the funnel are the OPS, sitting on top of the APS.


Investigation of respiratory disease: risk of aerosolisation from spirometry, peak flow, and other associated tests.

Supplement 2 - Results

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Results

Demographics

33 healthy volunteers and ten patients were recruited; with demographics and lung function results reported in Table S1. Volunteers were young (median age 32), of normal weight (median BMI 23.6) and had normal lung function; as would be expected from this cohort. 16 (48%) were male, with 17 being female (52%). 10 patients were recruited with a median age of 71. 3 were female and 7 were male. The clinical diagnoses were asthma (5 patients), bronchiectasis (3 patients), allergic bronchopulmonary aspergillosis (1 patient), asthma/COPD overlap (1 patient).

Table S1: Demographics of the study cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Volunteers (n = 33)</th>
<th>Patients (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight in kg</td>
<td>72 (64, 79)</td>
<td>73 (63, 81)</td>
</tr>
<tr>
<td>Height in centimetres</td>
<td>174 (164, 179)</td>
<td>161 (158, 162)</td>
</tr>
<tr>
<td>BMI</td>
<td>23.6 (22.0, 25.5)</td>
<td>28.1 (24.0 – 31.0)</td>
</tr>
<tr>
<td>Age</td>
<td>35 (32, 40)</td>
<td>71 (62, 76)</td>
</tr>
<tr>
<td>Female (n, %)</td>
<td>17 (52%)</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>FEV1 (litres)</td>
<td>3.50 (3.17, 4.07)</td>
<td>1.48 (1.20 – 1.65)</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>105 (91, 111)</td>
<td>59 (56 -67)</td>
</tr>
<tr>
<td>FVC (litres)</td>
<td>4.28 (3.98, 5.27)</td>
<td>2.43 (1.76 - 2.81)</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>110 (105, 118)</td>
<td>75 (72 -80)</td>
</tr>
<tr>
<td>1 Median (IQR), unless stated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Aerosol emission during respiratory function testing

Table 1 (in the main document) describes the geometric mean and geometric standard deviation of aerosol number concentrations generated during FEV1, peak flow, and FENO, in comparison with speaking, breathing and coughing for all patients and volunteers. Figure 1 (in the main document) reports the data as a boxplot, comparing volunteers and patients with lung disease. As all of those...
activities apart from breathing and speaking are sporadic activities, we report the maximum number of particles per cm$^3$ for those activities.

Patients with lung disease generated higher aerosol concentrations than volunteers when breathing (0.29 vs 0.04 particles/cm$^3$, p <0.01), and when speaking (0.20 vs 0.10 particles/cm$^3$, p = 0.04), but not when coughing (1.45 vs 1.61 particles/cm$^3$, p>0.2) although there was large individual variability, particularly in healthy volunteers. Figure S1 shows the aerosol emission for each individual patient.

Figure S1: Aerosol total concentration detected while speaking, breathing, and coughing for each patient with lung disease.

Of all the activities tested, voluntary cough produces by far the most particles, with an average of 1.61 particles/cm$^3$ in volunteers, and 1.45 particles/cm$^3$ in patients. However, adding a filter reduced this by an order of magnitude for both volunteers (0.76 unfiltered vs 0.09 particles/cm$^3$ filtered, p <0.01 ) and patients (0.37 unfiltered vs 0.01 particles filtered /cm$^3$, p <0.01). Therefore, compared to a filtered peak flow, voluntary cough produced factor of 18 more aerosol in volunteers (0.09 particles /cm$^3$ vs 1.61 particles/cm$^3$) and a factor of 145 more aerosol in patients (0.01 particles /cm$^3$ vs 1.45 particles/cm$^3$, both comparisons p<0.01).
For both patients and volunteers, filtered spirometry generated aerosol similar to a filtered peak flow (0.11 particles/cm³ in volunteers and 0.10 particles/cm³ in patients) at a concentration of one order of magnitude lower than a voluntary cough, with all participants except one healthy volunteer having >2-fold reduction in aerosol emission. On average, voluntary cough in healthy volunteers generated a factor of 56 more aerosol compared to spirometry in volunteers, with a factor of 22 in patients with lung disease (both comparisons p < 0.01).

**FENO device**

The FENO device does not have a clear exhalation port and the manufacturer does not make clear where exhaled breath leaves the device. We interrogated the device and measured aerosol concentration at all possible exhalation ports (see image in supplementary appendix). In all positions we did not find any significant aerosol emission from the FENO device.

**CPET mask**

We tested the use of a CPET mask with viral filter placed over the exhalation port to reduce aerosol emission on 5 healthy volunteers, with raw data shown in Table S2. Large reductions in aerosol emission during breathing (0.02 vs <0.001 particles/cm³, p = 0.08, paired t-test for all comparisons), speaking (0.1 vs <0.001 particles/cm³, p = 0.06) and coughing (1.12 vs 0.06 particles/cm³, p < 0.01) were measured when using the CPET mask with a filter. Because of the small numbers this only met significance testing for coughing, although the average reduction in aerosol emission was by more than a factor of ten for speaking, and a factor of 20 for coughing and breathing.

Table S2: Raw data from the CPET mitigation strategy.

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Breathe (mean)</th>
<th>CPET breathe (mean)</th>
<th>Speak (mean)</th>
<th>CPET speak (mean)</th>
<th>Cough (peak)</th>
<th>CPET cough (peak)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.044</td>
<td>0.006</td>
<td>0.044</td>
<td>0.002</td>
<td>1.23998</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.03</td>
<td>0.002</td>
<td>0.15</td>
<td>0.006</td>
<td>0.91998</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.01</td>
<td>0.002</td>
<td>0.056</td>
<td>0.002</td>
<td>1.05998</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.042</td>
<td>0</td>
<td>0.46599</td>
<td>0.002</td>
<td>7.29986</td>
<td>0.12</td>
</tr>
<tr>
<td>5</td>
<td>0.008</td>
<td>0</td>
<td>0.06162</td>
<td>0</td>
<td>0.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

All figures represent the raw reported aerosol number concentration from the APS, with peak number concentration for cough, and average for breathing and speaking.
This is visualised in Figure S2. This mask can be worn before, during, and after spirometry and may be a potential mitigation technique as induced coughing is common during lung function.

In summary, formal lung function testing using a spirometer with a filter did not generate significant aerosol in comparison to a cough; with peak levels similar to average emissions during speaking.
Figure S2: CPET mask as a mitigation strategy
**Peak flow with a filter**

To ensure peak flow results were reliable with a filter, we compared peak flow readings both with and without the protective filter for 9 participants. The measured flow rates are shown in Figure S3 and are strongly correlated with a R of 0.966. Therefore, although there may be a slight reduction in recorded FEV1 with the peak flow monitor when used with a filter, this is likely clinically insignificant.

Figure S3: Correlation between unfiltered and filtered peak flow
Aerosol emission pre and post-spirometry, and the effect of salbutamol

There is a concern that lung function testing itself may generate aerosol. Therefore, for all patients, we measured aerosol number concentrations from breathing, speaking, coughing both pre- and post-spirometry. Results are shown in Figure S4 and S5: no quantitative or qualitative difference in aerosol number concentration is reported between pre- and post-spirometry. For four patients, we assessed the impact of salbutamol nebulisation on aerosol emission, and again found no difference in aerosol emission post-salbutamol reversal (Figure S6).

Figure S4: Aerosol total concentration detected pre and post spirometry in patients with lung disease.

Figure S5: Aerosol size distribution detected pre and post spirometry in patients with lung disease.
Figure S6: Aerosol total concentration detected pre and post reversal in patients with lung disease.