Evaluating aerosol and splatter following dental

procedures

Running Title: Evaluating dental aerosol and splatter

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Abstract

Background

Dental procedures often produce aerosol and splatter which are potentially high risk for spreading pathogens such as SARS-CoV-2; existing literature is limited.

Objective

To develop a robust, reliable and valid methodology to evaluate distribution and persistence of dental aerosol and splatter, including initial investigations evaluating clinical procedures.

Methods

Fluorescein dye was introduced into irrigation reservoirs of dental equipment. High-speed airturbine, ultrasonic scaler and 3-in-1 spray were used on a mannequin. Procedures were in triplicate. Filter papers were placed in the immediate environment. The impact of dental suction and assistant presence were also evaluated. Fluorescein samples were analysed by photographic examination with image analysis, and spectrofluorometric analysis. Statistics were descriptive with Pearson's correlation for comparison of methods.

Results

All procedures were aerosol and splatter generating. Contamination was highest closest to the source, remaining high to 1-1.5 m. Contamination was detectable at the maximum distance measured (4 m) for high-speed air-turbine with maximum relative fluorescence units (RFU) being: 46,091 at 0.5 m, 3,541 at 1.0 m, and 1,695 at 4 m. There was uneven spatial distribution with highest levels of contamination opposite the operator. Very low levels of contamination ($\leq 0.1\%$ of original) were detected at 30- and 60-minutes post procedure. Suction reduced contamination levels by 67-75% between 0.5-1.5 m. Mannequin and operator were heavily contaminated. The two analytic methods showed good correlation (*r*=0.930, *n*=244, *p*=.000).

Conclusion

Dental procedures have potential to deposit aerosol and splatter at some distances from source, being effectively cleared by 30 minutes in our setting.

Keywords

COVID-19; Dental Infection Control; Aerosols, Dental Equipment; Dental High-Speed Equipment; Dental Scaling; Suction.

Introduction

The COVID-19 pandemic has had significant impact upon the provision of medical and dental care globally. In the United Kingdom, routine dental treatment was suspended in late March 2020¹⁻⁴, with care instead being provided through a network of urgent dental care centres⁵. During this period, it was advised that aerosol generating procedures (AGPs) were avoided unless absolutely necessary, leading to altered treatment planning and a negative impact on patient care⁶. As more routine dental services start to resume worldwide, the guidance in the UK and elsewhere is still to avoid or defer AGPs where possible⁷⁻¹³. Standard operating procedures (SOPs) have been published by a number of organisations to inform practice, however many of these acknowledge a limited evidence base¹⁴⁻¹⁸. Additionally, all face-to-face undergraduate and postgraduate clinical dental teaching is suspended¹⁹.

Many dental procedures produce both aerosol and splatter contaminated with saliva and, or blood^{20, 21}. Saliva has been shown to contain severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in infected individuals^{22, 23}, many of whom may be asymptomatic²⁴, with the salivary gland potentially being an early reservoir of infection^{25, 26}. Equally, however, preliminary data suggest that in asymptomatic carriers, the viral load may be low in saliva and these individuals may have faster viral clearance^{27, 28}. Early data suggest that SARS-CoV-2 can remain viable and infectious in aerosol for hours, and on surfaces for days²⁹. Hence, dental aerosols and splatter are likely to be a high-risk mode of transmission for SARS-CoV-2, and it is highly likely that international clinical protocols across the spectrum of dental practice will need to be significantly modified to allow a safe return to routine care.

A review of the impact of AGPs generally across healthcare (including dentistry) concluded that the existing evidence is limited³⁰. The current literature regarding the risks posed by aerosols and splatter in dental settings is particularly limited. A number of authors have used microbiological methods to study bacterial contamination from aerosol and splatter following dental procedures, either by air sampling^{20, 31, 32}, swabbing of contaminated surfaces^{33, 34}, or most commonly, by collection directly onto culture media³⁵⁻³⁸. These studies are limited in that they only detect culturable bacteria as a marker of aerosol and splatter distribution. A smaller number of studies have used various luminescent³⁹⁻⁴³ and non-luminescent tracers^{44, ⁴⁵ to measure aerosol and splatter distribution, although many of these have significant methodological flaws and major limitations. Many studies are small and report only one repetition of a single procedure and some have only examined contamination of the operator and assistant; many studies which have measured spatial distribution of aerosol and splatter have only done so to a limited distance from the source. Few studies have considered the temporal persistence of aerosol and splatter with sufficient granularity to inform clinical practice.}

Open plan clinical environments such as those common in dental (teaching) hospitals with multiple patients and operators in close proximity are problematic. The current lack of robust evidence about dental aerosol and splatter distribution and persistence will be a barrier to the reintroduction of routine dental services and dental education, which is likely to have a negative impact on the availability of care for patients, and on the future dental workforce if not addressed expediently¹⁹

The aim of the present study was to establish a robust, reliable and valid methodology to evaluate the distribution and persistence of aerosol and splatter following dental procedures. We present initial data on three dental procedures (high-speed air-turbine, ultrasonic scaler, and 3-in-1 spray use) and examine the effect of dental suction and the presence of an assistant on aerosol and splatter distribution.

Materials and Methods

Experiments were conducted in the Clinical Simulation Unit (CSU) at the School of Dental Sciences, Newcastle University (Newcastle upon Tyne, United Kingdom). This is a 308 m² dental clinical teaching laboratory situated within a large dental teaching hospital. The CSU is supplied by a standard hospital ventilation system with ventilation openings arranged as shown in figure 1; this provides 6.5 air changes per hour and all windows and doors remained closed during experiments. The temperature remained constant at 21.5 °C.

Dental procedures were conducted on a dental simulator unit (Model 4820, A-dec; OR, USA) with a mannequin containing model teeth (Frasaco GmbH; Tettnang, Germany). Polyvinyl siloxane putty (Lab-putty, Coltene/Whaledent; Altstätten, Switzerland) was added to the mouth of the mannequin to recreate the normal dimensions of the oral cavity as described by Dahlke et al.⁴¹ (figure 1). Fluorescein solution (2.65 mM) was made by dissolving fluorescein sodium salt (Sigma-Aldrich; MO, USA) in deionised water, and this was then introduced to the irrigation reservoirs of the dental unit and ultrasonic scaler. The procedures investigated were as follows: Anterior crown preparation – preparation of the upper right central incisor tooth for a full coverage crown using a high-speed air-turbine (Synea TA-98, W&H (UK) Ltd.; St Albans, UK); full mouth scaling using a magnetostrictive ultrasonic scaler (Cavitron Select SPS with 30K FSI-1000-94 insert, Dentsply Sirona; PA, USA); 3-in-1 spray (air/water syringe) use – washing of mesial-occlusal cavity in upper right first premolar tooth with air and water from 3-in-1 spray. Procedure durations were 10 minutes for anterior crown preparation and ultrasonic scaling, and 30 seconds for the 3-in-1 spray use (to represent removing acid etchant). Irrigant flow rate was measured at 29.3 mL/min for the air-rotor, 38.6 mL/min for the ultrasonic scaler and 140.6 mL/min for the 3-in-1 spray. We also investigated the following parameters: dental suction (measured at 6.3 L of water per minute) and the presence of an assistant.

Having developed the methods reported by other investigators^{36, 41, 43}, the present study used a reproducible, height adjustable rig. This rig was constructed to support cottoncellulose filter papers spaced at known distances from the mannequin (figure 1). 30 mm diameter grade 1 qualitative filter papers (Whatman; Cytiva, MA, USA) were used to collect aerosol and splatter. These were supported on platforms spaced at 0.5 m intervals along eight, 4 m, rigid rods, laid out at 45° intervals and supported by a central hub, thus creating an 8 m diameter circle around the mannequin; the centre of this circle was located 25 cm superior to the mouth of the mannequin, and in the same horizontal plane as the mouth of the mannequin (73 cm above the floor). Four filter papers were also placed on the body of the mannequin: two at 40 cm from the hub and two at 80 cm. In addition, filter papers were placed on the arms, body, and legs of the operator and assistant as well as on their full-face visor (28.0 x 27.5 cm) and the vertex of the head. For one condition (anterior crown prep with suction and assistant) we also placed three filter papers on the mask of the operator/assistant (beneath a full-face visor). Two operators conducted the procedures: RH conducted the high-speed air-turbine and ultrasonic scaler procedures (operator height = 170 cm); JA conducted the 3-in-1 spray procedure (operator height = 175 cm). There was a single assistant with a height of 164 cm.

Before each procedure the mannequin, rig and filter paper platforms were cleaned with 70% ethanol and left to fully air dry. A period of 120 minutes was left between each procedure to allow for clearance of aerosol and splatter. Following each procedure, the filter papers were left in position for 10 minutes to allow for settling and drying of aerosol and splatter, before being collected with clean tweezers and placed into a single-use, sealable polyethylene bag. For the anterior crown preparation without suction, additional filter papers were placed at 30 minutes and again at 60 minutes to examine persistence of aerosol and splatter. At both of these time points, the risk of fluorescein transfer was minimised by placing the new filter papers on new platforms, and filter papers were then left for 10 minutes before collection. All experimental conditions were repeated three times.

Image Analysis

Aerosol and splatter deposition were measured using photography and image analysis software. Filter papers were placed on a glass slide on a black background and covered by a second glass slide. Samples were illuminated by two halogen dental curing lights (QHL75 model 503; Dentsply, NC, USA) with 45 mW/cm² output at 400-500 nm wavelength; these were positioned at 0 and 180 degrees, 5 cm from the centre of the sample horizontally, and 9 cm vertically, with both beams of light focussed on the centre of the sample. Images were captured with a digital single-lens reflex camera (EOS 1000D, Canon; Tokyo, Japan) at 90 mm focal length (SP AF 90mm F/2.8 Di Macro, Tamron; Saitama, Japan) with an orange lens filter, positioned 43 cm directly above the sample (sample to sensor). Exposure parameters were f/10, 1/80 seconds and ISO 400. Images were saved in JPEG format at 3888 x 2592 pixel resolution. Image analysis was performed using ImageJ⁴⁶ (version 1.53b, U.S. National Institutes of Health; MD, USA) in a darkened room by one of four examiners blind to the experimental condition and sample position (JA, CC, DE, RH). Images were loaded into ImageJ and converted into 8-bit images. The pixel scale was set across the maximum diameter of the sample at 30mm. A manual threshold was then used to create a mask which selected all high intensity areas on the image. The analyse particles function was then used to identify particles from $0 - infinity mm^2$ in area and 0 - 1 in circularity. The number of particles, total surface area, and average particle size were calculated. Total surface area was selected as the primary outcome measure, representing contamination levels of the samples. Examiners underwent calibration prior to formal analysis by independently analysing 10 images and then discussing to reach consensus. Following this, examiners then independently analysed 30 images to assess inter-examiner agreement. Examiners re-examined the same 30 images one week later to assess intra-examiner agreement.

Spectrofluorometric Analysis

For one experimental condition (anterior crown preparation without suction, samples at 0-10, 30-40 and 60-70 minutes after procedure) we completed additional analysis of the filter paper samples using spectrofluorimetric analysis. This allowed validation of the image analysis technique for the measurement of fluorescein contamination and allowed us to pilot this new analytical technique, which has the potential to be more sensitive than image analysis. Building on the methods reported by Steiner et al.⁴⁷, fluorescein was recovered from filter papers by addition of 350 µL deionised water. Immersed filter papers were shaken for 5 min at 300 rpm using an orbital shaker at room temperature. The fluorescein was then eluted by centrifugation at 15,890 *g* for 3 min using a microcentrifuge. 100 µL of the supernatant was transferred to a well of a black 96-well microtitre plate with a micro-clear bottom (Greiner Bio-One; NC, USA) in triplicate in order to measure fluorescence. Fluorescence measurements were performed using a Synergy HT Microplate Reader (BioTek; VT, USA) at an excitation wavelength of 485 ± 20 nm and an emission wavelength of 528 ± 20 nm with the top optical probe.

For background correction, negative controls (n = 26) were included in the measurements for all runs. These included fresh filter papers out of the box and filter papers that had been placed on platforms in CSU for 10 minutes exposed to air. The negative control filter papers were processed for imaging and fluorescent measurements in the same manner as the remainder of sample. The negative control mean + 3SD (164 RFU) was used as the limit of detection; hence a zero reading was assigned to values below 164 RFU. For very high readings that were above the detection limit of the instrument (>100,000 RFU), a value of 100,000 RFU was assigned.

Statistical Methods

Data were collected using Excel (2016, Microsoft; WA, USA) and analysed using SPSS (version24, IBM Corp.; NY, USA) using basic descriptive statistics and Pearson's correlation

(to compare analytical techniques). Heatmaps demonstrating aerosol and splatter distribution were generated using Python 3⁴⁸. A two-way mixed effects model was used to assess inter-, and intra-examiner agreement by calculating interclass correlation coefficient (ICC) using STATA release 13 (StataCorp; TX, USA).

Results

Examiner Calibration for image analysis

Inter-examiner ICC for 30 images showed excellent agreement for total surface area (ICC 0.98; 95% CI 0.97-0.99), good agreement for total number of particles (ICC 0.88; 95% CI 0.80-0.93), and moderate agreement for average particle size (ICC 0.63; 95% CI 0.47-0.78). Intra-examiner agreement at one week for the same 30 images was excellent for total surface area (ICC 0.97 - 0.99), good to excellent for total number of particles (ICC 0.82 - 0.97), and good for average particle size (ICC 0.75 - 0.97)⁴⁹.

Aerosol and splatter distribution

Aerosol and/or splatter deposition (assessed by image analysis and calculation of total surface area) on filter papers was highest at the centre of the rig and decreased with increasing distance from the centre of the rig. Table 1 presents summarised data for the total surface area outcome measure, by distance from the centre. The majority of the contamination was within 1.5 m but there were smaller readings up to 4 m for some conditions. The spatial distribution of the contamination is shown by heatmaps in figure 2 and 3.

For one experimental condition (anterior crown prep with <u>no</u> suction, representing a presumed worst-case scenario) we also completed spectrofluorimetric analysis in order to validate the image analysis methods for assessing contamination of the filter papers (table 1). The particle count was found to be weakly correlated with the spectrofluorimetric

measurements (r = 0.344, n = 244, p = .000), average particle size was found to be moderately correlated (r = 0.555, n = 244, p = .000) and total surface area was found to be very strongly correlated (r = 0.930, n = 244, p = .000), supporting our use of total surface area as the main outcome measure from the image analysis (supplementary appendix 1). Data were logarithmically transformed and are presented in the heatmap in Figure 3. Using serial dilution of fluorescein, we derived a standard curve with highly robust linear range that covered 50 nM to 102 μ M. The equation y = 700.42 x -

1449.5 was derived from the standard curve for calculating the concentration of fluorescein from fluorescence readings (y = fluorescence, RFU, x= fluorescein concentration, μ M). For illustrative purposes we detail these for the 270 degree axis (mean values across three repetitions): 0 m = 132.6 μ M; 0.5 m = 26.3 μ M; 1 m = 5.25 μ M; 1.5 m = 3.02 μ M; 2 m = 3.44 μ M; 2.5 m = 3.30 μ M; 3 m = 2.79 μ M; 3.5 m = 2.86 μ M; 4 m = 3.09 μ M.

The mannequin, operator and assistant were all heavily contaminated (data presented in supplementary appendix 2). The operator's left (non-dominant) arm, left body and lower visor were the most contaminated sites. Generally, levels of contamination were much lower for the assistant, being highest on the left arm and left chest (the assistant used their left hand to hold the suction tip). All areas of the mannequin were heavily contaminated. The operator and assistant's masks (only assessed in one condition) showed low but measurable contamination, usually at the lateral edges.

Effect of dental suction (with and without assistant)

The use of dental suction, held by the operator, reduced the contamination of filter papers at each distance (table 1), although image analysis still detected contamination up to 2 m. Between 0.5-1.5 m there was a 67-75% reduction (central site contamination was unaffected). The spatial distribution was altered as demonstrated in figure 2. When an assistant was present and held the dental suction this further reduced contamination

readings within the first 1m, however, we noted a marked increase at the 1.5 m reading behind the assistant (0°).

Procedure type

Three clinical procedures (anterior crown preparation, ultrasonic scaling and 3-in-1 spray use) were assessed while the same operator held dental suction. Overall, the highest readings were obtained from the anterior crown preparation, but each procedure gave a unique pattern of contamination (table 1, figures 2 and 3). The ultrasonic scaler produced high levels of contamination at the centre, reducing markedly at 0.5 m, but with low levels of contamination being detectable up to the 4 m limit of our measurements. The 3-in-1 spray procedure produced high levels of contamination at 0.5 m (higher than the other two procedures) but little beyond 1 m.

Effect of time

Image analysis demonstrated no detectable fluorescein contamination of the filter papers at 30-40 and 60-70 minutes post procedure. Additionally, spectrofluorometric analysis of these samples demonstrated very low levels of fluorescein contamination. The overall contamination across the 8 m diameter experimental area at 30-40 minutes was 0.02% of the original level, and at 60-70 minutes it was 0.10% of the original level (Table 1).

Particle size

Average particle size measurements were combined for the 0, 0.5, 1 and 1.5 m readings for each condition to give an indication of the nature of the particles in this initial proximity. The anterior crown preparation without suction produced the largest particles (mean \pm SD: 0.49 \pm 2.98 mm²) which were similar to when suction was added by the operator (0.56 \pm 3.34 mm²). There was a size reduction when an assistant provided suction (0.11 \pm 0.69 mm²). The ultrasonic scaling produced the smallest particles (0.05 \pm 0.24 mm²) followed by the 3-in-1 spray (0.08 \pm 0.25 mm²). Figure 5 presents images of all samples for one repetition of a single experimental condition to demonstrate the distribution of particles and size.

Discussion

Dental aerosol and splatter are an important potential mode of transmission for many pathogens, including SARS-CoV-2. Understanding the risk these phenomena pose is vitally important in the reintroduction of dental services in the current COVID-19 pandemic. Our study is novel in that we are the first to measure aerosol and splatter distribution at distances up to 4 m from the source, and the first to apply image and spectrofluorometric analysis to the study of dental aerosol and splatter. This has allowed us to gather urgently needed data relevant to the provision of dental services during the COVID-19 pandemic, and to future pandemics. Specifically, we have demonstrated the relative distribution of aerosol and splatter following different dental procedures, the effect of suction and assistant presence, and the persistence of aerosol and splatter over time.

Previous investigators have used various tracer dyes and visual examination techniques to evaluate 'dental aerosol' and have demonstrated positive readings at up to 1.2 m^{41, 43}. Our study further optimises these methods and we have demonstrated positive readings at up to 2 m (and low levels at up to 4 m in the case of ultrasonic scaling). This is consistent with the findings of other investigators using bacterial culture methods to detect contamination at up to 2 m^{36, 37, 50}. Importantly, our spectrofluorometric analysis demonstrates that some fluorescein contamination may occur beyond this on filter papers that appear clean by image analysis. We propose that studies which use dye tracers assessed by visual examination or image analysis techniques are assessing primarily splatter (particles >5 µm) rather than aerosol (<5 µm); this is because in order for a fluorescein deposit to be visible to the eye or camera it has to be relatively large in size (i.e. splatter). Previous research using these methods should therefore be interpreted in this context. It is, however, worth noting that

larger particles are likely to contain a greater viral load and given the risk of SARS-CoV-2 transmission through contact with mucosal surfaces⁵¹, from a cross infection perspective, splatter is likely to be highly significant. Reassuringly in our study splatter was greatly reduced through the use of suction, particularly when held by an assistant rather than the operator.

Findings from both of our analytical techniques and from a number of clinical procedures demonstrate fluorescein contamination at a distance from the source (i.e. 4 m), although levels of contamination were lower at greater distances; this shows the potential for pathogens to travel a similar distance, although our methods replicate a worst-case scenario. Within closed single clinic environments this reinforces the requirement to have minimal clutter and thorough disinfection procedures. Within open clinic environments, such as those in teaching hospitals, further research is required to investigate parameters such as the impact of partitions on aerosol and splatter distribution in these settings.

We demonstrated significant contamination of the operator, assistant and mannequin for all procedures, which is consistent with the findings of other investigators^{33, 37, 40, 43}. This is unsurprising and underscores the need for adequate personal protective equipment (PPE), for the operator and assistant. This also highlights the importance of enhanced PPE⁵² during the peak of a pandemic for AGPs, because of the high likelihood clinicians may be treating an asymptomatic carrier. Coverage of the operator and assistant's exposed arms with a waterproof gown or other covering would protect against this contamination, although scrupulous hygiene of uncovered forearms and hands with an effective antiseptic (povidone-iodine or 70% alcohol^{53, 54}) would be a minimum requirement if this were not used. It is also important to note that PPE for patients' clothes do not feature in many dental clinical guidelines relating to COVID-19, and our findings would suggest that there is likely to be significant contamination of the patient during AGPs; this may present a risk of onward

cross-contamination by contact with surroundings, and it is therefore important to provide sufficient waterproof protection for patients' clothes.

Our findings demonstrate that use of an air-rotor, ultrasonic scaler and 3-in-1 spray are all AGPs. 3-in-1 use is not currently included in the list of defined healthcare related AGPs recently updated by Health Protection Scotland³⁰, which only details high speed devices such as ultrasonic scalers and high-speed drills. The highest levels of contamination were from the air-rotor, although the ultrasonic scaler demonstrated contamination at further distances, in keeping with the findings of Bennett *et al.*²⁰. Dental suction was effective at reducing fluorescein contamination, with reduction of 67-75% between 0.5-1.5m (central site contamination was unaffected). This is consistent with the mitigating effect of suction demonstrated by other investigators^{36, 55}.

When dental suction was provided by an assistant this was more effective in reducing contamination, although increased readings were seen at 1.5 m, potentially indicating that an additional barrier in the form of an assistant may have a more complex aerodynamic effect. High-volume dental suction is recommended in most dental guidelines and SOPs relating to COVID-19, as an essential mitigation procedure when conducting AGPs. However, we are not aware of any that provide a definition or basic minimal requirements for effective high-volume dental suction. National guidelines⁵⁶ classify suction systems based on air flow rate (high-volume systems: 250 L/min at the widest bore size of the operating hose). We did not have a suitable device available to measure air flow rate of the system used in the present study and hence we chose to use the term 'dental suction' as we were unable to confirm whether it met this definition. We did, however measure water flow rate (6.3 L/min) which we found to be similar to that reported by other investigators³⁸. Our findings highlight the importance of suction as a mitigation factor in splatter and aerosol distribution following dental procedures, and future research should examine the impact of this effect in relation to different levels of suction based on air flow rate.

Safe times following procedures, after which contamination becomes negligible have rarely been investigated robustly in the previous literature. In studies using tracer dyes we are only aware of a single paper which reports considerable contamination at 30 minutes after the procedure⁴³. This is in conflict with our findings which found <u>no</u> contamination by image analysis at the 30 and 60 minute time points, and only very low levels were detected by spectrofluorometric analysis ($\leq 0.10\%$ of original levels). It is unclear from the methods of Veena et al.⁴³ whether new filter papers were placed immediately following the procedure and collected at 30 minutes, or placed at 30 minutes and collected thereafter; in the prior case, any contamination found on the samples could have arisen at any time from the end of the procedure up to 30 minutes, and it cannot therefore be determined when contamination actually occurred. In addition, the authors do not report whether the tape they used to support filter papers was replaced following the initial exposure, and if not, it is possible that existing contamination was transferred to filter papers placed subsequently. Finally, the investigation reported by Veena et al.43 was a single experiment and did not appear to use multiple repetitions. Fluorescein can be detected at very low concentrations, hence we ensured our methodology had minimal risk of contamination by developing extensive cleaning protocols which we confirmed by spectrofluorometric analysis. It is important to note that the findings of this study relate to our environmental setting with ventilation of 6.5 air changes per hour; air exchange rate in dental surgeries are likely to vary, and our findings should be interpreted in light of this.

Our study has a number of limitations and our results need to be interpreted in the context of these. Our methods of detecting fluorescein deposition on filter papers serve as a model for aerosol and splatter contamination, and further work is required to confirm the biological validity of this technique. As our knowledge of the infective dose of SARS-CoV-2 required to cause COVID-19 develops, the clinical relevance of our findings need to be put into context; our understanding of this is still too basic to be able to draw definitive conclusions as to the

risks posed by aerosol and splatter from dental procedures. Our experimental set up incorporated the tracer dye within the irrigation system of the dental units and represents a **worst-case** scenario for distribution of biological material.

In reality, a small amount of blood and saliva will mix with large volume of water irrigant creating aerosol and splatter with diluted pathogen concentration compared to blood or saliva, and a likely reduced infective potential¹⁹. For example, it has been estimated that during a 15-minute exposure during dental treatment with high-speed instruments, an operator may be exposed to $0.014 - 0.12 \mu$ L of saliva²⁰. Early data suggest a median SARS-CoV-2 viral load of 3.3×10^6 copies per mL in the saliva of infected patients^{22, 23}; taken together, this suggests that an operator **without** PPE (at around 0.5 m from the source) may be exposed to an estimated 46 – 396 viral copies during a 15 minute procedure. These data were collected from hospital inpatients, and recent data suggest that asymptomatic carriers may have lower salivary viral loads^{27, 28}; similarly the average concentration of fluorescein detected by spectrofluorometric analysis past 2 m in the present study was almost two orders of magnitude lower than at 0.5 m, and so at distances beyond 0.5 m this risk is likely to be lower still. Importantly, we still do not yet know what the infective dose of SARS-CoV-2 required to cause COVID-19 is.

Conclusions

Within the limitation of this study, dental aerosol and splatter has the potential to be a cross infection risk even at a distance from the source. The high-speed air-turbine generated the most aerosol and splatter, even with assistant-held suction. Our findings suggest that it may be safe to reduce fallow times between dental AGPs in settings with 6.5 air changes per hour to 30 minutes. Future research should evaluate further procedures, mitigation strategies, time periods and aim to assess the biological relevance of this model.

References

 1.
 Bridgman C. Letter to all primary care dental teams in Wales, 23rd March 2020

 [Accessed:
 23/06/2020].

 Available
 from:

 https://www.fgdp.org.uk/sites/fgdp.org.uk/files/editors/2020.03.23%20CDO%20Wales%20C

 OVID-19%20advice%20letter.pdf

2. Donaldson M. COVID-19: Outline strategic plan for General Dental Services and updated guidance for General Dental Practice, 23rd March 2020 [Accessed: 23/06/2020]. Available from: <u>http://www.hscbusiness.hscni.net/pdf/HSCB_COVID-19_GDS-Strategic-Plan.pdf</u>

 3.
 Ferris T. Letter to NHS Dental Services (ref: POL/33888), 23rd March 2020 [Accessed:

 23/06/2020].
 Available
 from:

 https://www.fgdp.org.uk/sites/fgdp.org.uk/files/editors/2020.03.23%20CDO%20Scotland%20
 COVID-19%20advice%20letter.pdf

4. Hurley S, Neligan M. Letter to general dental practices and community dental services (ref: 001559), 25th March 2020 [Accessed: 06/06/2020]. Available from: https://www.england.nhs.uk/coronavirus/wp-content/uploads/sites/52/2020/03/issue-3-preparedness-letter-for-primary-dental-care-25-march-2020.pdf

5. Carter E, Currie CC, Asuni A, Goldsmith R, Toon G, Horridge C, et al. The first six weeks - setting up a UK urgent dental care centre during the COVID-19 pandemic. British Dental Journal. 2020;228(11):842-8.

6. Long L, Corsar K. The COVID-19 effect: number of patients presenting to The Mid Yorkshire Hospitals OMFS team with dental infections before and during The COVID-19 outbreak. Br J Oral Maxillofac Surg. 2020.

7. Bridgman C. Letter to All Primary Care Dental Teams and Health Boards, 22nd May 2020 [Accessed: 23/06/2020]. Available from: <u>https://www.gdc-uk.org/docs/default-source/covid-19/2020-05-22---cdo-letter---restoration-of-dental-services---22-05-20.pdf?sfvrsn=3f9d26a4_2</u>

8. Donaldson M. Plans for the Restoration of General Dental Services, 2nd June 2020 [Accessed: 23/06/2020]. Available from: https://www.fgdp.org.uk/sites/fgdp.org.uk/files/editors/2020.06.02%20CDO%20NI%20Plans %20for%20the%20Restoration%20of%20General%20Dental%20Services.pdf

9. Ferris T. Remobilisation of NHS Dental Services in Scotland, 20th May 2020 [Accessed: 23/06/2020]. Available from: <u>https://www.gdc-uk.org/docs/default-source/covid-19/cdo-letter---remobilisation-of-nhs-dental-services---20-may-2020.pdf?sfvrsn=2ab26ee_2</u>

10.Hurley S, Neligan M. Resumption of dental services in England, 28th May 2020
[Accessed: 23/06/2020].Availablefrom:
from:
https://www.fgdp.org.uk/sites/fgdp.org.uk/files/editors/2020.05.28%20CDO%20England%20r
esumption%20of%20dental%20services.pdf

11. Danish Health Authority. COVID-19 management: Adaptation and progressively increased dental activity. Copenhagen, Denmark: Danish Health Authority; 2020.

12. Kenya Dental Association. Update of guidelines to prevent spread of COVID-19 in the management of patient requiring dental and oralmaxillofacial services. Nairobi, Kenya: Kenya Dental Association; 2020.

13. Centres for Disease Control and Prevention. Guidance for Dental Settings: Centres for Disease Control and Prevention; 2020 [Accessed: 25/06/2020]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/dental-settings.html

14. British Endodontic Society. BES COVID-19 Return to Work SOP. St Albans, Herts: British Endodontic Society; 2020.

15. College of General Dentistry, Faculty of General Dental Practice. Implications of COVID-19 for the safe management of general dental practice: A practical guide. London: College of Genreal Dentistry, Faculty of General Dental Practice 2020.

16. NHS England. Urgent dental care guidance and standard operating procedure. London: NHS England; 2020.

17. Office of The Chief Dental Officer England. Standard Operating Procedure Transition to Recovery. London: Office of The Chief Dental Officer England; 2020.

18. Scottish Dental Clinical Effectiveness Programme. Resuming General Dental Services Following COVID-19 Shutdown. Dundee: Scottish Dental Clinical Effectiveness Programme 2020.

19. Durham J, Bagg J, Bain S, Bissell V, Burgden D, Chadwick B, et al. COVID-19 – returning to student-led dental clinical treatments 2020 [22/06/2020]. Available from: https://www.dentalschoolscouncil.ac.uk/wp-content/uploads/2020/06/COVID-19-report-on-returning-to-student-leddental-clinical-treatments.pdf

20. Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV, Marsh PD. Microbial aerosols in general dental practice. British Dental Journal. 2000;189(12):664-7.

21. Miller RL. Characteristics of Blood-Containing Aerosols Generated by Common Powered Dental Instruments. American Industrial Hygiene Association Journal. 1995;56(7):670-6.

22. To KK-W, Tsang OT-Y, Yip CC-Y, Chan K-H, Wu T-C, Chan JM-C, et al. Consistent Detection of 2019 Novel Coronavirus in Saliva. Clinical Infectious Diseases. 2020;DOI: 10.1093/cid/ciaa149.

23. Wyllie AL, Fournier J, Casanovas-Massana A, Campbell M, Tokuyama M, Vijayakumar P, et al. Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs. medRxiv. 2020:2020.04.16.20067835.

24. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, Norddahl GL, et al. Spread of SARS-CoV-2 in the Icelandic Population. New England Journal of Medicine. 2020;382(24):2302-15.

25. Pascolo L, Zupin L, Melato M, Tricarico PM, Crovella S. TMPRSS2 and ACE2 Coexpression in SARS-CoV-2 Salivary Glands Infection. Journal of Dental Research. 2020:0022034520933589.

26. Xu J, Li Y, Gan F, Du Y, Yao Y. Salivary Glands: Potential Reservoirs for COVID-19 Asymptomatic Infection. Journal of Dental Research. 2020:0022034520918518.

27. Chau NVV, Thanh Lam V, Thanh Dung N, Yen LM, Minh NNQ, Hung LM, et al. The natural history and transmission potential of asymptomatic SARS-CoV-2 infection. Clinical Infectious Diseases. 2020.

28. Kawasuji H, Takegoshi Y, Kaneda M, Ueno A, Miyajima Y, Kawago K, et al. Viral load dynamics in transmissible symptomatic patients with COVID-19. medRxiv. 2020:2020.06.02.20120014.

29. van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and Surface Stability of SARS-CoV-2 as Compared with SARS-CoV-1. New England Journal of Medicine. 2020;382(16):1564-7.

30. Health Protection Scotland. Transmission Based Precautions Literature Review: Aerosol Generating Procedures. Glasgow: Health Protection Scotland; 2020.

31. Dutil S, Meriaux A, de Latremoille MC, Lazure L, Barbeau J, Duchaine C. Measurement of airborne bacteria and endotoxin generated during dental cleaning. J Occup Environ Hyg. 2009;6(2):121-30.

32. Zemouri C, Volgenant CMC, Buijs MJ, Crielaard W, Rosema NAM, Brandt BW, et al. Dental aerosols: microbial composition and spatial distribution. J Oral Microbiol. 2020;12(1):1762040.

33. Al-Amad SH, Awad MA, Edher FM, Shahramian K, Omran TA. The effect of rubber dam on atmospheric bacterial aerosols during restorative dentistry. J Infect Public Health. 2017;10(2):195-200.

34. Watanabe A, Tamaki N, Yokota K, Matsuyama M, Kokeguchi S. Use of ATP bioluminescence to survey the spread of aerosol and splatter during dental treatments. J Hosp Infect. 2018;99(3):303-5.

35. Holloman JL, Mauriello SM, Pimenta L, Arnold RR. Comparison of suction device with saliva ejector for aerosol and spatter reduction during ultrasonic scaling. J Am Dent Assoc. 2015;146(1):27-33.

36. Miller RL, Micik RE, Abel C, Ryge G. Studies on Dental Aerobiology: II. Microbial Splatter Discharged from the Oral Cavity of Dental Patients. Journal of Dental Research. 1971;50(3):621-5.

37. Rautemaa R, Nordberg A, Wuolijoki-Saaristo K, Meurman JH. Bacterial aerosols in dental practice - a potential hospital infection problem? J Hosp Infect. 2006;64(1):76-81.

38. Timmerman MF, Menso L, Steinfort J, van Winkelhoff AJ, van der Weijden GA. Atmospheric contamination during ultrasonic scaling. J Clin Periodontol. 2004;31(6):458-62.

39. Bentley CD, Burkhart NW, Crawford JJ. Evaluating Spatter And Aerosol Contamination During Dental Procedures. The Journal of the American Dental Association. 1994;125(5):579-84.

40. Chanpong B, Tang M, Rosenczweig A, Lok P, Tang R. Aerosol-Generating Procedures and Simulated Cough in Dental Anesthesia. Anesthesia Progress. 2020;DOI: 10.2344/anpr-67-03-04.

41. Dahlke WO, Cottam MR, Herring MC, Leavitt JM, Ditmyer MM, Walker RS. Evaluation of the spatter-reduction effectiveness of two dry-field isolation techniques. J Am Dent Assoc. 2012;143(11):1199-204.

42. Harrel SK, Barnes JB, Rivera-Hidalgo F. AEROSOL AND SPLATTER CONTAMINATION FROM THE OPERATIVE SITE DURING ULTRASONIC SCALING. The Journal of the American Dental Association. 1998;129(9):1241-9.

43. Veena HR, Mahantesha S, Joseph PA, Patil SR, Patil SH. Dissemination of aerosol and splatter during ultrasonic scaling: a pilot study. J Infect Public Health. 2015;8(3):260-5.

44. Chiramana S, O S, Kadiyala K, Prakash M, Prasad T, Chaitanya S. Evaluation of Minimum Required Safe Distance between Two Consecutive Dental Chairs for Optimal Asepsis. Journal of Orofacial Research. 2013;3:12-5.

45. Harrel SK, Barnes JB, Rivera-Hidalgo F. Reduction of Aerosols Produced by Ultrasonic Sealers. Journal of Periodontology. 1996;67(1):28-32.

46. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 2012;9(7):671-5.

47. Steiner S, Majeed S, Kratzer G, Hoeng J, Frentzel S. A new fluorescence-based method for characterizing in vitro aerosol exposure systems. Toxicol In Vitro. 2017;38:150-8.

48. Van Rossum G, Drake FL. Python 3 Reference Manual. Scotts Valley, CA: CreateSpace; 2009.

49. Koo TK, Li MY. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. Journal of chiropractic medicine. 2016;15(2):155-63.

50. Tag El Din AM, Ghoname NAH. Efficacy of rubber dam isolation as an infection control procedure in paediatric dentistry. La Revue de Santé de la Mediterranée Orientale. 1997;3:530-9.

51. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, et al. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. International journal of oral science. 2020;12(1):8.

52. Verbeek JH, Rajamaki B, Ijaz S, Sauni R, Toomey E, Blackwood B, et al. Personal protective equipment for preventing highly infectious diseases due to exposure to contaminated body fluids in healthcare staff. Cochrane Database Syst Rev. 2020;4:CD011621.

53. Kratzel A, Todt D, V'kovski P, Steiner S, Gultom M, Thao TTN, et al. Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 by WHO-Recommended Hand Rub Formulations and Alcohols. Emerging Infectious Disease journal. 2020;26(7):1592.

54. Bidra AS, Pelletier JS, Westover JB, Frank S, Brown SM, Tessema B. Rapid In-Vitro Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Using Povidone-Iodine Oral Antiseptic Rinse. Journal of Prosthodontics. 2020;DOI: 10.1111/jopr.13209(n/a).

55. Micik RE, Miller RL, Mazzarella MA, Ryge G. Studies on Dental Aerobiology: I. Bacterial Aerosols Generated during Dental Procedures. Journal of Dental Research. 1969;48(1):49-56.

56. NHS Estates. HTM 2022 - Supplement 1: Dental compressed air and vacuum systems. London: The Stationary Office; 2003.

Figure Legends

Figure 1. A, Schematic diagram of experimental set up. Position of air vents shown; square vents = air intake; long vents = air output. Experimental set up shown with collection positions labelled (note: degrees are relative to facing the mannequin). B, Photograph of experimental set up showing platforms spaced at 0.5m intervals to support filter papers. C, demonstration of polyvinyl siloxane addition to mouth of mannequin.

Figure 2. Heatmap showing surface area outcome measure for three clinical procedures. A, anterior crown preparation (without suction). B, anterior crown preparation with suction. C, anterior crown preparation with suction and assistant. For each coordinate the maximum value recorded from three repetitions of each clinical procedure was used as this was deemed most clinically relevant. Logarithmic transformation was performed on the data (Log₁₀). Note the scale is reduced to remove areas showing zero readings.

Figure 3. Heatmap presenting surface area outcome measure for two clinical procedures. A, ultrasonic scaling. B, 3-in-1 spray. For each coordinate the maximum value recorded from three repetitions of each clinical procedure was used as this was deemed most clinically relevant. Logarithmic transformation was performed on the data (Log₁₀). Note the scale is reduced to remove areas showing zero readings in panel B.

Figure 4. Heatmap presenting spectrofluorimetric analysis of the samples from the anterior crown preparation (without suction) clinical procedure (surface area data shown in Figure 2A). For each coordinate the maximum value recorded from three repetitions of each clinical procedure was used. Logarithmic transformation was performed on the data (Log₁₀). Note the scale includes the full dimensions of the experimental rig.

Figure 5. Filter paper images demonstrating contamination pattern in first 1.5 m from anterior crown preparation (no suction) condition (images from one repetition). Colour balance and contrast adjusted to aid visualization of particles in this figure.

Data accessibility statement

Data available from the authors on reasonable request.

Author Contributions

JRA contributed to study design, design of the experimental rig and image analysis methods, data collection, analysis, and write-up; CCC contributed to study design, data collection, analysis, and write-up; DE contributed to study design, design of the experimental rig and image analysis methods, data collection, analysis, and write-up; CB contributed to data collection and analysis; JC contributed to data analysis; KP contributed to data collection; EK contributed data analysis; JD contributed to initial study conception and write-up; CJN contributed to study design, development of the spectrofluorometric methods, data analysis, and write-up; NJ contributed to initial study conception, study design of the image analysis methods, development of the spectrofluorometric methods, data analysis, and write-up; NR contributed to study design, development of the spectrofluorometric methods, data analysis, and write-up; RH contributed to initial study conception, study design, design, design of the experimental rig and image analysis methods, development of the spectrofluorometric methods, study design, design, design of the experimental rig and image analysis methods, development of the spectrofluorometric methods, data analysis, and write-up; RH contributed to initial study conception, study design, design, design of the experimental rig and image analysis methods, development of the spectrofluorometric methods, data analysis, and write-up; RH contributed to initial study conception, study design, design of the experimental rig and image analysis methods, development of the spectrofluorometric methods, data analysis, and write-up.

Declarations

The authors declare that there are no conflicts of interest.

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Min Mean (SD)	Distance from centre (m)									
Max	0	0.5	1	1.5	2	2.5	3	3.5	4	Total*
Sum [n]	·	0.0	-	2.0	-		Ū	0.0	-	
				Surfa	ce area (mn	1²)				
Anterior crown	668.54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
prep (no	690.07	77.81	1.47	0.03	0.00	0.00	0.00	0.00	0.00	25.32
suction) ⁺	(19.60)	(110.30)	(4.33)	(0.09)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(101.04)
	706.86 2,070.20	386.87 1867.32	21.00 35.40	0.42 0.75	0.08 0.11	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	706.86 5,241.05
	[3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[207]
Anterior crown	656.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
prep with	671.48	19.58	0.48	0.01	0.00	0.00	0.00	0.00	0.00	15.14
suction [‡]	(20.10)	(36.58)	(1.57)	(0.02)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(83.54)
	694.38	145.02	7.65	0.06	0.01	0.00	0.00	0.00	0.00	694.38
	2,014.44	470.01	11.60	0.13	0.01	0.00	0.00	0.00	0.00	3,133.33
	[3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[207]
Anterior crown	204.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
prep with	460.21	10.19	0.04	0.15	0.00	0.00	0.00	0.00	0.00	14.26
suction and	(227.75)	(21.87)	(0.11)	(0.73)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(76.13)
assistant §	641.29 1,380.64	100.54 244.51	0.47 1.07	3.58 3.60	0.02 0.06	0.01 0.01	0.01 0.03	0.01 0.02	0.01 0.03	641.29 2,952.17
	1,380.04 [3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	2,952.17 [207]
Ultrasonic	2.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
scaling with	129.11	3.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.76
suction ¶	(191.14)	(7.99)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(42.67)
	349.00	30.04	0.02	0.03	0.02	0.03	0.02	0.03	0.02	349.00
	387.32	75.59	0.06	0.06	0.05	0.07	0.06	0.08	0.05	1,605.91
	[3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[207]
3-in-1 spray	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
with suction #	0.78	20.47	0.02	0.00	0.00	0.00	0.00	0.00	0.00	10.30
	(0.13) 2.30	(47.32) 220.14	(0.05) 0.20	(0.00) 0.00	(0.00) 0.00	(0.00) 0.00	(0.00) 0.00	(0.00) 0.00	(0.00) 0.00	(53.19) 490.77
	2.30	491.29	0.20	0.00	0.00	0.00	0.00	0.00	0.00	2,131.64
	[3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[207]
				Fluor	escence (RF	U)				
Anterior crown	82,812	89	103	48	70	71	56	47	55	0
prep (no	91,406	11,438	889	319	381	239	388	243	242	4,056
suction) †	(12,153)	(14,907)	(932)	(390)	(600)	(330)	(555)	(342)	(437)	(14,997)
	100,000 182,812	4,6091 274,529	3,541 21,355	1,545 7,661	2,097 9,141	1,506 5,738	2,739 9,309	1,106 5,842	1,695 5,826	100,000 835,741
	[2]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[206]
30-40 min post-	0	0	0	0	0	0	0	0	0	0
procedure	Ő	ů 0	0 0	Ő	Ő	0 0	0 0	8	Ő	1
collection	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(39)	(0)	(13)
	0	0	0	0	0	0	0	191	0	191
	0 [3]	0 [24]	0 [24]	0 [24]	0 [24]	0 [24]	0 [24]	191 [24]	0 [24]	191 [207]
co 70										
60-70 min post- procedure	0 0	0 0	0 14	0 12	0 0	0 8	0 0	0 0	0 0	0 4
collection	(0)	(0)	(49)	(60)	(0)	8 (38)	(0)	(0)	(0)	4 (29)
Conection	(0)	(0)	177	294	(0)	184	0	0	0	294
	0	0	344	294	0	184	0	0	0	822
	[3]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[24]	[205]

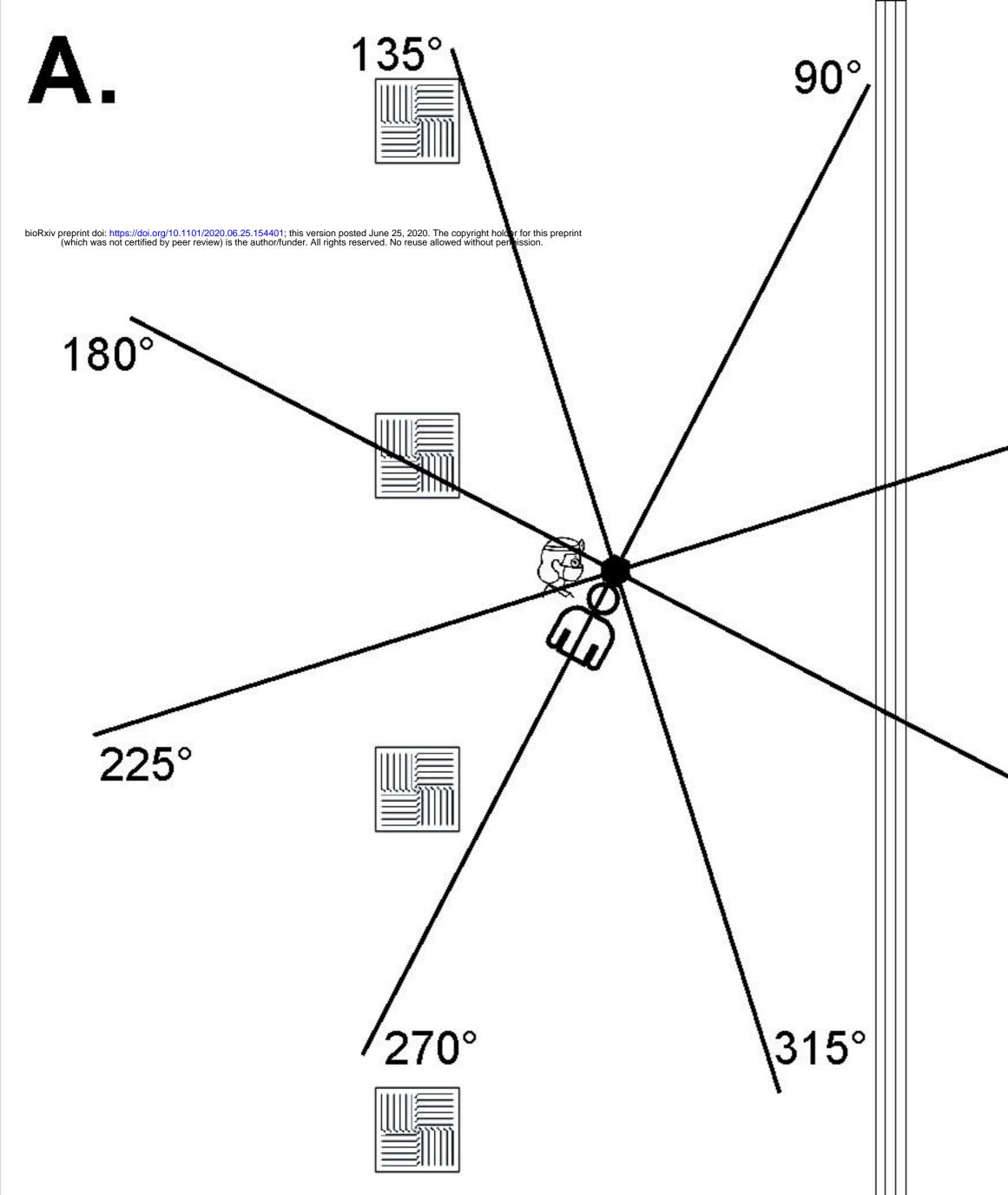
Table 1. Dental aerosol and splatter as measured by total surface area of filter paper contaminated or by spectrofluorometric analysis; grouped by distance from centre and total sum of all measurements. Data for each condition represents the combined data from three repetitions.

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⁺ Anterior crown preparation on upper right central incisor without suction or assistant. 10 minutes duration.

- [‡] Anterior crown preparation on upper right central incisor with suction. 10 minutes duration.
- § Anterior crown preparation on upper right central incisor with suction and assistant. 10 minutes duration.
- ¶ Full mouth ultrasonic scaling with suction. 10 minutes duration.
- # 3-in-1 spray with suction of a MO cavity in upper right first premolar tooth. 30 second duration to replicate washing acid etchant.

*All rig measurements with the addition of readings from the mannequin, representing a 8 m diameter experimental area.



Β.



