

# Dental Mitigation Strategies to Reduce Aerosolization of SARS-CoV-2

J.J. Vernon<sup>1</sup>, E.V.I. Black<sup>1</sup> , T. Dennis<sup>2</sup> , D.A. Devine<sup>1</sup> ,  
L. Fletcher<sup>3</sup>, D.J. Wood<sup>1</sup>, and B.R. Nattress<sup>4</sup>

Journal of Dental Research  
2021, Vol. 100(13) 1461–1467  
© International Association for Dental  
Research and American Association for Dental,  
Oral, and Craniofacial Research 2021



Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/00220345211032885  
journals.sagepub.com/home/jdr

## Abstract

Limiting infection transmission is central to the safety of all in dentistry, particularly during the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Aerosol-generating procedures (AGPs) are crucial to the practice of dentistry; it is imperative to understand the inherent risks of viral dispersion associated with AGPs and the efficacy of available mitigation strategies. In a dental surgery setting, crown preparation and root canal access procedures were performed with an air turbine or high-speed contra-angle handpiece (HSCAH), with mitigation via rubber dam or high-volume aspiration and a no-mitigation control. A phantom head was used with a 1.5-mL min<sup>-1</sup> flow of artificial saliva infected with Φ6-bacteriophage (a surrogate virus for SARS-CoV-2) at ~10<sup>8</sup> plaque-forming units mL<sup>-1</sup>, reflecting the upper limits of reported salivary SARS-CoV-2 levels. Bioaerosol dispersal was measured using agar settle plates lawned with the Φ6-bacteriophage host, *Pseudomonas syringae*. Viral air concentrations were assessed using MicroBio MB2 air sampling and particle quantities using Kanomax 3889 GEOα counters. Compared to an air turbine, the HSCAH reduced settled bioaerosols by 99.72%, 100.00%, and 100.00% for no mitigation, aspiration, and rubber dam, respectively. Bacteriophage concentrations in the air were reduced by 99.98%, 100.00%, and 100.00% with the same mitigations. Use of the HSCAH with high-volume aspiration resulted in no detectable bacteriophage, both on nonsplatter settle plates and in air samples taken 6 to 10 min postprocedure. To our knowledge, this study is the first to report the aerosolization in a dental clinic of active virus as a marker for risk determination. While this model represents a worst-case scenario for possible SARS-CoV-2 dispersal, these data showed that the use of HSCAHs can vastly reduce the risk of viral aerosolization and therefore remove the need for clinic fallow time. Furthermore, our findings indicate that the use of particle analysis alone cannot provide sufficient insight to understand bioaerosol infection risk.

**Keywords:** aerosol-generating procedure, bacteriophage, bioaerosol, high-speed contra-angle handpiece, fallow time, dentistry

## Introduction

The potential nosocomial spread of pathogens including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through oral fluid aerosolization provides a significant risk to patients, dentists, and oral health care teams. During the SARS-CoV-2 pandemic, extensive constraints have been placed on dentistry worldwide, with a particular focus on aerosol-generating procedures (AGPs) (World Health Organization 2020). These constraints affect widely upon dentistry delivery in practices/offices and on multioccupancy teaching clinics. There is a paucity of robust data supporting some of these restrictions; it is essential to investigate the efficacy of mitigation strategies and the requirement for fallow time between patients.

Various methodologies for determining aerosolization in dental environments have been implemented, including air particle measurement (Din et al. 2020; Allison et al. 2021), biological air sampling (Bennett et al. 2000; Dutil et al. 2009), culture of settle plates (Timmerman et al. 2004; Rautemaa et al. 2006; Holloman et al. 2015), and detection of fluorescent markers (Allison et al. 2021; Holliday et al. 2021; Llandro et al. 2021). However, each has limitations. For instance, settle plates cannot account for the smallest particles that will not settle out of the air, air particle data cannot distinguish particles from the dental unit waterline and those of biological origin,

and the use of fluorescent dyes cannot reveal viability of any biological component. Therefore, none of these methods alone can proffer robust findings regarding the dispersal of active SARS-CoV-2.

Here we report a novel viral model for bioaerosol enumeration, using the bacteriophage Phi6 (Φ6) as a surrogate for SARS-CoV-2. Structurally, the Φ6 virus particle is similar to SARS-CoV-2: it is a double-stranded RNA virus of ~80 to 100 nm in size, composed of a lipid membrane envelope and spike proteins (Adams 1959). Recent literature has suggested Φ6 is an appropriate surrogate for infectious enveloped viruses, such as coronavirus (Aquino de Carvalho et al. 2017; Prussin et al.

<sup>1</sup>Division of Oral Biology, School of Dentistry, University of Leeds, Leeds, UK

<sup>2</sup>Leeds Dental Institute, Leeds Teaching Hospitals Trust, Leeds, UK

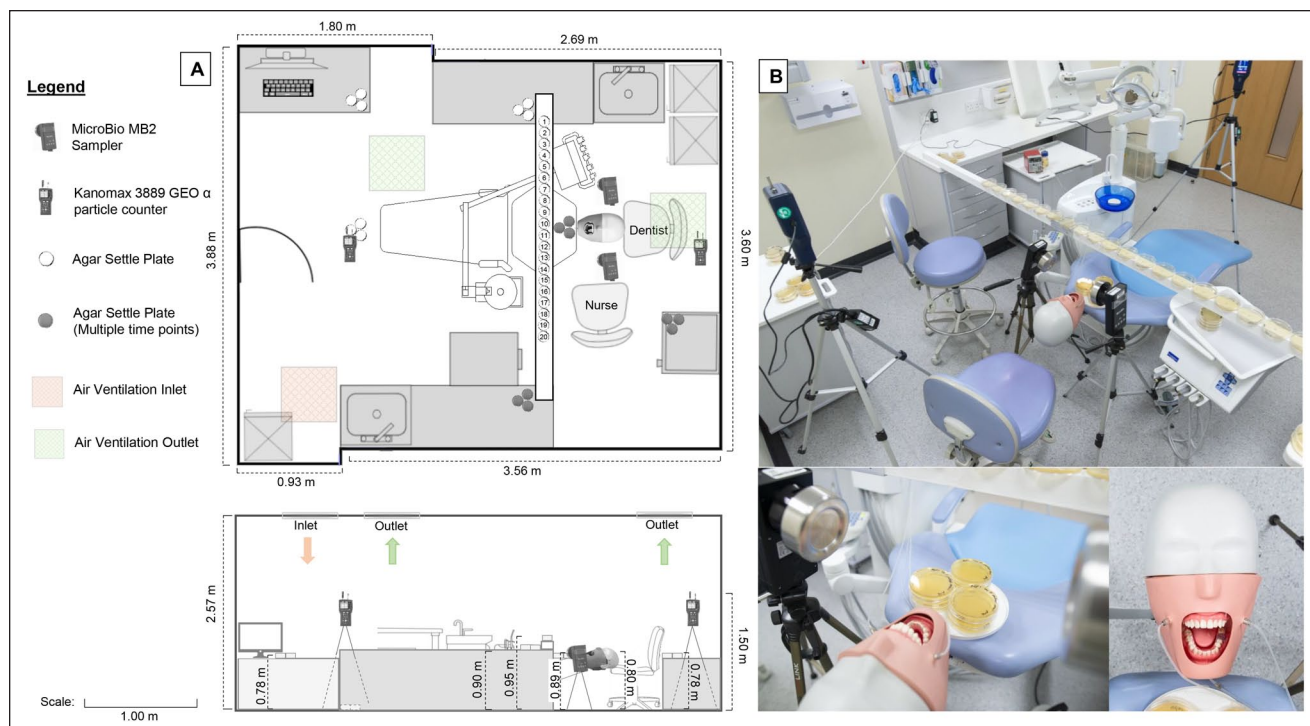
<sup>3</sup>School of Civil Engineering, University of Leeds, Leeds, UK

<sup>4</sup>Division of Restorative Dentistry, School of Dentistry, University of Leeds, Leeds, UK

A supplemental appendix to this article is available online.

### Corresponding Author:

D.J. Wood, Division of Oral Biology, School of Dentistry, University of Leeds, Worsley Building, Clarendon Way, Leeds LS2 9JT, UK.  
Email: d.j.wood@leeds.ac.uk



**Figure 1.** Experimental setup in the Leeds Dental Institute surgery. (A) Schematic of experimental setup (top, above; bottom, side view). (B) Top: photographic overview of surgical clinic layout. Bottom: closeup of the phantom head with saliva tubing inputs and oral cavity configuration.

2018; Buhr et al. 2020; Dubuis et al. 2020; Fedorenko et al. 2020). Comparing different bacteriophages for viral aerosolization models,  $\Phi 6$  was shown to behave comparably in nebulization experiments to influenza, another enveloped virus (Turgeon et al. 2014). Subsequently,  $\Phi 6$  behaved similarly to influenza virus in an investigation of viral recovery from hands (Casanova and Weaver 2015), and expected behavior of  $\Phi 6$  as a SARS-CoV-2 surrogate was reported when assessing viral survival in surface droplets (Fedorenko et al. 2020). Therefore,  $\Phi 6$  can be used in conjunction with its host bacterium, *Pseudomonas syringae*, to provide a valuable viral detection system. To our knowledge, this is the first study modeling, in the dental clinic, the aerosolization of a bacteriophage as an active biological marker and SARS-CoV-2 surrogate.

The aim of this study was to use a multifaceted approach to measure aerosol dispersal in a dental surgery to determine the potential infection risk to the dental team from bioaerosol exposure during routine dental procedures, optimal mitigation strategies, and the necessity of fallow time.

## Method

### Microorganism Strains and Culture Conditions

*P. syringae* (DSM 21482) and bacteriophage  $\Phi 6$  (DSM 21518) were acquired from the German Collection of Microorganisms and Cell Cultures (Leibniz Institute). *P. syringae* was cultured on Tryptic Soy Agar (TSA; Sigma) in 5% CO<sub>2</sub> for 48 h at 25°C (Forma Scientific) or in Tryptic Soy Broth (TSB; Oxoid) for 18 h at 25°C, 150 rpm.

Bacteriophage  $\Phi 6$  was propagated as previously reported, with modifications (see Appendix; Pinheiro et al. 2019; Whitworth et al. 2020).

### Experimental Model Design

Experiments were conducted at the Leeds Dental Institute, in a clinical surgery with a design airflow of 9 air changes per hour (ACH), measured by balometer (PH731 Capture hood; TSI AirFlow Instruments) at 8.3 ACH by the investigators. A dental phantom head (Nissin Dental Products), adapted to fit a dental chair, acted as a surrogate patient, and tooth preparations were performed on hard thermosetting plastic teeth (Frasaco) in the upper left 2 (UL2) and upper left 6 (UL6) positions. A continuous flow of artificial saliva (see Appendix), containing  $\Phi 6$  bacteriophage ( $\sim 10^8$  plaque-forming units, pfu mL<sup>-1</sup>), was introduced from 3 anatomical positions, 2× parotid, 1× sublingual (Fig. 1B). These positions were used for endodontic access procedures on the upper first molar tooth. For the anterior crown preparation, the saliva port from the upper left first molar was moved to over the apex of the upper left lateral incisor. Total salivary flow was 1.5 mL min<sup>-1</sup>, split equally across the 3 positions. Prior to commencing the procedures, the surfaces of the oral cavity were coated with artificial saliva containing bacteriophage.

### Aerosol-Generating Procedures

The AGPs investigated were root canal access of the upper left first molar and a full crown preparation on the upper left lateral

incisor, with an assistant providing high-volume aspiration where necessary. A KaVo EXPERTtorque LUX E680L air turbine handpiece (KaVo Dental GmbH) with an approximate cutting speed of 200,000 rpm (water flow rate 22 mL min<sup>-1</sup>, air pressure 36 psi) or a NSK S-Max M95L electric, high-speed contra-angle handpiece (HSCAH; Nakanishi) at 60,000 rpm (water flow rate 60 mL min<sup>-1</sup>) were used with the relevant diamond bur (Hidi-Once Diamond Bur Med; Dentsply). The NSK handpiece was used with the absence of “chip air” to reduce water atomization, as recommended in clinical guidelines (Sergis et al. 2020; Scottish Dental Clinical Effectiveness Program 2021). Mitigation strategies assessed included high-volume aspiration (with saliva ejection), rubber dam and aspiration, and an Aspi Jet 25 aerosol extraction device with a flute-shaped end piece (Cattani Air Technology), used as per the manufacturer’s instructions, in the 6-o’clock position. Each procedure was performed at least 3 times and comprised a 10-min settle period after setup, 20-min AGP, followed by 20-min fallow time. The AGP consisted of 4 min of active handpiece, followed by 1 min of rest, repeated 4 times for a total AGP time of 20 min. Settle plates were exposed during the 10-min pre-AGP settle period to determine bioaerosol carryover from the previous experiment. Fresh personal protective equipment (PPE) was donned for each procedure to prevent cross-contamination between experiments, and only the dentist and investigator (also acting as dental nurse) were present during AGPs. Postprocedure, a third investigator (wearing PPE) sealed settle plates as a final anti-cross-contamination measure.

### Bacteriophage Dispersal Detection

Passive and active sampling were undertaken to monitor the spread of bioaerosol. Settle plates and air sampling plates containing *P. syringae* were used to detect aerosol and droplets in the environment. Triplicate settle plates (lawns of *P. syringae* [OD<sub>600</sub> 0.6] on TSA containing 50 mg L<sup>-1</sup> cycloheximide; Fisher Scientific) were positioned in the dental surgery, at breathing zone, at bench height, or on the floor (Fig. 1A). In 3 locations, settle plates were exposed during AGP and post-AGP fallow period. Twenty settle plates arranged across the surgery, proximal to the oral cavity (Fig. 1), were used to collect bioaerosol and measure the distance traveled by splatter (particles >50 µm) (Harrel et al. 1998) and aerosolized droplets.

Furthermore, *P. syringae*-lawned settle plates were used with 2 MicroBio MB2 air sampling devices (Cantium Scientific), set 30 cm either side of the oral cavity (Fig. 1), to sample 400 L of air during each of the four 4-min periods of handpiece use. Two delayed air samples of 400 L were taken after 6 min of fallow period. Air sample counts were adjusted by a positive-hole correction factor (Macher 1989).

Procedures were performed in series, enabling mitigation strategies to be compared with each other and a no-mitigation baseline.

### Air Particle Enumeration and Size Determination

Two Kanomax 3889 GEOα particle counters, one positioned directly behind the dentist and another between the dental chair and the door (Fig. 1), monitored the size and quantity of particles in 6 size ranges simultaneously (diameters 0.3 µm, 0.5 µm, 1.0 µm, 3.0 µm, 5.0 µm, and 10 µm) in aerosols generated before, during, and post-AGP. Counters were situated at a height of 150 cm, corresponding to the average adult breathing zone. Measurements (particles/m<sup>3</sup>) were recorded in 1-min repeating periods and presented as baseline-standardized readings.

### Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics 26. Direct comparisons of air turbine and HSCAH and procedure location were performed using the Mann–Whitney *U* test. For bacteriophage data, the significance cutoff of Bonferroni corrections was *P* = 0.017 for multiple comparisons and *P* = 0.008 for particle data.

## Results

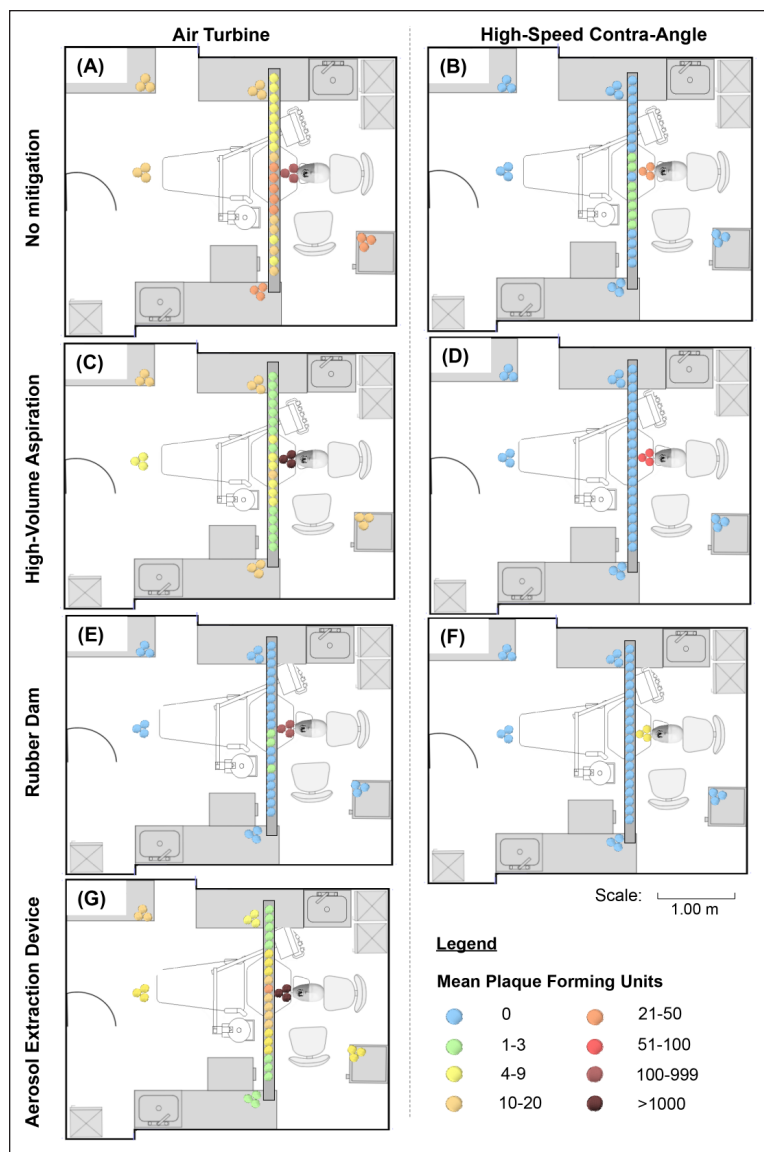
### Bioaerosol Dispersal during AGPs with Varying Mitigation Strategies

Bioaerosol was detected at all sampling points with an air turbine and no-mitigation control (Fig. 2). Each mitigation reduced levels of bioaerosol recovered from settle plates and air samples (Table 1), with minimal improvement on high-volume aspiration with the Aspi Jet 25. The use of a rubber dam greatly reduced aerosolized bacteriophage and splatter. Across all mitigations for anterior crown preparations, the HSCAH generated significantly less bioaerosol than the air turbine (*P* < 0.001). For high-volume aspiration procedures, the HSCAH reduced 100.00% of settled aerosol (*P* = 0.037) and 99.98% of bioaerosol recovered in air samples (*P* = 0.046) compared with the air turbine. For no-mitigation controls and rubber dam, settled bioaerosol counts were reduced with the HSCAH by 99.72% and 100.00%, respectively. Bacteriophage detection through air sampling was reduced by 99.49% and 100.00%, respectively, for the same mitigations (*P* < 0.001).

Procedures employing the air turbine with high-volume aspiration generated bacteriophage levels of 21 pfu and 11.25 pfu/m<sup>3</sup> for post-AGP settled bioaerosols and microbiological air samples, respectively (Table 1). Use of a HSCAH reduced both to zero (*P* = 0.037 and *P* = 0.037, respectively). The use of rubber dam for either handpiece also resulted in undetectable bioaerosol for the postprocedure, fallow period.

### Assessment of Handpiece and Mitigation Strategies through Air Particle Analysis

Particle counts of all size ranges recorded in anterior procedures were reduced with the HSCAH versus air turbine, except



**Figure 2.** Bacteriophage dispersal heat maps by handpiece and mitigation strategy. Data from anterior crown preparation procedures. (A, B) No mitigation, (C, D) high-volume aspiration, (E, F) rubber dam, and (G) Aspi Jet 25.

for 0.3-µm particles under rubber dam (Fig. 3, Appendix Table 1). Significant differences were observed comparing handpieces with no mitigation for several particle size ranges:  $P = 0.017$ ,  $P = 0.005$ ,  $P = 0.001$ ,  $P = 0.008$ ,  $P = 0.021$ , and  $P = 0.059$  for 0.3 µm, 0.5 µm, 1.0 µm, 3.0 µm, 5.0 µm, and 10.0 µm, respectively.

The time taken, post-AGP, for particle levels to return to preprocedure baseline levels was variable, with 22.2% of procedures not reaching baseline within 25 min. There were no discernible differences between air turbine and HSCAHs for the time to return to baseline for 0.3-µm and 0.5-µm particles, 16.3 versus 18.2 and 15.7 versus 17.2 min, respectively. Differences were greater for 1.0-µm, 3.0-µm, 5.0-µm, and 10.0-µm particles, with average times to reach baseline of 16.7

versus 7.4, 14.1 versus 4.3, 12.6 versus 3.9, and 12.8 versus 4.5 min, respectively.

### Bioaerosol Generation in Anterior versus Posterior Tooth Positions

Settled and air bioaerosol measurements demonstrated decreases of >92% bacteriophage counts when comparing UL6 versus UL2 positions, with aspiration reducing settle by 99.6% and air sample readings by 100.0% (Table 2). Particle detection was also greatly reduced in posterior endodontic experiments versus anterior procedures for all particle sizes and sampling positions (Appendix Tables 2 and 3).

### Discussion

To model bioaerosol spread during worse-case scenario AGPs, we performed experiments with a salivary bacteriophage concentration (~ $10^8$  pfu mL<sup>-1</sup>) close to maximum reported levels of SARS-CoV-2 in human saliva (To et al. 2020; Wyllie et al. 2020). Different mitigation strategies using both air turbine and HSCAHs were deployed for each dental procedure. There was a clear distinction between the amount of aerosolized saliva dispersed around the dental surgery using the air turbine and HSCAH. Bioaerosol levels were clearly diminished when using the HSCAH compared with the air turbine. No bacteriophage was detected on the pre-AGP settle plates for any of the experiments, indicating no cross-contamination between experiments.

Air sampling quantified active viral particles in the air, potentially too small to settle onto clinic surfaces (King et al. 2013). With high-volume aspiration, the differences comparing air turbine and HSCAH AGPs were large, 637.4 versus 0.1 pfu/m<sup>3</sup>, respectively. The latter represented a solitary pfu detected in 1 experimental replicate. For all mitigations, settle plates closest to the mouth indicated the highest quantities of bacteriophage

(Fig. 2), indicative of splatter rather than bioaerosol and considered a lower transmission risk. Our data were collected in an environment with mechanical ventilation, which is not available in many practices worldwide, and aerosol accumulation is greater in practices with poor ventilation (Ren et al. 2021).

These differences are clearly supported by the particle data, which indicated lower levels of all particle sizes for most mitigations (Fig. 3). By recording particle measurements from 2 locations, we saw that aerosols were not localized and displayed similar but slightly delayed trends toward the extremities of the clinic, further highlighting the necessity for good mitigation protocols.

Other studies have suggested the use of a rubber dam significantly reduces microbial aerosolization (Cochran et al.

**Table 1.** Mean Φ6 Bacteriophage Plaque-Forming Units (pfu) Collected with Upper Left Lateral Incisor Procedures on Settle and Air Sampling Agar Plates.<sup>a</sup>

Characteristic	Total pfu (Unless Stated, i.e., Air Samples)						
	Air Turbine			Aerosol Extraction Device (n = 3)	High-Speed Contra-Angle		
	No Mitigation (n = 8)	High-Volume Aspiration (n = 3)	Dam (n = 3)		No Mitigation (n = 7)	High-Volume Aspiration (n = 3)	Dam (n = 3)
Splatter zone	1,152.75 (345.50)	1,455.00 (190.05)	106.67 (35.53)	1,255.33 (245.19)	54.43 (20.59)	64.33 (61.84)	5.33 (3.53)
Settled aerosol	207.00 (98.22)	90.33 (26.09)	0.33 (0.33)	86.33 (22.64)	0.57 (0.57)	0.00 (0.00)	0.00 (0.00)
Average air (pfu/m <sup>3</sup> )	940.98 (56.29)	637.40 (142.79)	1.35 (0.27)	380.10 (68.74)	4.82 (1.29)	0.10 (0.04)	0.00 (0.00)
Fallow settle	24.75 (16.72)	21.00 (18.01)	0.00 (0.00)	2.00 (0.58)	0.57 (0.57)	0.00 (0.00)	0.00 (0.00)
Average fallow air (pfu/m <sup>3</sup> )	29.38 (2.80)	11.25 (0.29)	0.00 (0.00)	2.92 (0.17)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

<sup>a</sup>Data (SEM in parentheses) are delineated by handpiece and mitigation strategy. Splatter zone radius defined within 41 cm radius of mouth.

1989; Samaranyake et al. 1989). However, these studies reported bacterial air contamination rates, and it is important that we have documented the aerosolization of viruses. In addition to rubber dam use, preprocedural rinsing would likely reduce risk further. Although limited by a small sample size, Meethil et al. (2021) determined a moderately low risk of SARS-CoV-2 transmission during AGPs, with a 1% hydrogen peroxide rinse substantially limiting salivary microbiota transfer to the environment. The Aspi Jet 25, a specialist aerosol extraction device, was only marginally better than high-volume aspiration alone.

To improve the accuracy of the phantom head over previously published models (Allison et al. 2021; Holliday et al. 2021; Shahdad et al. 2021), we added an anatomical tongue model and a high-level physiological salivary flow of 1.5 mL min<sup>-1</sup>, spread across 3 positions. While an improvement, our model has limitations. It uses artificial teeth, which lack the anatomical intricacies of human teeth. However, previous particle analyses revealed little difference between plastic and real teeth (Shahdad et al. 2021). Our model does not imitate the effects of a patient breathing or other patient behaviors, such as talking or coughing, that would likely contribute to bioaerosol production. Nonetheless, by demonstrating reductions in active biological marker dispersal, we can make robust indications as to the value of dental mitigation approaches. The salivary bacteriophage levels used reflect those of symptomatic SARS-CoV-2 cases, which are unlikely to be encountered in a dental clinic that employs efficient triage protocols to exclude symptomatic patients. An inoculum based on lower levels of SARS-CoV-2 carriage in asymptomatic patients would be appropriate to assess true dispersal rates in a clinic; however, we focused on determining the efficacy of mitigation strategies for risk reduction, rather than establishing absolute viral spread, and employed conditions that were representative of a worse-case scenario.

Acknowledging the potential for operator-induced effects, our procedures were performed by a single operator to maintain consistency of findings between variables. As every clinic/surgery varies in airflow design, dispersal levels may not be directly translatable, particularly to environments lacking mechanical ventilation. However, our data indicating the efficacy of



**Figure 3.** Particle sizing and count data delineated by handpiece and mitigation. Top: mean particles/m<sup>3</sup> by size, produced during aerosol-generating procedures with air turbine and high-speed contra-angle hand-pieces (HSCAH) and various mitigation strategies. Data is standardized by the baseline control. Bottom: percentage differences in particles/m<sup>3</sup> produced during a series of procedures versus air turbine and no mitigation. Data from the behind dentist location and the anterior position.

aerosol reduction with rubber dam and the HSCAH remain pertinent. Since the ACH measured in the experimental setting was below the recommended figure, we present a worse-case scenario. As the fallow data suggest minimal/no bacteriophage detection in postprocedural air samples with the HSCAH, this finding may be applicable to surgical settings above the recommended ACH value with some confidence.

The requirement for, and length of, a period of fallow time is unclear, with estimates ranging from 2 to 180 min (Robertson et al. 2020; Ehtezazi et al. 2021; Shahdad et al. 2021). However, this is often based on particle data alone. We determined a wide

**Table 2.** Mean  $\Phi 6$  Bacteriophage Plaque-Forming Units (pfu) Collected during Procedures at the UL6 Position Compared with the UL2 Position.<sup>a</sup>

Characteristic	No Mitigation (n = 4)			High-Volume Aspiration (n = 3)			Dam (n = 3)		
	UL6 Total pfu (Unless Stated)	Percentage Difference to UL2	UL6 vs. UL2 (P Value)	UL6 Total pfu (Unless Stated)	Percentage Difference to UL2	UL6 vs. UL2 (P Value)	UL6 Total pfu (Unless Stated)	Percentage Difference to UL2	UL6 vs. UL2 (P Value)
Splatter zone	68.00 (51.88)	-94.1	0.01	37.67 (31.39)	-97.4	0.10	253.67 (215.38)	137.8	1.00
Settled aerosol	2.75 (1.11)	-98.7	0.01	0.33 (0.33)	-99.6	0.10	0.00 (0.00)	-100.0	0.70
Air (pfu/m <sup>3</sup> )	1.25 (0.51)	-99.7	0.01	0.00 (0.00)	-100.0	0.10	0.04 (0.04)	-92.3	0.40
Fallow settle	0.00 (0.00)	-100.0	0.02	0.00 (0.00)	-100.0	0.10	0.00 (0.00)	0*	1.00
Fallow air (pfu/m <sup>3</sup> )	0.13 (0.13)	-98.9	0.01	0.00 (0.00)	-100.0	0.10	0.00 (0.00)	0*	1.00

<sup>a</sup>Data (SEM in parentheses) are delineated by mitigation strategy. Splatter zone radius defined within 41 cm radius of mouth. P value based on Mann-Whitney U test.

\*Both pfu counts were zero.

variation in the time for particles of all sizes to return to pre-AGP levels, although larger particles required less time with the HSCAH. Shahdad et al. (2021) reported similar variability and suggested that fallow time estimates were longer for procedures where the handpiece was used in 5-min bursts, comparable to our protocol. A recent study indicated that <0.1% of aerosolized fluorescein dye was detectable after 30 min of fallow time (Allison et al. 2021). While such spectrofluorometric analysis can provide valuable information, it does not inform about the viability of the particles transferred. The bacteriophage data presented here demonstrated that with air turbine and high-volume aspiration, substantial amounts of both settle and aerosolized bacteriophage were detectable between the 6- and 10-min fallow period (Table 1). However, use of the HSCAH eliminated any bioaerosol within 6 min of procedure completion. This evidence strongly suggests there is no need for a prolonged fallow period with this handpiece. Where a HSCAH is not available, a rubber dam was equally effective in reducing air contamination shortly after conclusion of an AGP. Assessing the particle and bacteriophage fallow data together, it becomes clear that particle data alone cannot provide sufficient information to determine risk of airborne viral particles. Here we saw instances of baseline particle levels not reached post-AGP but no detectable active bacteriophage in the air.

We assessed the differences between anterior and posterior AGP positions. Both the bacteriophage and particle data highlighted the importance of procedural position on risk. When using an air turbine and high-volume aspiration, no airborne bacteriophage particles were detected during the posterior procedures, with a >84% reduction in all particle sizes observed from behind the dentist. Together, these data support the interpretation that endodontic procedures in the posterior of the mouth impart a lower risk of viral contamination and dispersal into the environment, with bioaerosols most likely trapped inside the oral cavity. Conversely, dental procedures in the anterior region pose the greatest risk.

Here, we report the first major study using bacteriophage aerosolization in a dental clinic, employing a model that mimics real dental procedures and timings and a bacteriophage surrogate that reflects the behavior of enveloped viruses (Turgeon et al. 2014; Casanova and Weaver 2015). Others have used

human coronavirus to assess the value of H<sub>2</sub>O<sub>2</sub> sprays for the decontamination of aerosols and to stress the importance of PPE (Ionescu et al. 2020, 2021). There is clear value to aerosolization studies in the use of a human coronavirus to more closely mimic the behavior of SARS-CoV-2, but these cannot be deployed safely in a real dental setting; hence, the studies of Ionescu et al. were completed in a closed-cabinet setting with a shorter period of handpiece time.

Through the combined use of novel and established methodologies, the data described here present a clear picture of how risk of SARS-CoV-2 and similar biological hazards can be greatly attenuated using HSCAHs. While detection of a single viral unit may not translate to an infective viral load, the reduction in levels with these mitigating approaches is clear. This study further suggests that with these handpieces and high-volume aspiration or the use of rubber dam, a prolonged fallow period is not necessary in the clinical setting used. Equipping our dental surgeries with these tools will be crucial to protecting the health, safety, and future of dental teams and services. Finally, the data presented here suggest that particle count data alone cannot provide accurate information regarding the dispersal and settlement of bioaerosols, with bacteriophage markers offering a greater insight into infection risk.

### Author Contributions

J.J. Vernon, contributed to design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; E.V.I. Black, contributed to design and data acquisition, critically revised the manuscript; T. Dennis, contributed to data acquisition, critically revised the manuscript; D.A. Devine, L. Fletcher, contributed to conception, design, data analysis, and interpretation, critically revised the manuscript; D.J. Wood, B.R. Nattress, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

### Acknowledgments

We thank the Leeds Dental Institute for accommodating the requirements for clinical space to perform these experiments, Dr. Jing Kang for advice on statistical analyses, and Tim Zoltie for photography.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by the British Endodontic Society, under the grant identification “The British Endodontic Society Grant—COVID-19”.

## ORCID iDs

E.V.I. Black  <https://orcid.org/0000-0003-1600-1094>

T. Dennis  <https://orcid.org/0000-0003-0737-0381>

D.A. Devine  <https://orcid.org/0000-0002-8037-9254>

## References

- Adams MH. 1959. Bacteriophages. New York: Inter-science Publishers.
- Allison JR, Currie CC, Edwards DC, Bowes C, Coulter J, Pickering K, Kozhevnikova E, Durham J, Nile CJ, Jakubovics N, et al. 2021. Evaluating aerosol and splatter following dental procedures: addressing new challenges for oral health care and rehabilitation. *J Oral Rehabil.* 48(1):61–72.
- Aquino de Carvalho N, Stachler EN, Cimabue N, Bibby K. 2017. Evaluation of Phi6 persistence and suitability as an enveloped virus surrogate. *Envir Sci Tech.* 51(15):8692–8700.
- Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, Marsh PD. 2000. Microbial aerosols in general dental practice. *Br Dent J.* 189(12):664–667.
- Buhr TL, Young AA, Borgers-Klonkowski E, Kennihan NL, Barnette HK, Minter ZA, Bohmke MD, Osborn EB, Hamilton SM, Kimani MB, et al. 2020. Hot, humid air decontamination of aircraft confirmed that high temperature and high humidity are critical for inactivation of infectious, enveloped ribonucleic acid (RNA) virus. *Front Bioeng Biotechnol.* 8:592621.
- Casanova LM, Weaver SR. 2015. Evaluation of eluents for the recovery of an enveloped virus from hands by whole-hand sampling. *J Appl Microbiol.* 118(5):1210–1216.
- Cochran MA, Miller CH, Sheldrake MA. 1989. The efficacy of the rubber dam as a barrier to the spread of microorganisms during dental treatment. *J Am Dent Assoc.* 119(1):141–144.
- Din AR, Hindocha A, Patel T, Sudarshan S, Cagney N, Koched A, Mueller J-D, Seoudi N, Morgan C, Shahdad S, et al. 2020. Quantitative particle analysis of particulate matter release during orthodontic procedures: a pilot study. [medRxiv.2020.2009.20191270](https://doi.org/10.2196/medRxiv.2020.2009.20191270). Preprint.
- Dubuis M-E, Dumont-Leblond N, Laliberté C, Veillette M, Turgeon N, Jean J, Duchaine C. 2020. Ozone efficacy for the control of airborne viruses: bacteriophage and norovirus models. *PLoS One.* 15(4):e0231164.
- Dutil S, Meriaux A, de Latremouille MC, Lazure L, Barbeau J, Duchaine C. 2009. Measurement of airborne bacteria and endotoxin generated during dental cleaning. *J Occup Environ Hyg.* 6(2):121–130.
- Ehtezazi T, Evans DG, Jenkinson ID, Evans PA, Vadgama VJ, Vadgama J, Jarad F, Grey N, Chilcott RP. 2021. SARS-CoV-2: characterisation and mitigation of risks associated with aerosol generating procedures in dental practices. *Br Dent J.* 1(169):1–7.
- Fedorenko A, Grinberg M, Orevi T, Kashtan N. 2020. Survival of the enveloped bacteriophage Phi6 (a surrogate for SARS-CoV-2) in evaporated saliva microdroplets deposited on glass surfaces. *Sci Rep.* 10(1):22419.
- Harrel SK, Barnes JB, Rivera-Hidalgo F. 1998. Aerosol and splatter contamination from the operative site during ultrasonic scaling. *J Am Dent Assoc.* 129(9):1241–1249.
- Holliday R, Allison JR, Currie CC, Edwards DC, Bowes C, Pickering K, Reay S, Durham J, Lumb J, Rostami N, et al. 2021. Evaluating contaminated dental aerosol and splatter in an open plan clinic environment: implications for the COVID-19 pandemic. *J Dent.* 105:103565.
- Holloman JL, Mauriello SM, Pimenta L, Arnold RR. 2015. Comparison of suction device with saliva ejector for aerosol and spatter reduction during ultrasonic scaling. *J Am Dent Assoc.* 146(1):27–33.
- Ionescu AC, Brambilla E, Manzoli L, Orsini G, Gentili V, Rizzo R. 2020. Efficacy of personal protective equipment and H<sub>2</sub>O<sub>2</sub>-based spray against coronavirus in dental setting. *Oral Dis* [pub ahead of print 28 Nov 2020]. doi:10.1111/odi.13736
- Ionescu AC, Brambilla E, Manzoli L, Orsini G, Gentili V, Rizzo R. 2021. Aerosols modification with h<sub>2</sub>o<sub>2</sub> reduces airborne contamination by dental handpieces. *J Oral Microbiol.* 13(1):1881361.
- King MF, Noakes CJ, Sleight PA, Camargo-Valero MA. 2013. Bioaerosol deposition in single and two-bed hospital rooms: a numerical and experimental study. *Build Environ.* 59:436–447.
- Llandro H, Allison JR, Currie CC, Edwards DC, Bowes C, Durham J, Jakubovics N, Rostami N, Holliday R. 2021. Evaluating splatter and settled aerosol during orthodontic debonding: implications for the COVID-19 pandemic. *Br Dent J* [pub ahead of print 8 Jan 2021]. doi:10.1038/s41415-020-2503-9
- Macher JM. 1989. Positive-hole correction of multiple-jet impactors for collecting viable microorganisms. *Am Ind Hyg Assoc J.* 50(11):561–568.
- Meethil AP, Saraswat S, Chaudhary PP, Dabdoub SM, Kumar PS. 2021. Sources of SARS-CoV-2 and other microorganisms in dental aerosols. *J Dent Res.* 100(8):817–823. doi:10.1177/00220345211015948
- Pinheiro LAM, Pereira C, Frazão C, Balcão VM, Almeida A. 2019. Efficiency of phage  $\phi$ 6 for biocontrol of *Pseudomonas syringae* pv. *Syringae*: an in vitro preliminary study. *Microorganisms.* 7(9):286.
- Prussin AJ, 2nd, Schwake DO, Lin K, Gallagher DL, Buttling L, Marr LC. 2018. Survival of the enveloped virus Phi6 in droplets as a function of relative humidity, absolute humidity, and temperature. *Appl Environ Microbiol.* 84(12):e00551-18.
- Rautemaa R, Nordberg A, Wuolijoki-Saaristo K, Meurman JH. 2006. Bacterial aerosols in dental practice—a potential hospital infection problem? *J Hosp Infect.* 64(1):76–81.
- Ren Y-F, Huang Q, Marzouk T, Richard R, Pembroke K, Martone P, Venner T, Malmstrom H, Eliav E. 2021. Effects of mechanical ventilation and portable air cleaner on aerosol removal from dental treatment rooms. *J Dent.* 105:103576.
- Robertson C, Clarkson JE, Aceves-Martins M, Ramsay CR, Richards D, Colloc T; CoDER Working Group. 2020. Aerosol generating procedures and their mitigation in international dental guidance documents—a rapid review. *Int Dent J* [pub ahead of print 3 Jun 2021]. doi:10.1016/j.identj.2021.04.002
- Samaranyake LP, Reid J, Evans D. 1989. The efficacy of rubber dam isolation in reducing atmospheric bacterial contamination. *ASDC J Dent Child.* 56(6):442–444.
- Scottish Dental Clinical Effectiveness Program. 2021. Mitigation of aerosol generating procedures in dentistry—a rapid review [accessed 2021 Jun 28]. <https://www.sdcep.org.uk/wp-content/uploads/2021/04/SDCEP-Mitigation-of-AGPs-in-Dentistry-Rapid-Review-v1.2-April-2021.pdf>
- Sergis A, Wade WG, Gallagher JE, Morrell AP, Patel S, Dickinson CM, Nizarali N, Whaites E, Johnson J, Addison O, et al. 2020. Mechanisms of atomization from rotary dental instruments and its mitigation. *J Dent Res.* 100(3):261–267.
- Shahdad S, Hindocha A, Patel T, Cagney N, Mueller J-D, Kochid A, Seoudi N, Morgan C, Fleming P, Din AR. 2021. Fallow time determination in dentistry using aerosol measurement. [medRxiv.2021.2001.2026.21250482](https://doi.org/10.2196/medRxiv.2021.2001.2026.21250482). Preprint.
- Timmerman MF, Menso L, Steinfurt J, van Winkelhoff AJ, van der Weijden GA. 2004. Atmospheric contamination during ultrasonic scaling. *J Clin Periodontol.* 31(6):458–462.
- To KK-W, Tsang OT-Y, Yip CC-Y, Chan K-H, Wu T-C, Chan JM-C, Leung W-S, Chik TS-H, Choi CY-C, Kandamby DH, et al. 2020. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis.* 71(15):841–843.
- Turgeon N, Toulouse M-J, Martel B, Moineau S, Duchaine C. 2014. Comparison of five bacteriophages as models for viral aerosol studies. *Appl Environ Microbiol.* 80(14):4242–4250.
- Whitworth C, Mu Y, Houston H, Martinez-Smith M, Noble-Wang J, Coulliette-Salmond A, Rose L. 2020. Persistence of bacteriophage Phi 6 on porous and nonporous surfaces and the potential for its use as an Ebola virus or coronavirus surrogate. *Appl Environ Microbiol.* 86(17):e01482-01420.
- World Health Organization. 2020. Transmission of SARS-CoV-2: implications for infection prevention precautions [accessed 2021 Mar 29]. Scientific Brief. WHO/2019-nCoV/Sci\_Brief/Transmission\_modes/2020.3. Available from: <https://www.who.int/publications/i/item/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations>.
- Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, Geng B, Muenker MC, Moore AJ, Vogels CBF, et al. 2020. Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. [medRxiv. doi:10.1101/2020.04.16.20067835](https://doi.org/10.1101/2020.04.16.20067835). Preprint.