Queen Mary University of London

Barts and The London School of Medicine and Dentistry

DclinDent Oral Medicine Research III

The Efficacy of 10 Air Changes Per Hour Ventilation in Controlling Air Contamination in Dentistry

2023

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Table of Contents

List of Figures

List of Table

Abbreviation List

- ACD: Air cleaner device
- ACH: Air change per hour
- ACS: Air cleaning system
- ADA: American Dental Association
- AGPs: Aerosol-generating procedures
- CDC: Centers for Disease Control and Prevention
- CEPH: Centre d'Étude du Polymorphisme Humain
- CFU/cm^2: Colony forming units per square centimetre
- CFU/m^3: Colony forming units per cubic metre
- CO_2: Carbon dioxide
- DSU: Dental stimulation unit
- DUWLs: Dental unit waterlines
- EOS: Extra-oral suction
- HEPA: High-efficiency particulate air
- NHS: National Health Service
- HSCAH: High-speed contra-angle handpiece
- HSS: National Services Scotland
- HVE: High volume evacuator
- HVS: High-volume suction
- ICU: Intensive Care Unit
- IPC: Infection Prevention and Control
- ISO: Isovac
- LEV: Local exhaust ventilation
- OPS: Optical Particle Sensor
- OR: Operating Room
- PA: Portable ambient
- PAC: Portable air cleaner
- PCO: Photocatalytic Oxidation
- PCR: Polymerase chain reaction
- PECO: Photo-electrochemical Oxidation
- PFU: Plaque-forming units
- PM_{10}: Particulate matter ten µm
- PN: particle number
- PPE: Personal protective equipment
- Qubit: Quantum bit
- RFU: Relative fluorescence units
- RH: Relative Humidity
- RLH: Royal London Hospital
- RT: Reverse transcriptase
- SARS-CoV-2: severe acute respiratory syndrome coronavirus 2
- SD: Standard deviation
- SE: Saliva ejector
- SLG: Sir Ludwig Guttmann Health & Wellbeing Centre
- SOP: Standard operating procedure
- T: Temperature
- TVC: Total viable count
- UL: Upper left

- ULPA: Ultra-Low Particulate Air
- UR: Upper right
- UV: Ultraviolet
- VLP: Virus-like particles
- VOC: Variant of concern
- WHO: World Health Organization
- WIBS: Wideband Integrated Bioaerosol Sensor

Abstract

Background: Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19) pandemic has heightened the need to protect healthcare workers, including dental. The use of mechanical ventilation, particularly high-efficiency particulate air (HEPA) ventilation, was recommended by UK Health Security Agency with a parameter of 6 air changes per hour (ACH) initially. The recommendation was changed to 10ACH for aerosol-generating procedures (AGPs). There have yet to be any previous studies investigating the effect of these two parameters on air contamination in dental hospitals outside the dirty zone. Therefore, this study aimed to fill this knowledge gap.

Methodology: MD8 airscan was used (Sartorius, Epsom, UK) with sterile gelatine filters (80 mm diameter and 3 µm pores; Sartorius) to enable RNA extraction for SARS-CoV-2 Polymerase Chain Reaction (PCR) and with polystyrol culture media plate measuring 116 x 24 mm (BACTair culture media, Sartorius, Germany) for bacterial and fungal colony forming unit (CFU) quantification. Sampling air was performed from outside the dirty zone in open clinical areas with ventilation of 6ACH and 10 ACH when AGPs and non-AGPs were performed in two different dental settings; Barts Health NHS Dental Hospital (RLH) and Sir Ludwig Guttmann (SLG) Dental Centre.

Results: The air contamination at 10ACH was significantly lower than 6ACH at baseline (13.83±5.4 vs 68.67±74.73; p=0.019), AGP (177.3±19.04 vs 288.5±108.6; p=0.023), and non-AGPs (114.7±23.69 vs 245.3±37.97; p=0.007) in RLH. In SLG, 10ACH maintained air contamination at 30.33±26.73 and 18.33±11.85 for non-AGP and AGP, respectively, compared to 192±34.64 for non-AGP in 6ACH (p=0.0003).

Conclusion: This study proves that 10ACH is an efficient intervention to improve the air quality in open bay dental clinics during all types of dental procedures (AGPs and non-AGPs) in different dental settings, large dental hospital and outreach dental clinics, which has close similarity to community dental clinic environment.

1. Introduction

1.1. Severe acute respiratory syndrome 2 (SARS-CoV-2)

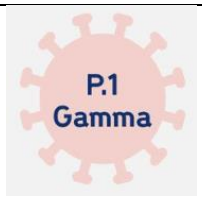
A new acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected in Wuhan, China, at the end of 2019. It has caused a worldwide coronavirus disease 2019 (COVID-19) pandemic. Because of that, in March 2020, the World Health Organisation (WHO) declared a global alert (Checchi et al., 2021). SARS-CoV-2 is a member of the pathogen family responsible for another respiratory infection that outbreak in 2002-2003 (SARS-CoV-1) (Komperda et al., 2021). Symptoms of COVID-19 vary from headache and sore throat to severe dyspnoea and acute pneumonia; most cases are mild, whereas 15% of the patients required hospitalisation in the first wave alone (Amato et al., 2020).

In September 2020, a new variant of SARS-CoV-2 was estimated to be up to 70% more transmissible, triggering further restrictions to control the second wave of the pandemic (Kirby, 2021). Introducing SARS-CoV-2 alpha, beta, and delta variants of concern (VOCs) was accompanied by new waves of infection, sometimes spanning the globe. For instance, the greater transmissibility of the delta VOC was related to an enormous viral load, a prolonged timeframe of infectiousness, and a high reinfection rate, owing to the delta VOC's capacity to evade natural immunity. The delta VOC continued to fuel fresh waves of infection and remained the primary VOC in several nations throughout the fourth wave.

On the 11th of November 2021, Botswana reported the first sequenced omicron case, and a few days later, Hong Kong reported another sequenced case in a tourist from South Africa (Karim and Karim, 2021). Omicron has a large number of mutations in the spike protein, making it more sophisticated and effective in overwhelming acquired

immunity; even vaccinated individuals were proven infected, demonstrating omicron's better infection capabilities and casting doubt on the usefulness of currently available vaccines (as shown in Table 1.1) (Okpeku, 2022). Other variations were de-escalated because the variant stopped spreading or had no significant effect for an extended period. Moreover, the updated global death reached almost 7 million cases worldwide on the 31st of March 2023 (Figures 1.1 and 1.2).

Table 1.1. The Variants of Concern for COVID-19 (WHO, 2022)

Virus variant					
First reported	United Kingdom in late December 2020	South Africa in December 2020	Brazil in early January 2021	India in December 2020	South Africa in November 2021
Transmission	Spreads easily	Spreads easily Some vaccine may be less effective against	Spreads easily Some vaccine may be less effective against	Spreads easily Symptoms may differ. Some vaccines may be less effective against	Spreads easily

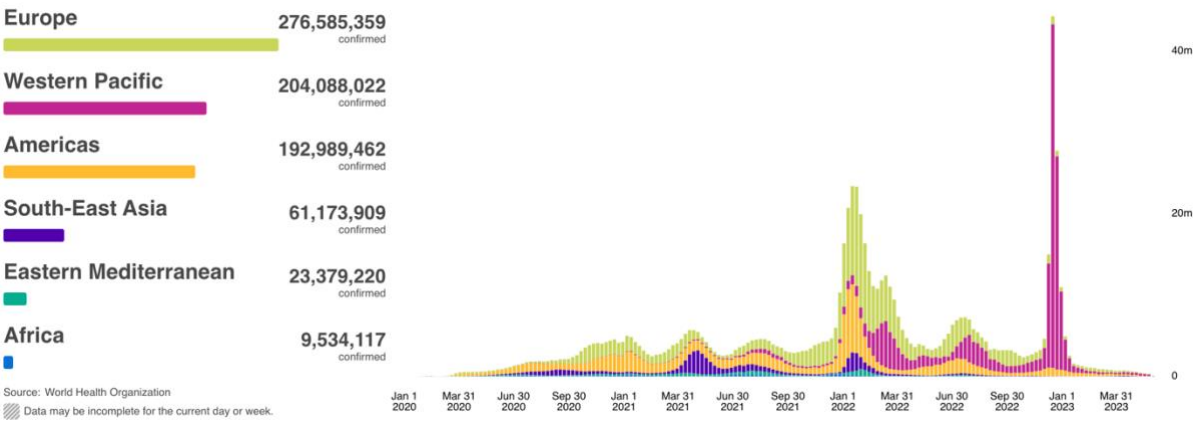


Figure 1.1. COVID-19 confirmed cases globally 31st of March (WHO, 2023)

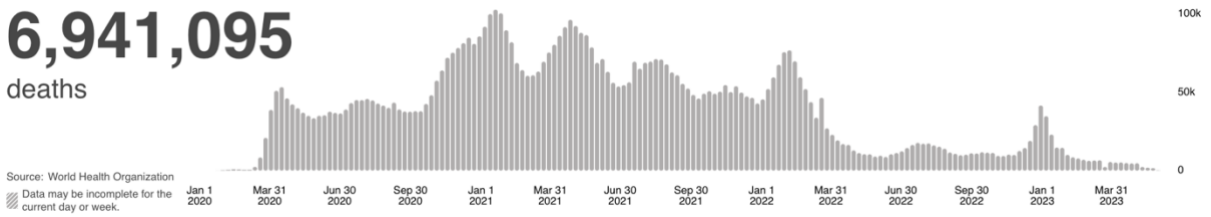


Figure 1.2. Global death caused by COVID-19 on the 31st of March (WHO, 2023)

The standard transmission route of a novel coronavirus from human to human is believed to be through close contact with people infected with SARS-CoV-2, even in their incubation period. The transmission is either by direct inhalation of respiratory droplets or contact transmission with the oral, nasal, and eye mucous membrane (Leung et al., 2020; Long et al., 2020; Peng et al., 2020; Shiu et al., 2019). Also, the Centers for Disease Control and Prevention (CDC) reported that SARS-CoV-2 is transmitted by contact with infectious virus-carrying respiratory fluids. Exposure can happen in three different ways. (1) breathing of air containing tiny respiratory droplets and aerosol particles; (2) deposition of aerosol particles on the nasal or oral mucosa of vulnerable individuals; and (3) contact with mucous membranes with hands contaminated with discharged respiratory fluid (Centers for Disease Control and Prevention, 2021). Additionally, the salivary glands have been associated with SARS-CoV-2 infection as possible storage, as angiotensin-converting enzyme 2 (ACE2) protein, which has proved to have a solid binding to SARS-CoV-2 protein S, is found frequently in the upper respiratory tract and salivary ducts (Holliday et al., 2021, Teichert et al., 2020). Also, the incubation period differs between 3 and 14 days; nevertheless, some cases have reported 24 days incubation period (Checchi et al., 2021).

SARS-CoV-2 variations raise worries regarding increased transmission and evasion of vaccination and natural infection defence, particularly considering concerns about a specific mink strain that caused a person's disease and the prospect of future

changes. While the majority of alterations are harmless, the virus occasionally acquires a mutation that gives it an advantage over other strains (Thirumal Kumar et al., 2022, Tai et al., 2020).

Notably, a comprehensive and systematic review of articles emphasised the high risk for patients with either chronic illness or immunosuppressants to get infected with COVID-19. The authors of these two articles concluded that immunosuppressant individuals or patients with chronic disease require more hospitalisation when they get infected than the general population as the result of having more comorbidities, such as hypertension and diabetes, and that leads to being at risk of having worse outcome (Belsky et al., 2021 and Triggle et al. 2021).

Despite the lack of effective treatments, several efforts have been made to create vaccinations against COVID-19. The other immunisations utilised in the conduit are live attenuated or inactivated viruses, virus-like particles (VLP), protein subunits, viral vectors (non-replicating and replicating), DNA, RNA, and nanoparticles. Each has its own set of strengths and drawbacks (Thirumal Kumar et al., 2022). Vaccination is a cost-effective method of reducing infectious illnesses that are epidemic or pandemic. New COVID-19 vaccines have been designed, studied, and registered at a breakneck rate. Presently, there are nine COVID-19 vaccines which are extensively used, significantly lowering infection, severe illness, and death rates around the world (Roozen et al., 2022). Actual evidence from Israel, the United Kingdom, Sweden, and the United States of America indicated that complete vaccination with either the BNT162b2 (Pfizer–BioNtech) or mRNA-1273 (Moderna) vaccines secured individuals against infection, hospitalisation, and death from viral variants by 61–92 %, 80–87 %, and 85 %, respectively (Roozen et al., 2022; Henry et al., 2021).

It was discovered that the COVID-19 pandemic is very similar to the 1918 Spanish influenza pandemic. Thus, different studies were conducted to compare these two. An H1N1 influenza virus causes the Spanish influenza of 1918. A virus of avian origin is hypothesised. It lasted from 1918 to 1920 and was divided into four waves. The first wave lasted around 15 February to 1 June 1918; the second wave lasted roughly 1 August to 2 December 1918; the third wave lasted approximately 3 December 1918 to 30 April 1919; and the fourth wave lasted around 1 December 1919 to 30 April 1920. It infected around 500 million people, almost one-third of the world's population, and killed 50 million (Liang et al., 2021).

To note, there are significant differences in the patient group. The Spanish flu killed a surprising amount of 25–40-year-olds, while COVID-19 primarily affects persons over 65, particularly those with comorbidities (Liang et al., 2021; He, 2020). The 1918 influenza patients mainly died from secondary bacterial pneumonia, whereas COVID-19 patients died primarily from a hyperactive immune response that resulted in organ failure (Liang et al., 2021). There is indeed a correlation between the weekly incidence of COVID-19-associated pneumonia mortality up to May 2020 in England and Wales and Influenza deaths up to May 1919 (He, 2020) (Figure 1.3). Spanish flu killed 50 million people worldwide, while COVID-19 killed roughly 6,941,035 people up to March 2023. The second and third waves of COVID-19 have subsequently been recorded worldwide, comparable to the flu epidemic of 1918 (Simonetti et al., 2021).

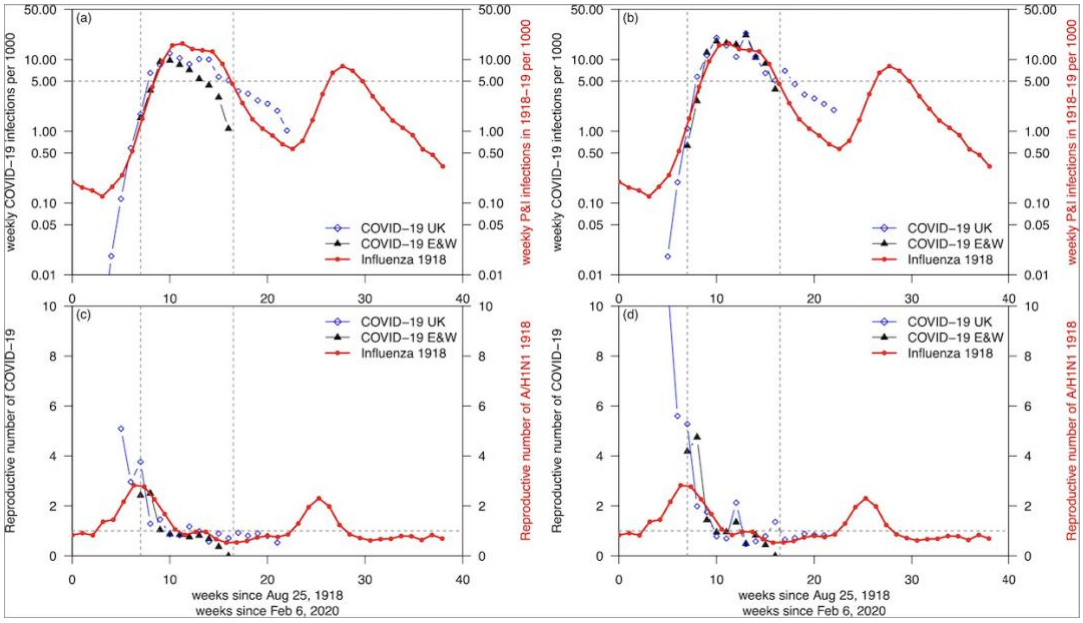


Figure1.3. Comparisons of COVID-19 and A/H1N1 1918 weekly infection according to He (2020)

After the wide spreading of the virus all over the world and due to the occupational hazards in the dental clinic, dentists in the UK had to treat only emergency and urgent cases in the period between March and June 2020 (Holliday et al., 2021, Long et al., 2020, Meng et al., 2020, Teichert-Filho et al., 2020). Due to the close contact and the high exposure to body fluids such as saliva and blood generated by frequent aerosol-generated procedures (AGPs), the transmission rate in the population might increase (Amato et al., 2020, Meng et al., 2020). Standard dental handpieces such as ultrasonic scalers and air rotors have been linked to significant respirable aerosol production. Because these AGPs are performed so often in dental clinics, they may serve as a critical mode of infection transmission (Fennelly et al., 2022). Not only can the air be contaminated by aerosol, but it can remain in the air for an extended period. Therefore, patients or clinicians might inhale it. Also, the aerosol may settle on surfaces in the dental clinic, causing a potential risk for indirect transmission if not adequately decontaminated (Barabari and Moharamzadeh, 2020). Moreover, there is a gap in the current knowledge regarding bio-aerosol contamination in hospital and

dental environments, which should be addressed by research urgently to reduce the risk for healthcare workers and patients (Zemouri et al., 2017).

1.2. Infection Prevention and Control in Dentistry

The pandemic caused by COVID-19 has increased the need to protect healthcare providers (Maurais et al., 2021). Preventive measures to minimise SARS-CoV-2 infection transmission in dental clinics areas include keeping distance and lowering the number of patients in the waiting room, hand hygiene, and the use of personal protective equipment (PPE) (fit tested filtering respirators such as FFP3, gloves, face shield, surgical glasses, and surgical gown). Other protective measures during dental treatments include four-handed dentistry, high-volume suction, placing the rubber dam, limiting the usage of high-speed handpieces, implementing fallow time after the AGPs and thorough decontamination for all contaminated surfaces on the dirty zone (Meng et al. 2020, Peng et al., 2020, Teichert et al., 2020, Harrel and Molinari, 2004). In addition, using an air purifying system is a technique for reducing the aerosol concentration. Such a system aims to lower the exposure risk during dental treatments for dental staff and other patients (Maurais et al., 2021). SARS-CoV-2 RNA concentrations were reduced in isolation and ventilated patient rooms but elevated in patient bathroom areas. Even though they could not evaluate the infection risk in these regions, they established that SARS-CoV-2 could potentially spread by aerosols in medical clinics (Liu et al., 2020).

Given that COVID-19 is transmitted chiefly by droplets and aerosols (droplet nuclei), it is reasonable to hypothesise that dentistry would have a high transmission rate and related death of SARS-CoV-2. Also, healthcare workers are vulnerable, especially when considering some medical procedures where a face mask is not feasible, such

as dentistry, consequently in aerosol production or creation in these situations (Komperda et al., 2021). Nevertheless, when the number of fatalities from March to December 2020 in England and Wales was analysed, there was no indication of increased mortalities among dentists induced by COVID-19 (Devlin and Soltani, 2021). This comes to the realisation that dentists' low infection rate might result from the robust safety standards in place.

The American Dental Association (ADA), and also the majority of European dental organisations, recommend pre-screening patients before their visit, allowing only one patient in the waiting room at a time, monitoring staff and patient temperatures, hand washing and sanitising, patient access to sanitisers, disinfection of surfaces, PPE for medical staff, and the use of UV lamps and other air purifiers and high-tech disinfectants (Goriuc et al., 2022). Another reason for the low mortality rate among dental staff can be a consequence of a triage strategy. The strategy included virtual appointments to consider the risk of spreading and initially limited treatment to advise, analgesia, and antibiotic therapy when justified. Patients with positive COVID-19 tests were sent only to the Urgent Dental Care unit for face-to-face care (Devlin and Soltani, 2021).

The standard operating procedure (SOP) guideline was updated by the National Health Service (NHS) in November 2021 to allow dental services to re-start delivering dental care in the community. The policy is divided into three sections: the first is about the fundamentals of patient care, the second is about methods for planning and delivering services, and the final is about supporting the dental staff. Starting with the recommendation for the fundamentals of patient care, all clinics should welcome referrals for urgent dental treatment. The dental team should continue to pursue

National Institute for Health and Care Excellence (NICE) recommendations for risk-based recall intervals to optimise their quality-of-service delivery. Regarding dental access, limited accessibility to dental treatment may unfairly target specific patient groups, which should be avoided to the maximum extent feasible (NHS SOP, 2021).

Moreover, Dental services were advised to ensure patients understand how to obtain dental care. Therefore, patients should be able to make in-person appointments at receptions, subject to risk management and social distancing restrictions. This ensures that patients with limited phone access or other communication devices are unaffected (NHS SOP, 2021).

The second section of the SOP focused on how to approach and plan the service to minimise the risk of transmission, and all practices were advised to screen patients before receiving care. Dental offices were advised to use the UK infection prevention and control (IPC) screening tool for COVID-19 in healthcare settings and check the uk.gov website regularly for any changes or adjustments to the screening questions. Following screening and risk assessment, the practice was expected to decide whether to place the patient on the respiratory or non-respiratory route and proceed with face-to-face dental care. Dental care may be provided to patients on the non-respiratory route utilising standard IPC precautions for both non-AGP and AGP treatments without the need for fallow time. On the other hand, individuals on the respiratory path need additional assessment to decide if routine care may be delayed until respiratory symptoms have resolved and any COVID-19 quarantine periods have ended. However, suppose the patients on the respiratory route require care that is judged emergency or urgent. In that case, they should receive face-to-

face care at any facility using transmission-based IPC precautions. Still, they must be isolated in place and time from other patients (NHS SOP, 2021).

Notably, a fallow time was advised to be observed between patients to permit ventilation to eliminate airborne contaminants (Fennelly et al., 2022). The fallow time was only required for patients treated on the respiratory pathway. The period of fallow time varies according to the clinic's ventilation characteristics, the length of the AGP, and the mitigating measures implemented. Globally it’s agreed that the patient spacing time durations range from non to 120 min (Dave M., 2020). For the majority of the vulnerable individuals to acquire severe COVID-19 infection, the probability of COVID-19 complications has been significantly lowered due to the immunisation campaign. Nonetheless, some patients may take further steps to ensure their safety, even if they are completely vaccinated against COVID-19 (Cascella et al., 2022).

Last but not least is the dental team support system. Since January 2021, all dental team members who interacted with patients, including dental professionals and non-clinical personnel (e.g., receptionists and domestic workers), have been entitled to the COVID-19 vaccination. Most dental staff will have had all recommended vaccinations, consistent with the expectation that healthcare workers fulfil their duty of care to patients by making all reasonable efforts to protect themselves and their patients from infectious illnesses. A booster vaccine was also offered for health workers who had the vaccine for the second time at least six months ago. Also, to protect health workers and reduce the risk of infection, dental offices were advised to review risk assessments for all employees regularly. Primary care workers interacting with high-risk patients were required to self-test twice weekly and submit the results to UK Health Security Agency (NHS SOP, 2021).

The air changes per hour (ACH) is used to calculate air turnover or flow (Vonci et al., 2019). ACH is computed by dividing the air volume in the operating room per hour (m3/hr) by the operating room size (Gupta et al., 2015). According to recent CDC standards, ACH in the patient waiting area should be 6, 12 in the radiography section, and 15 in the surgery room (Rathi et al., 2022; Ninomura and Bartley, 2001).

A technical report by National Services Scotland (NSS) focused on the ventilation and environmental cleaning associated with COVID-19. After a literature review, NSS reported that a minimum of 10 ACH is recommended in dental surgery. The American Society of Heating Air-conditioning and Refrigeration Engineers recommendation for ACH in clinics to be a minimum of 6 ACH in patients' rooms, 12 ACH in isolation rooms, and 15 ACH in procedure rooms for surgeries or interventional cardiology settings need (Ren et al., 2021). Moreover, the present-day evidence advises that the higher bacterial contamination during dental treatments may take around 30 min to disappear but could vary according to the risk reduction strategy implemented and the number of ACH. However, evidence regarding determining the ideal fallow time and its benefits is limited (National Services Scotland Short Life Working Group SBAR, 2020). Most of the evidence is based on simulations, and none investigated the microbial load in air samples at different time points and places around the dental chair. In addition to this, the association between ACH and airborne contamination have not been fully covered previously (Vonci et al., 2019).

A study that analysed the control of aerosol particle clearance in dental clinics by the usage of a numerical method came to the conclusion that using air cleaner could be a possible technique to minimise environmental contamination in the dental clinic—it

also added that the air cleaner position significantly reduces the exposure to droplets (Chen et al., 2009). Another study supported this statement by calculating the fallow time using high-efficiency particulate air (HEPA) mechanical ventilation. There was a significant reduction in the fallow time requirement (Shahdad et al., 2021).

Overall, it is crucial to identify the effect of the risk, as mentioned earlier, reduction strategies, especially ventilation parameters, on reducing environmental contamination and the potential risk of infection transmission to health workers and patients. Especially investigating the air level of contamination in dental hospitals has yet to be done.

1.3. Research Question

What is the effect of changing ACH from 6 to 10 on the microbial contamination of air when AGPs and non-AGPs are performed in an open dental clinical area in a dental hospital and outreach dental facility?

1.4. Research Hypothesis

Using ventilation of 10 ACH have a better effect on reducing the microbial contamination of air when AGPs and non-AGPs than 6 ACH in open clinical areas at different dental settings.

1.5. Null Hypothesis

There is no difference between the ventilation of 6ACH and 10ACH in the microbial contamination of air when AGPs and non-AGPs performed in open clinical areas at different dental settings.

Keywords*:* aerosol; splatters; airborne; SARS-CoV-2; COVID19; dental clinic; contamination; ventilation

2. Literature Review

Aerosol and splatters are terms that were used by Micik et al. (1969) during their work on aerobiology. Their size defines both. Aerosol is a particle that is less than 50 micrometres in diameter, while splatters are more significant than 50 micrometres in diameter (Ionescu et al., 2020, Harrel and Molinari, 2004). Moreover, aerosols are defined by the CDC as a mixture of the micro (<5μm) aerosolised particles or droplet nuclei in the air that can occasionally produce negative health impacts on workers. The public health literature today recognises an obsessive focus on the size limitations of particles able to penetrate far into the lungs, and bigger particles up to and exceeding the inhalable limit of 100 μm are acknowledged to spread illness through the air (Randall et a., 2021). Also, 100 μm is the recognised bar for droplets to be classified as aerosols (Prather et al., 2020). Recently, more information complied, leading to differentiating aerosol (droplet nuclei) (<5μm) from droplet (>5μm) and splatter (>50μm) (Johnson et al. 2021).

Former research demonstrates that dentists and patients are exposed to more than 10,000 germs per cubic metre, and there may be considerable danger of breathing infectious aerosols during dental treatments (Kumar et al., 2020; Dutil et al., 2008). Dental health professionals might inhale up to 0.014 μl of saliva during 15 min in maximum exposure and up to 0.12 μl of saliva in severe cases (Bennett et al., 2000).

2.1. Aerosol and Splatters

Aerosols or droplet nuclei are tiny particles that could be solid or liquid, that may remain in the air for up to 30 min and are easily inhaled. In contrast, splatters are large particles that cannot stay in the air for long because of the heavy particle weight. Aerosol can travel for 1 to 3 meters in the distance after a dental procedure, unlike

splatters that settle directly within 1 meter and could contaminate eyes, hair, skin, clothing, and dental working area (Ionescu et al., 2020, Zemouri et al., 2020, Harrel and Molinari, 2004). A straightforward way to differentiate between droplets and droplet nuclei, the latter does not fully evaporate. Hence, they can remain airborne for longer, and the travel distance is wider (Verma et al., 2020; Duguid, 1946). Additionally, due to their tiny size, they may penetrate surgical masks by up to 70% (Zangmeister et al. 2020).

Other particles can pass through other shows and alternative materials, such as fabric masks and gaiters (Lindsley et al. 2021; Morais et al. 2021). Up to 98% of the bigger particles that pass through would settle in the pharynx and upper respiratory system (Yeh and Schum 1980). The outstanding small particles would enter the respiratory system further since the upper airways are ineffective at filtering them. For comparison, 90% of particles less than 1 mm in diameter are anticipated to enter the lungs (Brown et al., 2013). These particles will represent a severe risk to one's health if they carry any respiratory infections.

Ren et al. (2021) studied aerosol particles 0.3, 0.5 and 1.0 μm in size. They were generated by burning sticks of incense in the dental clinic. They noted that after 5 min of burning, the concentration of the aerosols would reach the greatest extent and continued at that rate until 30 min after the incense was terminated. The route of transmission and the characteristics of droplet nuclei, droplets and splatters, along with expected particle size from different activities, are illustrated in Figure 2.1 and Table 2.1.

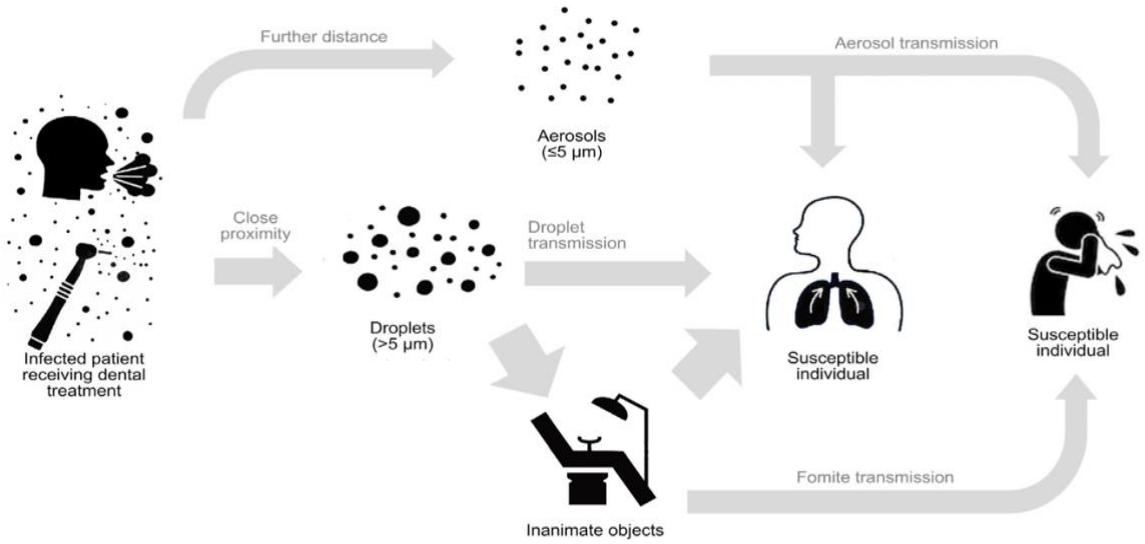


Figure 2.1. Route of Transmission in Dental Settings: Aerosols (droplet nuclei), Droplets, and Splatters (Ge et al., 2020)

Table2.1. The Comparison of Characteristics Between Droplet Nuclei and Droplets

	Size	**Distance**	**Remaining in the Air**	**Route of Infection**
Droplet nuclei (aerosols)	<5 μm	≤3 meters	Up to 30 min	Easy to be inhaled
Droplet And Splatters	>5 μm	<1 meter	Contaminates the surfaces around it.	Direct or indirect contact, and in close contact with an infected person.

Aerosol and splatter can be contaminated by bacteria and viruses from patients with airborne diseases. Pathogen determines the size of the particles required for its transmission. For example, bacteria need a large particle to carry; however, viruses would be held by a small one (Ionescu et al., 2020). The prevention of disease transmission by droplets can be prevented by using sufficient PPE and routine cleaning and disinfection. Aerosol particles are much harder to regulate and remove because of their tiny size (Yang et al. 2021).

Consequently, an outbreak can happen from airborne spreading through coughing, sneezing, or even speaking, as it can travel up to 2 m until it settles on any surrounding surface (Prather et al., 2020). Splatter and droplet nuclei have previously been linked to spreading illnesses such as SARS, measles, and herpetic viruses (Harrel and Molinari, 2004). According to WHO, the COVID-19 virus primarily transmits between people in proximity (1 m). It can also circulate in weakly ventilated and congested locations because pathogenic aerosols persist floating in the air and can travel more than 1 m (WHO, 2021). To date, 19 bacterial species were noted with aerosol around the patients, and most species were from *Staphylococcus* spp (Zemouri et al., 2020) (Table 2.2).

Table 2.2. Particle Sizes from Natural Respiratory Activities (Gralton et al., 2011)

Author	Date	Method of sizing	Microorganism	Particle size range (μm)
Heymann et al.	1899	Solid impaction	*Mycobacterium tuberculosis*	Coughing: 30-500
Buckland et al.	1964	Liquid impaction	Bacterial unspecified species	Sneezing: 80-180
Fabian et al.	2008	Optical technology	Viral unspecified species	Breathing: 0.3-0.5
Wainwright et al.	2009	Solid impaction	Bacterial unspecified species	Coughing: <3.3
Lindsley et al.	2010	Solid impaction	Flu virus	Coughing: <1.0

2.1.1. Aerosol Generation Procedures (AGP)

AGPs are defined as: “dental procedures using high-speed devices such as ultrasonic scalers and drills” (Johnson et al., 2021). Another definition by the WHO, AGPs are any medical, dental, or patient care operation that produces airborne particles that

enhance the possibility of infectious transmission (WHO, 2014). In dental clinics, instruments such as ultrasonic scalers and contra-angle handpieces operating with speeds higher than 60,000 rpm produce variable levels of aerosol contamination (Table 2.3). These procedures raise a concern about IPC (Ionescu et al., 2020, Zemouri et al., 2020, Hallier et al., 2010, Harrel and Molinari, 2004). The CDC does not presently classify rubber cup polishing as an AGP (CDC, 2020). A comparison between AGP and non-AGP is presented in (Table 2.3).

Table 2.3. Comparison Between AGP and Non-AGP (WHO, 2014)

Type of Procedure	Examples	Characteristics
Non-AGP In dental clinic procedures that do not cause any spraying or do not produce much of it.	Manual scaling Examination Ortho bonding Manual excavating Fluoride application X-ray Denture fitting Extraction	Slow Does not generate aerosols which is safer.
AGP The procedures that produce a big amount of spraying like the usage of drills (high speed) or air water syringe.	Ultrasonic scaling Crown preparation Cavity preparation Air tooth polisher Air water syringe	Fast Generates airborne which leads to an increase in contamination.

AGP: aerosol generating procedure.

Concerns among dental practitioners about AGPs potentially placing dental professionals and their patients vulnerable to infection have been intensified by COVID-19 (Zemouri et al., 2020). Thus, the SARS-CoV-2 pandemic highlighted the need for studying the topographic distribution of AGP, as there is a limited number of studies (Ge et al., 2020, Ionescu et al., 2020, Peng et al., 2020, Zemouri et al., 2020). Studies have reported that viruses and bacteria can be transmitted to the environment via AGPs on patients' noses, throats, and respiratory tracts. Moreover, dental unit

waterlines (DUWLs) can contribute to the contaminated aerosols (Ionescu et al., 2020, Zemouri et al., 2020, Hallier et al., 2010, Harrel and Molinari, 2004).

2.2. Environmental Microbial Contamination

2.2.1. Dental Chair Unit Contamination

Depending on what type of dental procedure is being done, a different load of contamination and even the distance that could be contaminated can be different (Zemouri et al., 2017). Dental treatments include procedures performed by using an ultrasonic scaler, high-speed handpiece, tooth extraction (third moral), slow handpiece, air-water syringe, and hand scaling. These treatments can all lead to contamination which was studied by Innes et al. (2021) in their systematic review.

It was proved that the ultrasonic scaler could produce more droplet contamination within 1 m of the patient. The operator's face (mask) and areas closest to the patient (operator's nearest arm) were significantly polluted. Contamination was discovered on the assistant's face and arm during an examination. The patient was extensively contaminated, with the chest being one of the most contaminated locations. With increasing distance from the mouth, contamination levels decreased. Contamination was discovered at the maximum observed length of 3 m.

Similarly seen with a high-speed handpiece, contamination was detected at a maximum height of 3 m from the patient. A higher number of contaminations were noted in front of the patient and got lower as the distance from the patient increased. The contamination of the operator, nurse and patient was the same as in the ultrasonic scaler.

Interestingly, in both procedures, the aerosol needed two hours to return to the baseline level. Coming to the air-water syringe, contamination was identified after using it. However, the generation of aerosol dose varies when the air and water are used separately. It generates less contamination than using them together. In addition, tiny particles can stay in the air for up to 6 h and more, and travel distance may reach 6 feet from the patient (Innes et al., 2021).

Moving to non-AGPs, there was a risk of contamination in the oral surgery department, but it's more limited to less than 1 m from the patient. That might be due to the use of motorised handpieces and irrigation. The splatters mostly contain blood and bacterial microorganism. Even though minimal information was found regarding slow handpiece contamination, an increase in bacterial load compared to the baseline level during deboning and enamel cleaning was spotted. Also, microbiological contamination was noticed more at a distance of two feet from the operatory location compared to one and three feet. Moreover, microorganisms such as yeasts and Gram-negative bacteria were found in the aerosol created during denture polishing and trimming. Even hand scaling was proven to produce an aerosol that is not more than what is generated during speaking (Innes et al., 2021).

According to an experiment by Ionescu et al. (2020), a biological tracer was used on the dental unit in 22 different locations while the manikin head was oriented in an everyday work setting with resin teeth. All sites in the dental clinic were contaminated. The indicator showed the highest contamination when using the air turbine and the lowest during contra-angle handpiece usage. The right side of the dental chair showed lower contamination, and no difference was noted when using the contra-angle handpiece and the ultrasonic scaler (Ionescu et al., 2020). Increased microbial

contamination was shown in the patient's chest and near the patient's head during dental treatment (Zemouri et al., 2020). That was also demonstrated when Zemouri et al. (2020) did a passive sampling during dental treatment, 30 min before, 30 min during, and the last 30 min after the treatment.

A systematic review by Johnson et al. (2021) concluded higher contamination using higher power settings than ultrasonic scaler. The chest is the central area of contamination for the operator, assistant, and patient. The arms of the operator were also contaminated. The contamination was noted in the head and neck region of the operator and the assistant, even inside the full-face shield and the face mask. Maurais et al. (2021) studied the risk of contamination that the dentist and his assistant may encounter during dental procedures like AGPs and non-AGPs. By measuring particles matters (PM) less than ten μm in size or smaller, in the study, they found that the contamination before using any interventions was 1334 PM_{10} ($\mu g/m^3$) in AGPs and 1227 PM_{10} ($\mu g/m^3$) in non-AGPs for the dentist, and for the dental assistance were slightly higher than the dentist (1748 PM_{10} ($\mu g/m^3$) in AGPs and 1634 PM_{10} ($\mu g/m^3$) in non-AGPs) both producers were ongoing for 5 min.

Fennelly et al. (2022) measured the areole concentration by two different devices, the first one is Optical Particle Sensor (OPS), and the other one is Wideband Integrated Bioaerosol Sensor (WIBS). A professional dentist has conducted two procedures (scaling and drilling) on the right upper first moral (UR6) and right lower first moral (LR6) with no suction used. UR6 drilling was linked to the most significant rise in particle counts measured by the OPS and the second most considerable elevation measured by the WIBS. The average OPS and WIBS were 28.08 particle counts /cm^3 and 69.37 particle counts /cm^3, respectively. The WIBS found that LR6 drilling caused

the highest rise in particle counts. The mean number of particles per process was 24 times greater than the background observed by the WIBS, at 139.15 particles/cm^3. However, the OPS for the LR6 was only 7.89 particles/cm^3. Only WIBS showed a significant elevation in particle number during scaling of the lower jaw. The baseline was 4.06 particles/cm^3 for OPS and 3.63 particles /cm^3 for WIBS.

2.2.2. Operative Clinic Contamination

A study was conducted to test the operative clinic contamination, where 68 biological tracers were used around the clinic in various locations. These tracers were closed, and the co-worker opened them when the doctor started the procedure and closed them 26 min after the procedure had finished allowing the aerosols to settle (Ionescu et al., 2020). There was evidence of widespread contamination of the dental operative room to a maximum distance of 360 cm. A low level of contamination was noticed on the ceilings and walls, with evidence of more contamination on the ceiling directly above the dental unit (Ionescu et al., 2020, Zemouri et al., 2020). Splatter and droplet contamination generated by the ultrasonic device was a tremendous amount near the patient and decreased while moving further from the patient.

Research has shown that dental unit contamination is more common on the left and front sides of the patient when the operator is positioned on the right (Johnson et al., 2021). A study conducted on a mannequin in a dental clinic revealed that using high-speed handpieces and low-speed electric-driven handpieces for just 5 min resulted in ambient air contamination. Before interventions were installed, AGPs had 2036 PM_{10} ($\mu g/m^3$), and non-AGPs had 1704 PM_{10} ($\mu g/m^3$) (Maurais et al., 2021). The air turbine, contra-angle handpiece, and ultrasonic scaler all produced air contamination, but the variation was in the distance it travelled. The air turbine had the longest distance at

360 cm, the contra-angle handpiece had 300 cm, and the ultrasonic scaler had the shortest distance at 240 cm (Ionescu et al., 2020). The summary is shown in (Table 2.4.).

A study conducted by Hallier et al. (2010) evaluated the level of air contamination during various dental procedures within a 20 cm radius of the dental chair. The study found that cavity preparation produced the highest air contamination at 105.1 cfu/m^3. Examination, tooth extraction, and ultrasonic scaling produced similar levels of air contamination at 69.2 cfu/m^3, 66.1 cfu/m^3, and 70.9 cfu/m^3, respectively. Another study conducted by D. Grenier in 1995 also compared air contamination during operative treatment and ultrasonic scaling. The study found that at a distance of 122 cm, ultrasonic scaling produced significantly more contamination than operative procedures, with levels of 216 cfu/m^3 and 75 cfu/m cfu/m^3, respectively. All studies were summarised in (Table 2.4).

Table 2.4. AGPs and contamination of the dental clinic

Author Name	Procedure	Length of Contamination	Technique of Measurements	TVC or Total Contamination
Fennelly et al., 2022	UR6 drilling LR6 drilling	WIBS :30cm from the mouth OPS: outside the enclosure	particles/cm^3	UR6: WIBS is 69.37 and OPS is 28.08 LR6: WIBS is 139.15 and OPS is 7.89
Holliday et al., 2021	Crown preparation	5 meters	RFU	700,000 RFU
Maurais et al., 2021	AGPs and Non-AGPs	Chairside	10 µm particulate matter in size or smaller (PM_{10})	1- Nos-AGPs 1704 PM_{10} (µg/m^3) 2- AGPs 2036 PM_{10} (µg/m^3)
Ionescu et al., 2020	1. Air turbine 2. Contra angle handpiece 3. Ultrasonic scaler	1. 360 cm 2. 300 cm 3. 240 cm	Contamination in cm^2	1. 0.51CFU/cm2 2. 0.47 CFU/cm2 3. 0.41CFU/cm2
Teichert-Filho et al., 2020	Standardized simulated dental procedures.		Using ultraviolet flashlight illumination, the dye was observed.	1. Mannequin's face 2. Surgical gloves 3. Apron (chest, legs, fists) 4. Face shield, 5. Dental chair (backrest, light reflector) 6. Floor 7. Operator's clothes under the apron
Barlean et al., 2010	Ultrasonic scaling	30cm and 2.5m	cfu/m^3	429.6 cfu/m^3
Hallier et.al., 2010	1. Cavity preparation 2. History and examination 3. Ultrasonic scaler 4. extraction	20 cm	cfu/m^3	1. 105.1 cfu/m^3 2. 69.2 cfu/m^3 3. 70.9 cfu/m^3 4. 66.1 cfu/m^3
Timmerman et al., 2003	Ultrasonic scaling	1. 40 cm 2. 150 cm	Total CFU during conventional dental suction	Before: 0.6 0-5 min 40 cm: 2.5 20-25 min 40 cm: 1.8 0-5 min 150 cm: 4.3 20-25min 150 cm: 6.3 0-40min 150 cm: 4.0
Kedjarune et al., 2000	1. Endodontic 2. Operative 3. scaling	30-35 cm	The mean level of contamination before/during cfu/m^3	1- 264.16 / 270.29 2- 188.28 / 186.23 3- 245.10 / 182.57
D. Grenier 1995	Ultrasonic scaling	122 cm	The mean level of contamination cfu/m^3	Before: 12 During: 216 At the end:44 2 h after 10 4 h after 6
D. Grenier 1995	Operative treatment	122 cm	The mean level of contamination cfu/m^3	Before: 14 During: 75 At the end: 51 2 h after 12 4 h after 9

UR: upper right. LR: lower right. WIBS: wideband integrated bioaerosol sensor. OPS: optical particle sensor RFU: relative fluorescence units. CFU/m^3: colony forming units per cubic metre. SD: standard deviation. CFU/cm^2: colony forming units per square centimetre. TVC: total viable count

2.2.3. Air Contamination

Air contamination was previously examined by opening agar plates in different places in all the dental operative rooms (Ionescu et al., 2020). Active and passive air sampling was also used. The former was measured by BioSampler®, which determined the microbial load per cubic meter, whereas the latter was measured using blood agar or Reasoner's 2A (R2A) agar. The air samples were placed on the patient's chest, next to dental instruments, and 150 cm away from the patient's oral cavity (Zemouri et al., 2020). Interestingly, it is known that the pathogen's size determines the size of the particle. For example, particle size is much more prominent when it carries bacteria than viruses. However, in dental practice, it may not be the same, as the mechanical production of aerosols and spatters depends on the function of each handpiece. The ability of these particles to carry any pathogens in the nose, throat, and respiratory tract (Ionescu et al., 2020). Regarding the aerobic and anaerobic contamination in the dental clinic, Zemouri et al. (2020) found no significant difference between the aerobic and anaerobic counts during the treatment. However, unsurprisingly, the aerobic count was significantly higher than the anaerobic before and after the treatment.

A scoping review including some of the studies we have mentioned and many others that assess the amount of aerosol generated in dental clinics. Fifty-one studies were included in Nóbrega et al. (2021) scoping review. Microorganisms were discovered in most experiments. The article searches also included searches for bloodstains, aerosol spills, and airborne particles. Several dental setting types for dental treatment were utilised in the included investigations. Hospital dental clinics, dental offices or

clinics with or without ventilation systems, probable open bay dental clinics, single dental chair clinics, and in laboratories for vitro research.

In most of the studies, they have used standard units of volume of particles or colonies of microorganisms to measure the aerosol generated. The measurements were done before, during, and after the treatment. Most studies identifying the bacterial colonies found the number of staphylococcus and streptococci species to be significantly higher. Compared to what was seen before and during treatment, the microbial contamination of the air caused by dental work was more substantial. The contamination from aerosol and splatter varies depending on the technique, instrument, and suction device type. High-speed dental drills, ultrasonic scalers, piezoelectric ultrasonic scalers, mechanical scalers, and triple syringes produced more significant amounts of oral bacteria (Nóbrega et al., 2021).

It was discovered that the aerosol dispersal is stronger and more uniform on neighbouring surfaces from the mouth, and it may be felt up to two metres away from the patient's mouth and 40 cm from the work area. The type of dental care affected how quickly bacteria spread. Colony forming unit (CFU) counts during endodontic and restorative dental procedures were substantially higher at 0.5 than at 2 m. Microbes were discovered on PPE, such as sleeves, masks, scrub jacket chests, and face shields. This demonstrates the need to use PPE and cleaning techniques to avoid contaminating dental employees (Nóbrega et al., 2021).

By dispersing microorganisms throughout the dental clinic's environment with the help of the air conditioning system, bacterial aerosol could travel far from its original location, posing a severe risk to dental staff members as well as to patients, operators,

assistants, and the area where operations are being performed (Nóbrega et al., 2021)—summary of the included studies in (Table 2.5.).

Table 2.5. Summary for the included studies for a scoping review extracted from (Nóbrega et al., 2021)

Author, Year	Study type	Collected Data
Adhikari, 2017	Cross-sectional	Bacteria
Aguilar-Duran, 2020	Cross-sectional	Haemoglobin
Allison, 2021	Experimental model	Splatters of the aerosol
Azari, 2008	Cross-sectional	Bacteria
Bennett, 2000	Cross-sectional	Bacteria
Bentley, 1994	Experimental model	Bacteria
Chuang, 2014	Experimental model	Bacteria
Cristina, 2008	Cross-sectional	Haemoglobin
Discacciati, 1998	Experimental model	Splatters of the aerosol
Divya, 2019	Cross-sectional	Bacteria
Dutil, 2009	Cross-sectional	Bacteria
Ghiabi, 1998	Cross-sectional	Bacteria
Greco, 2008	Cross-sectional	Bacteria
Grenier, 1995	Cross-sectional	Bacteria
Guida , 2012	Cross-sectional	Bacteria
Gund, 2020	Cross-sectional	Bacteria
Harrel, 1998	Vitro	Splatters of the aerosol
Holliday, 2021	Experimental model	Splatters of the aerosol
Huntley, 1998	Cross-sectional	Bacteria and Fungi
Ishihama, 2008	Cross-sectional	Blood
Jimson, 201	Cross-sectional	Bacteria
Kedjarune, 2000	Cross-sectional	Bacteria
Kobza , 2018	Cross-sectional	Bacteria and Fungi
Krogulski , 2010	Cross-sectional	Fungi
Labaf , 2011	Cross-sectional	Not specified
Legnani, 1994	Cross-sectional	Bacteria
Manarte-Monteiro, 2013	Cross-sectional	Bacteria
Matys, 2020	Experimental model	Aerosol
Micik, 1969	Cross-sectional	Bacteria
Motta, 2007	Cross-sectional	Bacteria
Nejatidanesh, 2013	Cross-sectional	Face shield contamination
Nunes, 2018	Cross-sectional	Fungi
Osorio, 1995	Cross-sectional	Bacteria and Fungi
Pasquarella, 2012	Prospective cohort study	Bacteria
Perdelli, 2008	Cross-sectional	Haemoglobin
Pina-Vaz, 2008	Cross-sectional	Bacteria
Polednik, 2014	Cross-sectional	Bacteria and Fungi
Prospero, 2003	Cross-sectional	Bacteria
Rautemaa , 2006	Prospective cohort study	Bacteria
Rupf, 2015	Cross-sectional	Bacteria
Shivakuma, 2007	Prospective cohort study	Bacteria
Singh, 2016	Cross-sectional	Bacteria
Smolik, 2011	Prospective cohort study	Aerosol
Sotiriou, 2008	Cross-sectional	Particles
Szymanska, 2005	Prospective cohort study	Bacteria
Timmerman, 2004	Prospective cohort study	Bacteria
Toroglu, 2001	Case-control	Bacteria
Veena, 2015	Pilot study	Splatters of the aerosol
Watanabe, 2018	Cross-sectional	Bacteria
Yen-Tseng, 2013	Cross-sectional	Bacteria
Zemouri, 2020	Cross-sectional	Bacteria

2.3. Reduction of air contamination

Decreasing the air microbial contamination in dental clinics is crucial. That could be by adequate air ventilation and lowering the contamination of DUWLs (Zemouri et al., 2020, Harrel and Molinari, 2004). One of the significant ways of mitigating air contamination is air management techniques. The techniques are divided into two criteria, the first one is quantitative, and the second one is qualitative (Rathi et al., 2022). The quantitative management is laminar airflow, transparent extraoral barrier, controlled air pressure handpiece, and high-volume evacuator (HVE). The qualitative managements are ion-based air purifiers, photon-based air purification, filter-based air purification, gas-based air disinfectant, and aerosol-based air disinfection (Rathi et al., 2022). Reduction of aerosols by the usage of the HVE rather than using a saliva ejector (SE) is recommended by different studies, as it could reduce the concentration of the particles by up to 90% (Agostini-Walesch et al., 2021, Harrel and Molinari, 2004). Fennelly et al. (2022) have studied different mitigation processes to reduce air contamination. They compared the use of HVE and local exhaust ventilation (LEV) in non-mechanically ventilated clinics to minimise the dissemination and persistence of inhalable aerosol particles during dental AGPs. Table 2.6. sums up some of the risk reduction strategies to reduce aerosol contamination.

Low-volume suction systems can show a significant reduction in the level of PM_{10} with a real-time monitor. While the efficiency with ultrafine particles (< PM_{10}) was not that noticeable (Rexhepi et al., 2021). The current investigation by Rexhepi et al. (2021) shows that numerous parameters in daily clinical practice, such as ventilation, operation type, or standard saliva ejectors, might impact the overall concentration of PM generated during dental treatments.

Table 2.6. The Effectiveness of Risk Reduction Strategies

Author Name	Year of Publication	Procedure	Risk Reduction Strategy	Technique of Measurements	Decreases in TVC or Total Contamination
Fennelly et al.	2022	Drilling UR6 and LR6 Scaling upper and lower jaw	HVE	Particles / cm^3	UR6 -OPS: from 28.08 to 3.37 -WIBS: from 69.34 to 1.63 LR6 -OPS: from 7.98 to 3.18 -WIBS: from 139.15 to 1.92 Scaling -Upper from 7 to 1.33 -Lower from 3.78 to 1.58
Fennelly et al.	2022	Drilling UR6 and LR6 Scaling upper and lower jaw	LVE	Particles / cm^3	UR6 -OPS: from 28.08 to 3.72 -WIBS: from 69.34 to 1.61 LR6 -OPS: from 7.98 to 4.01 -WIBS: from 139.15 to 2.18 Scaling -Upper from 7 to 1.44 -Lower from 3.78 to 1.91
Holliday et al.	2021	Crown preparation	Medium volume suction + cross ventilation	RFU	From 700.000 RFU to 350.000 RFU
Maurais et al.	2021	Non-AGPs (Using low-speed hand piece electric-driven)	Air cleaner device (ACD) and portable ambient (PA) ACD.	10 µm particulate matter in size or smaller (PM_{10})	1. Chairside ACD 1259 PM_{10} ($\mu g/m^3$) 2. Chairside ACD + PA ACD 925 PM_{10} ($\mu g/m^3$)
Maurais et al.	2021	AGPs (Using high-speed hand piece air-driven)	Air cleaner device (ACD) and portable ambient (PA) ACD.	10 µm particulate matter in size or smaller (PM_{10})	1- Chairside ACD 1671 PM_{10} ($\mu g/m^3$) 2- Chairside ACD + PA ACD 1206 PM_{10} ($\mu g/m^3$)
Teichert-Filho et al.,	2020	Standardized simulated dental procedures	Translucent acrylic chamber	Using ultraviolet flashlight illumination, the dye was observed	1- The surgical gloves 2- Apron, 3- Internal walls of the acrylic chamber.
Hallier et al.	2010	1- Cavity preparation 2- History and examination 3- Ultrasonic scaler 4- Extraction	Air Cleaning System (ACS)	Cfu/m^3	1- 38.4 cfu/m^3 2- 59.8 cfu/m^3 3- 38.5 cfu/m^3 4- 37.0 cfu/m^3
Timmerman et al.	2003	Ultrasonic scaling	High volume evacuation (HVE)	Total CFU during high volume evacuation	Before: 0.2 0-5 min 40 cm: 0.4 20-25 min 40 cm: 1.6 0-5 min 150 cm: 5.4 20-25min 150 cm: 2.7 0-40min 150 cm: 8.1
Kedjarune et al.	2000	Endodontic	Rubber dam	The mean level of contamination before/during cfu/m^3	264.16 / 270.29

2.3.1. High Volume Evacuator

HVE and extraoral suction are designed to control water and reduce aerosols to avoid spreading disease (Kohn et al., 2003). It might be more practical to eliminate the contaminated airborne as soon as they leave the mouth of the patient by using HVE as recommended by Harrel and Molinari (2004). The aerosol-containing air evacuated after they leave the oral cavity is disinfected by HVE equipped with HEPA filters and UV light. They feature a large bore, which allows them to remove more air in less time, reducing bioaerosols by up to 98% (Hallier et al., 2010; Lee et al., 2004). They pointed out that the opening of HVE should be 8 millimetres or more and ought to remove a large volume of air (which might reach 100 cubic feet of air/min). The saliva ejector (SE) cannot be confedered as HVE because of the small opening that will limit its ability to evacuate enough air. With four-handed dentistry, it is easier to use the HVE and to handle it by the assistant correctly to eliminate as many particles as possible during the procedure. The only disadvantage that might encounter with HVE is when the dental hygienist is working alone, it is hard to handle the HVE, and it is necessary to attach the HVE to an instrument or a dry field device (Harrel and Molinari, 2004). Nevertheless, hands-free HVE has been found to prevent operation site contamination by over 90% (Fennelly et al., 2022).

Comparisons between SE and HVE were made by Agostini-Walesch et al. (2021). They used dental stimulation unit (DSU) and mixed methylene blue dye with water to detect the contamination around the dental unit during the ultrasonic scaling procedure. The procedure ran 5 min for the two tests, the first ultrasonic scaling with SE and the second with HVE. Collection papers were placed around the dental unit and left for 10 min more after the procedure to allow the particles to settle down and

dry. Agostini-Walesch et al. (2021) noticed significant differences in the particle count, travel distance, and direction.

First, starting with the regular employment of SE, 166,137 particles were detected in the distance up to 43.5 - 45.6 inches. Regarding the direction of the contamination, the opposite side to the operator recorded the highest number of particles concentration as it is on the direction of the fluid and airflow. However, only 10% of the engagement was detected on the operator side. The distribution declined with further distance. Near the unit, the particles were higher in number. On the other hand, when HVE was used alone, more particles were removed, 1.655 particles were recorded, and the reduction is near 99% compared to SE.

Additionally, the contamination of the particles in respect of the travel distance of almost half the length was reduced (22.5 – 25.5 inches). Finally, the particle concentration was slightly different from the SE. The highest is the same as in the SE on the opposite side of the operator. Nevertheless, a higher concentration was found on the operator side (21%). In both cases, staining was detected on the operator's face mask and face shield, and higher concentrations of particles were recorded on the patient’s head between the four and six o’clock positions. Over four feet in distance from the patient mouth, no evidence of contamination was found in both tests (Agostine-Walesch et al., 2021).

A similar study also has compared the effectiveness and the difference in aerosol elimination by using HVE and LEV during drilling and scaling. Fennelly et al. (2022) have used two different devices for counting the particles produced during dental

treatment. He used OPS and WIBS when drilling for UR6 and LR6 and scaling for the upper and lower jaws.

The HVE had shown a significant reduction in particle removal compared to no suction during drilling of UR6 and LR6 to 99% and 90%, respectively, when OPS was used. With WIBS, the reduction was 94% and 95%, respectively. The drop of particle counts with HVE in the scaling procedure comparing no suction was 82% in the upper jaw and 50% in the lower one by WIBS. Likewise, when LEV was installed, the elimination of particles was close to HVE with no significant difference. The OPS count for UR6 was 99% and 93% for LR6, and the WIBS for both was 95%. During scaling, the WIBS counts were lower than HVE but significant, 74% reduction in upper arch scaling and 41% in the lower one compared to no suction (Fennelly et al., 2022).

For two fundamental reasons, extra-oral suction (EOS) or Isovac (ISO) is more suitable than HVE. The first reason is that they can be used effectively without a dental assistant and are helpful when the dental nurse is unavailable, like in a dental school. The second one is reducing the number of people in the dental clinic; these self-operating devices may reduce the risk of disease transmission between dental staff and patients (D'Antonio et al., 2022).

Employing EOS or ISO during dental treatment to mitigate air contamination can be, at any rate, equal to HVE. To support this statement D'Antonio et al. (2022) conducted a study to demonstrate the effectiveness of these devices individually. He experimented with two phases; the first was on a manikin during different dental treatments like high-speed handpiece, air-water syringe, ultrasonic scaler, and rubber

cup polishing. Each procedure was set for 10 min: using various ventilation controls like HVE, ISO, and EOS. The second phase was done at an open dental clinic with varying occupancy rates, the first day at 25%, the second day at 50%, and the last day at 100%. Even though they couldn't reach 100%, they stimulated the number by repeating the 50% and getting them closer to each other. Thus, they ended up with 63%. Two separate respirable samplers were used, Sidepak and pDR.

The main finding was that the rubber cub polisher generated a lower concentration in contrast to other procedures, and a higher number was seen in the ultrasonic scaler. However, no significant difference was seen between the ventilation controls. That was regarding the first phase. In the second phase, an interesting effect was discovered when they studied the relationship between dental unit occupancy and contamination in open dental clinics. The aerosol concentration when the occupancy was 25%, 50%, and stimulated 100% as follows, 0.09, 1.43, and 2.97, respectively, all in $\mu g/m^3$ of the background and 0.13, 0.82, and 2.62, respectively, during the procedures. The dissimilarities in the aerosol concentration as a consequence of the number of EOS were used, as there were 13 active EOS when the occupancy was 25% and only 20 EOS at 50% occupancy. Additionally, in background-adjusted methodology, when patients were clustered together without any open operating rooms in between them, respirable aerosol concentrations were more sumptuous than when they were spaced apart (D'Antonio et al., 2022).

Most of the mentioned trials were conducted outside actual patient care and needed the requisite real-time particle observation to establish the impact of these therapies. Therefore, Choudhary et al. (2022) experimented with the effect of aerosol mitigation

strategies during different dental treatments in different departments configurations in paediatric, general dental operatories had a single dental unit in the room, endodontic and periodontic departments had a semi-private clinic with partial barrier divider separating the dental units and a large open bay clinic for orthodontic. Choudhary et al. (2022) examined the risk to the dentist and the dental assistance during treatment using various evacuators. This study used HVE, conical tip HVE, and ISOVAC HVE as mitigating techniques. Also, for particle observation, he utilises MINIMA wearable sensor that the doctor wore to measure the contamination directed to the dentist; and an optical aerosol spectrometer 20 cm from the patient's mouth.

Starting with higher emission of aerosol was seen during high-speed drilling in orthodontics (340 particles/cm^3) than using the same handpiece in endodontics (170 particles/cm^3) and paediatrics clinics (30 particles/cm^3). The lowest was in the periodontics clinic during ultrasonic scaling (15 particles/cm^3) and using standard-tip HVE. On the other hand, when they used conical HVE, a decline in the particles was recorded versus standard-tip HVE. Like conical HVE, ISOVAC with HVE showed a significant decrease in aerosol emissions, even more than conical HVE alone (Choudhary et al., 2022).

Another interesting finding from Choudhary et al. (2022) experiments is that intermittent ultrasonic scaling can be lower than continuous scaling. Hence, any physician or dental hygienist who expects to do ultrasonic scaling for an extended time may want to switch the scaler off occasionally during regular treatment to enable aerosols to disperse. Apart from this, the dental clinic configuration can also affect contamination exposure. Closed single dental units have higher $PM_{2.5}$ concentration

(350 µg/m^3) compared to open bay dental clinics (50 µg/m^3) during high-speed drilling (Choudhary et al., 2022).

Graetz et al. (2021) also studied how effective EOS can be during dental treatment and which particle size would be eliminated. A Manikin head with artificial teeth was used in this experiment. They have studied the effectiveness of the following particle sizes (0.1, 0.15, 0.20, 0.25, 0.3, 0.5, 1.0, 5.0 µm) using SE and HVE with and without EOS. There is no baseline level for particle assessment. As a result, only the difference in PN concentration is significant. The EOS showed a substantial reduction in smaller particles with diameters of 0.1 to 0.3 µm rather than particles sized 0.5 µm and above. This finding could be not because the EOS is not competent to eliminate larger particles but due to the usage of SE and HVE. According to Rexhepi et al. (2021), the HVE are more effective in reducing larger particles as they are immediately concentrated as they leave the mouth and can't travel long distances, unlike smaller particles <0.5 µm.

2.3.2. Rubber Dam

The rubber dam is a one-use sheet wrapped over the treated tooth to free the treatment zone saliva. Most dental clinics and hospitals regard using a rubber dam during restorative and endodontic procedures as the standard of care. Its usage has been linked to increased rates of dental treatment success (Al-Amad et al., 2017).

Balanta-Melo et al. (2020) have discussed in their experiment the effectiveness of the rubber dam in mitigating fine and ultrafine particles produced during dental treatments. The investigation was a complete anterior crown preparation on a mannequin's mouth.

They read the particles during three different scenarios using a laser diffraction technique. The first scenario was while using standard suction, the second one was by using a rubber dam and standard suction, and the last method was using the second intervention plus HVE.

Bleanta-Melo et al. (2020) studied particle sizes $PM_{0.1}$, $PM_{2.5}$, and PM_{10} in this experiment. They concluded a significant reduction of ultrafine ($PM_{0.1}$) and coarse (PM_{10}) particles when the rubber dam was used alone or with HVE, compared to standard suction on the vestibular side. However, on the palatal side, only rubber dam with HVE has shown a significant difference in particle mitigating (Bleanta-Melo et al., 2020).

A previous study was also conducted by The University of Sharjah in 2017 on rubber dam efficiency in lower air contamination. In this study, they examined the contamination of the female dental students' headscarves during dental procedures with and without placing a rubber dam. Forty-seven students were assigned to this study, dividing them into two groups. The first group did a dental treatment with a rubber dam (25 students), and the other group without it (22 students). Sterilised headscarves were given just before the procedures to ensure no contamination could happen (Al-Amad et al., 2017).

Five points were localised for contamination, as shown in (Figure 2.2.). A swab will be taken from these areas and sent to Microbiology Department. After a restorative procedure or inlay preparation, a swab was taken from the assigned points (A, B, C, and D). Unpredictable funding was seen in this experiment. Significantly higher CFUs

were experienced in procedures with rubber dams than without them. Despite the effectiveness of the rubber dam during dental procedures and its crucial rule in obtaining a good restoration by isolating from saliva and blood, this study has found that the rubber dam group has higher bacterial aerosol levels than the non-rubber dam group. Nevertheless, the study has some limitations as all the participants were students with limited experience. Also, the treatment type or location if it was in the maxilla or mandible jaw (Al-Amad et al., 2017).

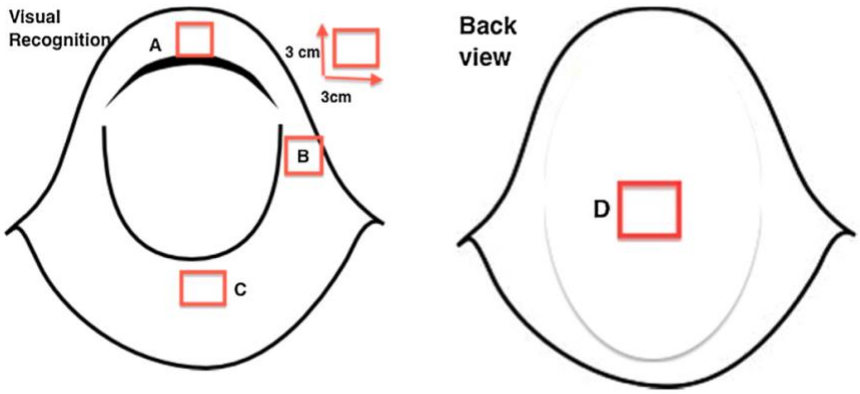


Figure 2.2. The visual guide used by researchers to collect samples from the scarves using swabs

2.3.3. Air Cleaning System

Without a doubt, ventilation systems significantly limit airborne microbial transmission in dental operating rooms (Chen et al., 2009; Gao et al., 2016). Various elements must be addressed in the design and installation of the ventilation system to provide successful infection control. The ideal temperature setting for clinical spaces is 21°C to 24°C, with relative humidity between 40% and 60%, ACH between 6 and 12, and airflow passing from clean to less clean places. Humidity is crucial in preventing the spread of droplets and certain airborne viruses (Yang and Marr, 2011). The most effective air movement pattern for minimising contamination in the operating room is to provide air from the ceiling through the laminar unidirectional downward flow to the

operation area and then to multiple exhaust/return opening grills situated low on opposing walls. Dual low and high exhaust grills may outperform single low or single high exhaust grills (Memarzadeh, 2012).

Although portable air cleaners utilise a variety of technologies, including fibrous media air filters, which are commonly referred to as HEPA or Ultra-Low Particulate Air filters (ULPA), ultraviolet air filtration, and electronic air cleaners, which include electrostatic precipitators and ionisers, either alone or in conjunction (EPA, 2022), particles are captured on fibrous filter materials and removed using fibrous media air filters. Electrostatic precipitators and ionisers use an active electrostatic charging technique to remove particles. Ultraviolet (UV) air filtering kills or deactivates live airborne bacteria. Furthermore, some examples of gas-phase air-cleaning technologies include adsorbent air filters like activated carbon, chemisorbed media air filters, photocatalytic oxidation, plasma, and ozone producers. These remove or transform gaseous air contaminants into harmless forms (EPA, 2022).

According to several studies, the performance of various portable air cleaners varied from 12 to 99% based on the technology employed, the location, and the results evaluated (Secretariat, 2005). Theoretically, it stands to reason that reducing indoor airborne particle concentrations and microbial populations would decrease illness rates.

HEPA filters are designed to remove at least 99.97% of dust, pollen, fungus, bacteria, viruses, and other airborne particulate particles (Vijayan et al., 2016). These filters resemble a pleated fibre net and are manufactured in grades ranging from 10 to 17,

based on their filtering rate and particle size. 13 and 14 are considered medical grade filters, with retention percentages of 0.05 and 0.005 for 0.1µm particles/litre of air, respectively (First, 1998). Tiny particles, such as corona viridian, diffuse through Brownian motion, striking the fibres and being lodged in the filter (Van Durme et al., 2007). Air-purifying systems with HEPA filters capture particulate matter, but there is no disposal. As a result, HEPA systems are typically equipped with UV light for viral disinfection (Secretariat, 2005). Compared to HEPA, ionic and electrostatic room air purifiers give relatively little advantage (Wang WH., 2003). Thus, HEPA filters are becoming more popular for obtaining clinical benefits (Sublett, 2011).

HEPA filters and UV chambers are the most mentioned method for helping the ventilation system and lowering contamination. Despite the effectiveness of both scenarios, their cost could be unaffordable for most dental offices. Also, both approaches need a long period to filter or treat the air through the UV system in dental clinics (Harrel and Molinari, 2004). Furthermore, HEPA filters are solely effective after infective particles have travelled a certain distance in the air. Eliminating airborne at the source may be more advantageous (Fennelly et al., 2022).

Hallier et al. (2010) investigated an air cleaning system (ACS) device, significantly reducing airborne contamination during dental treatments, such as cavity preparation, ultrasonic scaling, and tooth extraction. A study was conducted by Maurais et al. (2021) to evaluate the effectiveness of using air cleaner devices (ACD) and portable ambient (PA) ACD, both located by the chairside. They compared the air contamination in different scenarios, the first with the personal exposure (dentist and dental assistant) in AGPs and non-AGPs while both ACDs are off, chairside ACD only,

and chairside ACD + PA ACD. The findings when both ACDs were off are mentioned in point (2.2.1). For the latter two, when only ACD was on, the results were as follows: during AGP for the dentist exposure, the contamination was 1104 PM_{10} ($\mu g/m^3$), and for the assistant, 1244 PM_{10} ($\mu g/m^3$), while during non-AGP the count was 908 PM_{10} ($\mu g/m^3$) for the dentist and 1386 PM_{10} ($\mu g/m^3$) for the assistant.

In the last experiment, when both ACDs were active, there was a significant reduction in air contamination for the dentist (739 PM_{10} ($\mu g/m^3$) in AGP and 544 PM_{10} ($\mu g/m^3$) in non-AGPs) but slightly lower for the assistant (1244 PM_{10} ($\mu g/m^3$) in AGP and 1201 PM_{10} ($\mu g/m^3$) in non-AGP). The second scenario is ambient air contamination, with the same tenancies and interventions. The finding with both ACDs was off, are uttered in point (2.2.2.). The count of air contamination, while only ACD is on in AGP, was 1671 PM_{10} ($\mu g/m^3$) and 1259 PM_{10} ($\mu g/m^3$) in non-AGP, whereas both ACD and PV ACD were on the count were highly reduced to 1206 PM_{10} ($\mu g/m^3$) in AGP and 925 PM_{10} ($\mu g/m^3$) in non-AGP. It is worth noting that returning ambient air PM_{10} levels to background levels by reactivating the portable ambient air and chairside ACDs took 7.5 min after an AGP, which might help in reducing the fallow time. In contrast, without any ACDs, it took over 30 min for the ambient air PM_{10} levels to return to the background levels (Maurais et al., 2021). Using portable air cleaners (PAC) is

promising as it has dramatically impacted aerosol concentration in dental clinics, with or without air ventilation.

He et al. (2022) have studied the usefulness of increasing ACH to 15 by employing an air purifier with a HEPA filter with no air circulation but in a single-patient treatment clinic and on a mannequin. At first, an ultrasonic scaler and high-speed handpiece were examined to determine which would generate more aerosols. The experiment occurred during AGPs with HPEA and HVE working together or separately. Then the finding was compared with the control group, where no AGPs were running, only the existence of the dentist and the assistant. Aerosol concentration was measured using a particle counter (Optical Particle Sizer 3330, TSI Incorporated, USA).

After examining which instrument would generate more aerosol, the high-speed handpiece produces 181.5 particles/cm^3. This number was significantly higher than the control group, with only 5.4 particles/cm^3. In contrast, the ultrasonic scaler generated only 4.4 particles/cm^3. Thus, the high-speed handpiece was chosen for this study (He et al., 2022).

The procedure was crown preparation for the right lower first molar. The reduction when the HVE was used reached 94.8% (9.8 particles/cm^3), and similar results were seen with the air purifier. Noteworthy, combining HVE and air purifiers would reduce the contamination to 99.6 % (0.8 particles/cm^3) (He et al., 2022).

An experiment done by Ren et al. (2021) showed that mechanical ventilation with high ACH (ACH ≥15) would decrease the aerosol concentration faster along with PAC, especially when the ACH is low (ACH ≤6) significant decrease in the concentration would be seen when the PAC is on. Measuring the effectiveness of mechanical ventilation alone has indicated a reduction in aerosol concentration depending on the ACH in each clinic. At 15 ACH and higher, 100% of aerosol particles were removed after 30 min. However, only 96.4% removed the aerosols in clinics with 6 ACH after the same time. A significant difference was noted when removing the aerosol particles after implementing an additional device with mechanical ventilation.

A PAC was used alongside the air ventilation at different rates (poor ventilation 3-4 ACH, high ventilation 6-13 ACH, and most increased ventilation 15-32 ACH). They examined the efficacy of PAC alone compared to 15-32 ACH; they noted that PAC needed only 5-11 min to remove the aerosols while the mechanical ventilation was off. Unlike the highest mechanical ventilation, the aerosols were drawn for 10 to 30 min. Figure 2.3 explains the differences when different rates of mechanical ventilation were working alone or in combination with PAC. The low rate of ACH (less than 6 ACH) will never reach 100% of removing aerosols by itself, but with PAC, 100% removal of 0.3 μm aerosols was done in the first 5 min.

In contrast, a higher ACH rate will reach 100% removal of aerosols, which would take 10 to 30 min depending on the rate of the ACH (Ren et al., 2021). PAC will be more efficient when used with a low ACH rate, showing a significant difference in the time and percentage of removing the aerosol particles. Moreover, the location of the PAC

and air vents will play an essential role in aerosol removal. The ventilation effect upon air contamination is shown in (Figure 2.3).

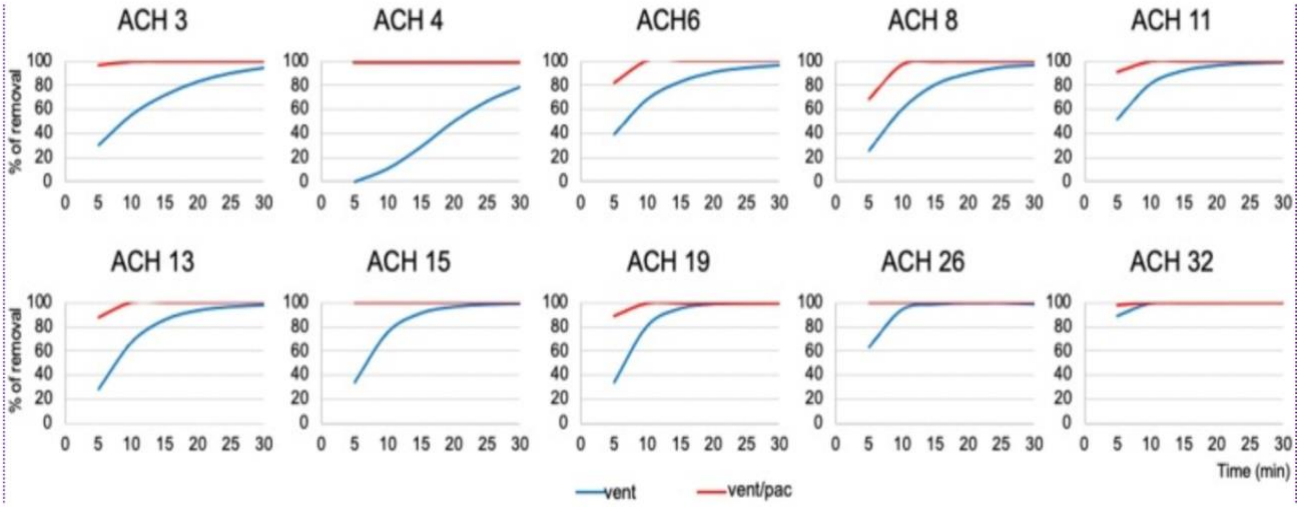


Figure 2.3. The efficiency of removing aerosol particles is 0.3μm by mechanical ventilation alone or with PAC in a different ACH in 5, 10, 15, 20,25, and 30 min (Ren et al., 2021).

One study has examined the usage of PAC but not in dental or hospital settings. It was in the rooms of patients who tested positive for SARS-CoV-2. For air sampling, an MD8 Airport Portable Air collector (Sartorius AG, Gottingen Germany) with gelatine membrane filters was used, and swabs were done. Dyson PAC with HEPA filter was used according to the manufacturer instructions, which claimed that the device could clean up to 99.95% of air particles as low as 0.1 μm in a room measured 27 m^2 in size or less. The device was placed in the centre of the room, which did not have adequate ventilation when the heaters were on, and all the doors and windows were closed

(Rodríguez et al., 2021). Rodríguez et al. (2021) placed the device in different rooms that varied in size (13 to 60 m^2). All had positive SARS-CoV-2 after 2 h of sampling.

Interestingly, the rooms were subsequently cleaned, and the sampling for SARS-CoV-2 was negative, but only one room was still positive. This room was over the recommended size (60 m^2). Therefore, the PAC could have been more effective in that situation. As a consequence of this experiment, consider using PAC with HEPA filter in places without sufficient air ventilation or when it is not easy to achieve the needed ventilation, for instance, in schools, commercial buildings or meeting rooms. The use of PAC is promising, but more and more extensive studies should be considered in the future for more evidence.

There is even a difference in the type of ventilation used at the dental clinic and their effect on air contamination. Vonci et al. (2019) did a cross-sectional study to note the differences between laminar airflow ventilation systems and turbulent flow ventilation in operatory rooms. The laminar flow pattern occurs once the airflow is smooth, exhibiting a parabolic velocity profile (Rathi et al., 2022). Despite the limitation in this study, as the amount of collected data was restricted for laminar airflow, the CFU per cubic meter of air for laminar airflow was 30 and 50 in turbulent flow ventilation. Both ventilations showed contamination lower than 180 CFU/m^3 with 15 ACH. However, for the laminar airflow to be sufficient in the operatory room, they should increase the ACH where the CFU remain below 20 (Vonci et al., 2019).

The remarkable impact of the PAC is not limited to the reduction of the particle number but also from the microbiological point of view, as confirmed during a study conducted

by Cappare et al. (2022). This study found that the PAC device reduced particle contamination by 64-85% at rest, 49-73% during professional dental hygiene activities, and 76-83% during minor surgical procedures for particle size (0.3, 0.5, 1.0, and 5.0 µm). Moreover, the PAC's efficacy is also proven microbiologically, decreasing from 69% to 80% in professional oral hygiene activity and 62 66% in essential surgical activity. The microbiological contamination decreased during dental hygiene from 161 CFU/m^3 for proper plates to 49 CFU/m^3 and from 204 CFU/m^3 to 40 CFU/m^3 for left plates when the PAC was employed (Cappare et al., 2022).

The amount of carbon dioxide (CO_2) in inhabited interior spaces is a significant measure of ventilation. CO_2 is a by-product of human metabolism that may be found in high concentrations in exhaled air. Because CO_2 is inert, its indoor emission source (human), and measurement is simple and reliable, it is frequently employed as a proxy for indoor air quality and a risk marker for transmission of airborne infections (Batterman 2017). Poor ventilation and overpopulation are commonly connected with elevated CO_2 levels (Huang et al., 2021). Huang et al. (2021) studied different ventilation parameters in dental treatment clinics. The lowest ACH was 3.9, and 35 ACH was the highest. No treatment was done, only rooms occupied by people.

The data revealed that CO_2 levels in rooms with ventilation rates less than 6 ACH can regularly remain over 800 ppm, especially when three or more people (including the patient not wearing a mask) are present during dental treatment. In clinical teaching, it was noticed that CO_2 levels remained above 1,000 ppm and surpassed 1,600 ppm when 3 to 6 people were in a room with 3.9 ACH. CO_2 levels remained consistently below 700 ppm in most dental operatories with ventilation rates greater than 10 ACH

occupied by three people in the room. Hence, crowding and a poor ventilation rate were linked with CO_2 build-up (Huang et al., 2021). Moreover, Azuma et al. (2018) stated that at 700 ppm, CO_2 tends to have negative health consequences, and respiratory symptoms may arise once indoor CO_2 levels exceed 1,000 ppm.

Several extraoral HVS has been brought to the market in recent years, including ADS and Tokyo Giken. The ADS unit features a motor-driven high-power suction, a HEPA filtering system, and a disinfectant system by UV-C light, providing extra aerosol reduction and air disinfection in the dental operatory (Yang et al., 2021).

A mock treatment using a high-speed handpiece on an actual patient to imitate dental aerosols, which are made of compressed air, water, and patient saliva, was conducted by Yang et al. (2021). They aimed to study aerosol concentration differences with and without HSV as an external air cleaner. The baseline averages 250 pts/cm^3 with (PTrack) and 144 pts/cm^3 (AeroTrack 0.3 channel). Various locations for contamination were detected, like the patient's chest, 3 feet above the patient, the shoe cover of the dentist, the floor by the dentist's stool, the assistant's chest and the dentist's chest. The dentist's chest showed the most apparent drop. The aerosol decreased from 967 without to 274 with points per cubic centimetre (PTrack) and from 712 without HSV to 107 with HSV points per cubic metre (AeroTrack 0.3 channel). All the other locations detect a lower level of contamination, even at the baseline level or even lower, regardless of the use of HSV. In some circumstances, employing HVS can reduce aerosol concentration even lower than the baseline. In addition, it was noteworthy to learn that the assistant side had far less aerosol than the dentist. This might be because both SE and HVS were approached from the nurse's side, and the

slanted angle of the SE tip and HVS suction mouth resulted in a somewhat different suction power (Yang et al., 2021).

Altogether, evaluating the use of portable air-cleaning systems in medical facilities and the outcome from different studies, Alvarenga et al. (2022) conducted a scoping review regarding these matters. They have included 24 articles in the review; various microbes were detected, including SARS-CoV-2. Many different techniques were embraced in the study with the significant outcome of reducing airborne in medical and dental facilities. The healthcare facilities evaluated were dental clinics, patient wards, operating rooms, intensive care units, single-bed patient rooms, and emergency rooms. Teaching hospitals and clinics in various countries were also included. Also, all the included experiments were conducted in situ in a real-life setting, assessing microbial airborne or aerosol particles. The studies, methods, and outcomes are summarised in (Table 2.7.).

2.3.4. Other Techniques

Installing a low-cost device with an aspiration and filter system provides a negative pressure, decreasing the dental clinic's environmental contamination (Teichert-Filho et al., 2020). The main idea of this device is to isolate the patient from the external (operatory) environment. The device is made of translucent acryl, designed to cover the patient's head, neck and chest and fit in the dental chair. For operator access, three oval-shaped openings allow the dentist to be ergonomic (9 to 3 o'clock). Two pipes are attached to the chamber for aspiration and filtering of the air. Then the aspirated air is forced to go through an outside box to be treated with an antiseptic solution (2% NaOCl) to be returned to the external environment without toxic particles.

Table 2.7. Summary of the scoping review for reducing aerosol contamination in medical and dental facilities, extracted from (Alvarenga et al., 2022)

Author, Year	Settings	Methodology	Indoor parameter	Outcome
Capparè et al.,2022	Dental OR (AGPs)	HEPA		A significant decrease in airborne (bacterial and fungal counts)
Oberst et al., 2021	OR	HEPA; activated carbon; Plasma		A significant reduction in particles count for all sizes measured
Arikan et al., 2021	ICUs	HEPA; activated carbon; plasma	T (°C): 20 - 25 RH: 30 - 60	A significant decline in airborne (bacterial totals)
Corrêa et al., 2021	Emergency care unit	UV-C lamps		A significant decrease in airborne (bacterial and fungal counts)
Maurais et al., 2021	COVID patients and procedures in dental office	HEPA; activated carbon	ACH: 6 - 13	A significant decline in particles values in all circumstances evaluated
Tzoutzas et al., 2021	Dental OR	UV lamp; silver Ion; plasma	ACH: 7 T (°C): 23 -26 RH: 30 - 60	A significant drop in particles for the length of the experimental period
Morris et al., 2021	ICU	HEPA; UV-C lamp		A significant drop of airborne (SARS-CoV-2 and other airborne pathogens detected)
Buising et al., 2021	Patient's ward (hospital)	HEPA; activated carbon		A significant drop of particles count
Lee et al., 2021	Hospital single room	HEPA; activated carbon	ACH: 13.9	The speed of airborne particle clearing was greatly increased (3 times faster)
Razavi et al., 2021	Dental OR	HEPA; UV-C lamps; activated carbon, PCO	ACH: 7.23 – 14.73 T (°C): 22 – 25 RH: 49 - 60	The speed of airborne particle clearing was greatly increased (at least 6.3 times faster)
Ren et al., 2021	Open dental bay	HEPA	ACH: 3 – 45 T (°C): 22 – 23 RH: 34 - 52	a considerable decrease in the build-up of airborne particles and a quicker clearance. Particularly noticeable in spaces with insufficient ventilation.
Verbeure et al., 2021	Room for oesophageal HRM	HEPA/ molecular		a marginal decline in the levels of airborne particles
Messina et al., 2020	ISO	HEPA; UV-C lamps; PCO	ACH: 15	a considerable drop in the values of all airborne particle sizes
Pouvaret et al., 2020	12-bed adult haematology unit	ULPA; UV-C lamps	ACH: 2 T (°C): 35	A significant decrease in airborne (bacterial and fungal counts)
Rao et al., 2020	Paediatric wards	PECO		Non-significant correlation between the utilisation of the ICU, intubation, nebulizer, and non-invasive ventilation and the shorter total period of hospitalisation.
Anis et al., 2019	OR	C-UVC chamber		A non-significant decline in airborne bacterial counts but a considerable drop in the values of all airborne particle sizes
Bischoff et al., 2019	Emergency rooms	C-UVC; chamber PCO		A significant decline in airborne (bacterial totals)
Ozen et al., 2016	Haematology ward	HEPA; UV-C lamps		substantial correlation with much lower infection rates overall.
Le et al., 2015	ICU	UV-A lamps; activated carbon; PCO		A significant decrease in airborne (bacterial and fungal sums)
Abdul Salam et al., 2010	Hospital	HEPA		Clearly correlated with the much lower frequency rate of aggressive aspergillosis infection
Hallier et al., 2010	Three separate dental OR	HEPA	T (°C): 21-24	A significant decline in airborne (bacterial totals)
Chotigawin et al., 2010	A renal unit	HEPA; PCO	RH: 74-76	A significant decrease in airborne bacterial counts only a non-significant reduction in airborne fungal
Pelleu et al., 1970	Three dental OR	HEPA		A significant decline in airborne (bacterial totals)

HEPA: High-efficiency Particulate Absorbing filter; OR: Operating Room; ICU: Intensive Care Unit; PCO: Photocatalytic Oxidation; UV: Ultraviolet; PECO: Photo-electrochemical Oxidation; C-UVC: Crystalline UV-C; ULPA: Ultra-Low Particulate Air filter; ACH: Air Change per hour; T: Temperature; RH: Relative Humidity; AGP: Aerosol Generator Procedure.

Teichert-Filho et al. (2020) measured the contamination by using dye added to the water of the dental unit. The dye was spotted on the surgical gloves, apron, the internal walls of the acrylic chamber, and inside the pipe system, significantly reducing contamination in the dental clinic environment. Even though the usage of this device will substantially lower the contamination of the operative environment but the major disadvantage of it is the limitation of the movement and clear vision for the dentist while dental procedures (Teichert-Filho et al., 2020).

An introduction to the use of a high-speed contra-angle handpiece (HSCAH) instead of an air turbine handpiece was discussed by Vernon et al. (2021). Besides using known mitigation strategies like high-volume aspiration with saliva ejection and rubber dam, an HSCAH was used in the dental clinic with 9 ACH. They substituted SARS-CoV-2 with bacteriophage Phi6, which is a like SARS-CoV-2, with a double-stranded RNA virus of 80 to 100 nm in size and constituted of a lipid membrane envelope plus spike proteins (Fedorenko et al., 2020). They also imitate the salivary gland with an artificial salivary flow of 1.5 ml/min. Endodontic access for the upper right molar and crown preparation for the upper left second incisor was done. Each procedure was repeated thrice for 20 min followed by 20 min fallow time.

For bacterial spreading detection, they have used passive and active sampling. Plates containing P.syringae were used for air sampling, and settled leaves were used to recognise either aerosol or droplets. The plates were positioned at the breathing zone, on the clinic floor, and as high as the bench in triplicate. Moreover, 2 MicroBio air sampling devices were used at 30 cm at either side of the oral cavity. The samples were taken during the AGPs and 6 min after the fallow time. Finally, Fedorenko et al.

(2020) utilised Kanomax 3889 GEOα as a particle counter. The device can monitor six different sizes simultaneously (0.3 μm, 0.5 μm, 1.0 μm, 3.0 μm, 5.0 μm, and 10.0 μm in diameter). They have used two, one positioned behind the dentist and the second between the door and the dental unit at 150 cm in height.

An exciting finding was provided form Fedorenko et al. (2020). Each mitigation decreased the bioaerosol levels collected from settle plates and air samples. High reduction in aerosol and splatter by using a rubber dam, which opposes the results from Al-Amad et al. (2017). It is worth mentioning that HSCAH reached a 100% reduction in settled aerosol and 99.98% in air samples compared with air turbines when used with high-volume aspiration. Combining the HSCAH with a rubber dam almost have the same effect as the high-volume aspiration, but settle plates and air sampling showed a 100% reduction in the aerosol sampling. In addition, post-AGP sampling was 21 plaque-forming units (pfu) and 11.25 pfu/m^3 for settle plates and air sampling, respectively, employing air turbine and high-volume aspiration. In contrast, both were reduced to zero when HSCAH was used.

Fedorenko et al. (2020) have also studied the mitigation concerning particle sizes. With the HSCAH versus air turbine, particle counts across all size ranges observed in earlier treatments was decreased, except for particle size 0.3 μm.

Apart from this, Fallow time can be implemented, reducing airbornes in the clinic environment until it is back to the baseline level (Fennelly et al., 2022). Also, the fallow time is designated to settle the suspended particles produced by AGPs. Estimating the length of fallow time, which is crucial for reducing the exposure hazards for dental

staff and patients, is a difficulty in the setting of a dental procedure (Clarkson et al., 2020). Uncertainty surrounds the necessity and duration of a fallow phase, with approximations ranging from 2 to 180 min (Ehtezazi et al., 2021; Shahdad et al., 2021). Furthermore, fallow time might differ depending on what type of procedure and what handpiece has been used. Fedorenko et al. (2020) measured the post-AGP period when air turbines and HSCAH were used. Rely upon particle size fallow time can differ. There was no difference in fallow time when 0.3 μm and 0.5 μm particle size were generated by either handpiece. Nevertheless, significant differences were seen for other particle sizes. The post-AGP period to reach baseline when air turbine was employed for particle size 1.0 μm, 3.0 μm, 5.0 μm, and 10.0 μm were 16.7, 14.1, 12.6, and 12.8, respectively. At the same time, the post-AGP period for HSCAH for the same particle size was 7.4, 4.3, 3.9, and 4.5, respectively.

According to a study by Ehtezazi et al. (2021), the fallow time can reach zero min when HVS companies with ACS are used. Even though the study was done on a phantom, they could return the particle to the baseline concentration after the operations when the ACS was equivalent to 24 ACH. Different procedures and dental instruments were used, such as an air turbine handpiece, a contra-angle handpiece, a three-in-one syringe, and an ultrasonic scaler (Ehtezazi et al., 2021).

Fennelly et al. (2022) during their experiment for mitigating airhorns in the dental clinic. He counted how long the fallow time should be to return to the baseline count using OPS and WIBS. It was found that unmitigated particle number ($PN_{2.5}$) needed 71 min to return to baseline, and with WIBS, it needed 126 min to return to baseline. However, with no suction, $PN_{2.5}$ never returned to the baseline level. In addition, they observed

that smaller particles (0.3-0.5 μm) took longer than larger particles after the procedure. Also, small-size particles will not return to the baseline level without the implementation of HVE and LEV, which was confirmed by this study.

Procedures in a confined, single-unit room had greater aerosol intensities than those in a more extensive, open, ventilated clinic bay. Nonetheless, regardless of the dental clinic design, we noticed that dental aerosol, when present, appeared momentary. As a result, fallow time can be cut to 5 min, which is most commonly seen during routine patient care (Choudhary et al., 2022).

Many international organisations, including the CDC, the ADA, and the Australian Dental Association, have recommended using a preprocedural mouth rinse or gargling in addition to recommendations like strictly enforced infection control, patient screening, and donning appropriate personal protective equipment (CDC, 2022; Ather et al., 2021). The main aim of using pre-procedural oral rinsing is to reduce oral microbes, which will mitigate microbial transition by aerosol, splatter, or even close contact (Ather et al., 2021). Different types of mouth rinsing showed a reduction in the load of microbes in the oral cavity, such as chlorhexidine gluconate, a very efficient antibacterial agent, one of the most often used preprocedural mouth rinses in dentistry. Alternative mouth rinses with comparable antibacterial activity include iodine-based [povidone-iodine (PVP-I)] or essential oil-based (Listerine) or oxygenating agents [hydrogen peroxide (H2O2)] (O'Donnell et al., 2020).

Ather et al. (2021) conducted a systematic review to study the efficacy of mouth rinsing against SARS-CoV-2. Despite most of the studies being in vitro, a reduction in viral

titters was more significant in PVP-I than in other types of mouth rinsing when COVID-19 infected patients rinsed with 0.33-1.5% PVP-1 in vitro and 1% in vivo for 60 seconds. A decrease in viral load was observed for three h. Admittedly, the PVP-I was more effective in patients with high viral load. On the other hand, 0.2% chlorhexidine mouthwash and 3% hydrogen peroxide did not show any significant defence in reducing the viral titer. It should be noted that 3% hydrogen peroxide had an excellent effect on SARS-CoV-2 Polymerase Chain Reaction (PCR), which was negative for 72h after rinsing.

2.4. Limitation of Previous Studies

So far, there are no records of microbial air contamination (bacterial and viral) when AGPs are performed in open clinical areas in different ACH parameters (Holliday et al., 2021, Zemouri et al., 2017). Besides, most of the previous studies have yet to be done when actual patients are present, and some of the experiment designs may restrict the movement and visualisation of the dentist during the treatment. Not only that, but also other drawbacks should be mentioned. For instance, air particle records cannot distinguish between particles from the dental unit waterline and those of biological origin, the settle plates cannot account for the smallest particles that remain in the air, and fluorescent dyes cannot show the biological component viability. Therefore, more than these approaches alone are needed to produce reliable results about the distribution of active SARS-CoV-2.

It should be noted that there is a need for more data, particularly for treatments; realistic simulations of mechanical therapy settings have been performed, but the interaction with the breathing patient has yet to be examined. Such a circumstance

arises in real life in preclinical dentistry training courses; however, the treatment situation varies in that manikin heads are used to model the oral cavity (Graetz et al., 2021).

2.5. Systematic review

2.5.1. Introduction

The COVID-19 pandemic has intensified the pre-existing worries among dental practitioners about the potential risk of contagion to dental professionals and their patients from AGPs (Zemouri et a., 2020). According to the WHO, AGPs are medical, dental, and patient care procedures that generate airborne particles, which increase the risk of transmitting infectious diseases (WHO, 2014).

Microorganisms such as bacteria, fungi, and viruses that can potentially cause infection can spread through droplets expelled from an infected person via breathing, talking, or coughing. These droplets can propel through the air and infect others by inhaling or settling on the skin, mucosal surfaces, or infrastructure (Jayaweera et al., 2020). Several case studies have demonstrated that SARS-CoV-2 can survive in aerosols for extended periods and remain suspended in the air for several hours. Additionally, some studies have detected significant amounts of SARS-CoV-2 RNA in saliva samples from infected patients (Yang et al., 2021; Chin et al., 2020; Van Doremalen et al., 2020).

Dental personnel are advised to wear PPE to minimise the risk of aerosol exposure. Rubber dams and suction tubes protect patients and the people in the clinic, although rubber dams are limited to specific dental procedures (Balanta-Melo et al., 2020; Rupf et al., 2015). Various aerosol-removing measures have been suggested for use in dental procedures, including HVS and ACSs, which are designed to filter, purify, and recirculate room air, and ventilation systems (Peng et al., 2019; Hallier et al., 2010; Noro et al., 1995). Also, during the period of inactivity, known as a fallow time, it is

implemented between patients to facilitate the removal of airborne particles through ventilation.

Regular implementation of fallow times may reduce the ability to provide dental services to a full extent. Nonetheless, there needs to be more uniformity in defining AGPs or determining the need for and duration of fallow time following AGPs (Shahdad et al., 2021). According to a recent quick evaluation of dental guidance documents worldwide, most did not mention fallow times. When a fallow time was suggested, the duration varied widely from 2 to 180 min. The median fallow time was 15 min for patients without COVID-19 symptoms and 20 min for patients with confirmed or suspected COVID-19 (Clarkson et al., 2020).

Therefore, this systematic review aims to understand the effect of ventilation on fallow time in dental or hospital settings. The study was guided by the following PICO question: "Does improved ventilation in a clinical setting (dental or hospital) effectively reduce contamination and decrease the required fallow time?"

2.5.2. Material and Method

2.5.2.1. Search Strategy

A systematic search was carried out in the following databases: Pubmed, ScienceDirect, and Google Scholar. The search was conducted on March 20, 2023, and included all published articles until that date. The Keywords were as follows: ("fallow time" OR "disinfection time" OR "sterilisation time") AND ("dental clinics" OR "dental offices" OR "dental practices" OR “hospital settings” OR “hospital”) AND ("ventilation" OR "air exchange" OR "airflow")

2.5.2.2. Data `Collection and Analysis

During the initial selection stage, the articles were assessed based on their title and abstract to identify studies that directly reported on the effectiveness of ventilation on reducing fallow time. The full-length articles were evaluated in the second stage using the inclusion and exclusion criteria outlined below.

Inclusion:

- Databases were searched for eligible English language publications.
- Mitigation only by using air ventilation or extra-oral ejection.
- Procedures are done on actual patients or in a phantom.
- Studies reported a direct relationship between ventilation and fallow time.
- There is no restriction on the date of publication up until 20 March 2023.

Exclusion:

- Any other mitigation technique is different from air ventilation.
- Studies not directly related to the theme of this review and duplicates
- articles published in languages other than English.

2.5.2.3. Data Extraction

The following information from each included study was extracted: first author's name, publication year, study design, devices used for detecting and counting, type of ventilation, dental procedure, duration of dental procedure, the clearance rate, and fallow time before and after.

2.5.2.4. Quality Assessment

The Joanna Briggs Institute (JBI) Critical Appraisal tools for quasi-experimental studies assessed the quality of each article. This tool is a checklist with nine questions (appendix) that can be answered with Yes, No, Unclear, or Not applicable.

2.5.2.5. Risk of Bias

As all included studies were in human environmental epidemiology studies, the OHAT risk of bias tool was used. The tool consists of six types of bias: selection, confounding, performance, attrition, detection, and, finally, selective reporting. The total of questions is 11.

2.5.3. Results

The search strategy identified a total of 169 eligible for further screening. Sixteen studies were excluded due to duplication. The remaining 153 articles undergo additional screening for title and abstract. One hundred twenty-five articles were excluded, 96 after title reading, four were not in English, and 25 were not fulfilling the inclusion criteria.

After a full-text assessment of the left 28 articles, 24 were eliminated after full-text reading, as they used different mitigation techniques or the fallow time weren't mentioned. The four remaining articles were included in the data synthesis. All studies are experimental in vitro, 3 in the dental clinic and 1 in the endoscopy room. As shown in the PRISMA flow chart (Figure 2.4.).

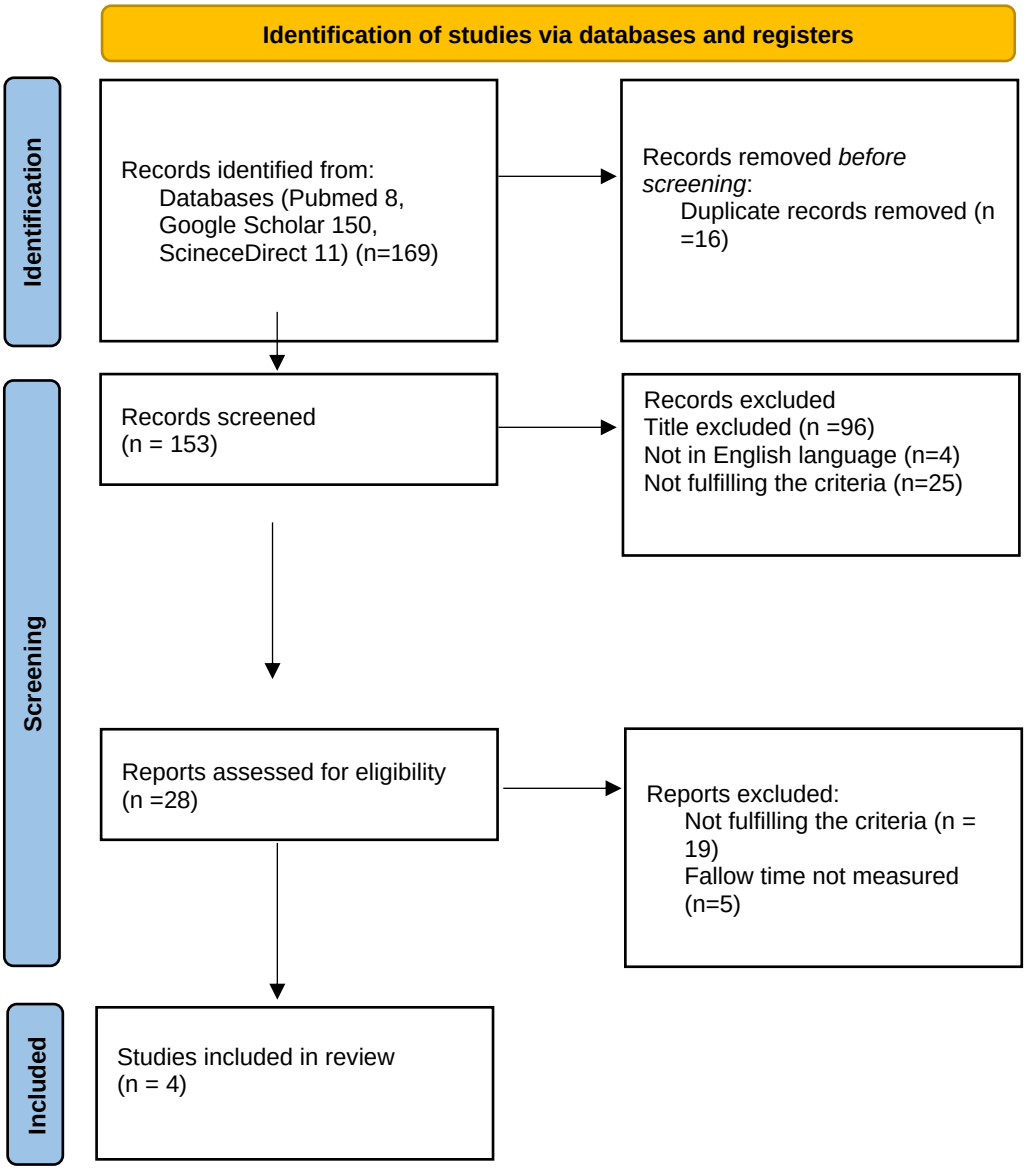


Figure 2.4. Flow chart of review synthesis

2.5.3.1. Quality Assessment

After applying the JBI checklist for Quasi-Experimental Studies to all included studies, shown in (Table 2.8.). Most of the questions were answered positively, with only one concern about the follow-up as it was not applicable.

Table 2.8. JBI Checklist for Quasi-Experimental Studies

JBI Critical Appraisal Checklist		Shahdad et al., 2021	Ehtizazi et al., 2021	Fennelly et al., 2022	Phillips et al., 2022
1- Is it clear in the study what is the 'cause' and what is the 'effect'?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
2- Were the participants included in any comparisons similar?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
3- Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
4- Was there a control group?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
5- Were there multiple measurements of the outcome both pre and post the intervention/exposure?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
6- Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?	Yes No Unclear N/A	N/A	N/A	N/A	N/A
7- Were the outcomes of participants included in any comparisons measured in the same way?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
8- Were outcomes measured in a reliable way?	Yes No Unclear N/A	Yes	Yes	Yes	Yes
9- Was appropriate statistical analysis used?	Yes No Unclear N/A	Yes	Yes	Yes	Yes

2.5.3.2. Risk of Bias

After utilising OHAT for environmental epidemiology studies, overall results for all studies were low. The entire risk of bias assessment is demonstrated in (Figure 2.5.).

Figure 2.5. OHAT for environmental epidemiology studies

2.5.3.3. Study Design and Settings

A different setting was found in the included studies. Three were dental (Fennelly et al., 2022; Ehtizazi et al., 2021; Shahdad et al., 2021), and one was in the hospital (Phillips et al., 2022). All of the included studies are experimental studies in vitro. The Shahdad et al. (2021) experiment was done in two different settings. The first was conducted in a multi-chair dental clinic with 6 ACH, and the second was in a private dental clinic with no mechanical ventilation. Ehtizazi et al. (2021) did their study in a dental surgery clinic having all non-experimental air ventilation turned off. The last dental study done by Fennelly et al. (2022), the study was done in a non-mechanical ventilated single dental unit with an open doorway. Regarding the only hospital setting study done by Phillips et al., 2022), a standard endoscopy room was the place for the experiment with 15 – 17 ACH.

2.5.3.4. Devices for Particle Count and Ventilation

The devices used to detect and count the particles in the environment in the included studies differed. OPS (Optical Particle Sizer 3330, TSI Inc., Minnesota, USA) was used by two studies (Fennelly et al., 2022; Shahdad et al., 2021) in addition to other devices. Fennelly et al. (2022) also used WIBS-4a (Droplet Measurement Technologies, Colorado, USA), and Shahdad et al. (2021) added a spectrometer particle scanner (NanoScan SMPS Nanoparticle Sizer 3910, TSI Inc., Minnesota, USA). On the other hand, Ehtizazi et al. (2021) employed a high-resolution electrical low-pressure impactor particle sizer (HR-ELPI: 'ELPI+', Dekati, Kangasala, Finland). The experiment was done in a standard endoscopy room. They analysed and measured the particles using an AeroTrak portable particle counter (TSI, Shoreview MN, model 9500-01) (Phillips et al., 2022).

Various devices and techniques were followed to increase ventilation and mitigate air contamination. An extraoral scavenger was employed by Shahdad et al. (2021), and a smellier technique was used by Fennelly et al. (2022) but in the non-mechanical ventilated clinic. They used a local exhaust ventilator. In contrast, Ehtizazi et al. (2021) and Phillips et al. (2022) have increased the ACH in the experimental setting to 24 ACH and 300 ACH, respectively. The former used an air-clearing system, and the latter used a laminar flow theatre.

2.5.3.5. Procedure and Duration

Three of the included studies used high-speed air turbines for cavity and crown preparation in different acrylic teeth. Ultrasonic scalers were also employed in two

studies for upper and lower arches. Only one study examined the contamination by electric contra-angle handpiece and three-in-one syringe. Moreover, an oral gastroscopy was done in one study in 3 separate rooms. Each procedure was repeated three times. The duration of the treatment varies from 12 to 20 min.

2.5.3.6. Fallow Time Without Mitigation

With no mitigation technique implemented, most of the studies showed that the particles would persist for extended periods before returning to the baseline lever. The time for particles to settle may range from 23.8 – 280 min depending on the ACH of the room before the additional ventilation. It was noticed that when the ACH in the clinic was around 15 – 17 ACH, it took less time for the particle to return to the pre-procedure level. In contrast, when Fennelly et al. (2022) turned off all non-experimental ventilation, the particles remained in the air for 49- 280 min before reaching the baseline level. Included studies were summarised in (Table 2.9.).

2.5.3.7. Fallow Time After Intervention

A considerable impact on fallow time can be observed after applying different extraoral or mechanical ventilation techniques, which enhanced ACH—after the intervention, the fallow time ranged from 0-16.8 min. In the following studies, the contamination level was restored to the baseline level immediately after finishing the procedure, like in studies conducted by Fennelly et al. (2022) and Ehtizazi et al. (2021), where the former employed LVE with 3000l/ min air clearance, and the latter has used ACS reaching 24 ACH.

Table 2.9. Fallow time without mitigation

Author	Date	Study design	Settings	ACH	Device for particle's detecting	Ventilation device
Shahdad et al.	2021	Experimental study in vitro	1- a multi-chair open clinic and closed surgery in a dental teaching hospital. 2- a private dental clinic	1- 6 ACH 2- The private dental clinic without mechanical ventilation	1- Optical Particle Sizer 3330, TSI Inc., Minnesota, USA 2- NanoScan SMPS Nanoparticle Sizer 3910, TSI Inc., Minnesota, USA	extraoral scavenging
Ehtizazi et al.	2021	Experimental study in vitro	a dental surgery	All non-experimental air-conditioning equipment was turned off	high-resolution electrical low-pressure impactor particle sizer	1- high-volume extraoral suction 2- an air cleaning system.
Fennelly et al.	2022	Experimental study in vitro	a floor-to-ceiling dental clinic, partitioned single patient enclosure with an open doorway	non-mechanically ventilated clinic	1- Optical Particle Sensor 2- Wideband Integrated Bioaerosol Sensor	local exhaust ventilation
Phillips et al.	2022	Experimental study in vitro	standard endoscopy room	15 -17 ACH	AeroTrak portable particle counter	1- portable HEPA filtration unit, 2- a laminar flow theatre with 300 air changes per hour

However, when the procedure was done in a laminar flow theatre, the particle clearance was faster than the detecting machine, resulting in zero fallow time. But in the same experiment, when authors used only HEPA, the fallow time rose to 16.8 min (Phillips et al., 2022). On the other hand, a minimal elevation in the fallow time was noticed in the experiment by Shahdad et al. (2021) by utilising extraoral scavenging. It took 10 min for the contamination to return to the pre-procedure concentration. The summary is illustrated in (Table 2.10.).

Table 2.10. Fallow Time After the Intervention

Author	Procedure	Duration of procedure	Evidence to support change	Fallow time before mitigation	Fallow time after mitigation	ACH with ventilation device
Shahdad et al., 2021	1- Cavity preparation of tooth 36 2- Crown preparation of 31 and 21 Air turbine at maximum flow was used.	20 min repeated 3 times	In non-mechanically ventilation clinic, the particle concentration never returned to the pre-operative concentration even after 1 hour. In a 6 ACH clinic with the use of extraoral scavenger, it took 10 min for the particles to returned to the pre-procedure concentration.	In the clinic with no mechanical ventilation even after 1 hour, the contamination didn't reach the baseline level.	10 min	6 ACH + extraoral scavenging
Ehtizazi et al., 2021	They have chosen different teeth from each quadrant using the following instruments: 1- air turbine handpiece 2- electric contra-angle handpiece 3- three-in-one syringe 4- ultrasonic scaler;	18 min repeated 3 times	The particles after the procedure remain above the baseline in the control group. However, with ACS upon completion of the procedures, the concentration of particles has been restored to the baseline range.	30 min	Reduce fallow time to zero.	Air cleaning system with 24 ACH
Fennelly et al., 2022	1- Ultrasonic scaling of the upper and lower arch 2- high-speed turbine handpiece on the upper right and lower right first moral	12 min for each treatment repeated 3 times with 10 min interval	In the control group with the OPS was used the $PN_{2.5}$ did not return to the baseline, and it needed 126 min to returned when it was measured with WIBS. In contrast, when LEV was employed, the particle number did not remain elevated over the baseline at the end of the procedure.	49 – 280 min	no particles remained airborne after the procedure.	3000 L/min
Phillips et al., 2022	Oral gastroscopy 1- standard endoscopy rooms (n=33) 2- Portable HEPA filtration unit (n=4) 3- Laminar flow theatre (n=4)	20 min	The particle clearance in non-mitigation group to reach the baseline needed more time comparing to the use of HEPA. When the procedure was done in a laminar flow theatre, the particle clearance was faster than the detecting machine.	23.8 min	1- portable HEPA filtration unit needed 16.8 min. 2- The fallow time is zero in A laminar flow theatre.	1- 15 -17 ACH 2- a laminar flow theatre with 300 ACH

2.5.4. Discussion and Conclusion

Based on the finding after conducting this review, it's possible to decrease the fallow time in the dental clinic or even the surgical theatre by implementing the correct type of ventilation. To reduce aerosol, mitigation has been mentioned in national and international guidelines and publications created by working groups. However, most regulations are based on out-of-date studies or information about splatter rather than actual aerosol (Shahdad et al., 2020). Regardless of the air change rate, the National Services Scotland technical assessment stated that droplets (>5-10 m) needed ten min to settle and the routine infection control methods, which are well-practised in dental practice, are sufficient to limit the risk of infection (NHS, 2021). However, in this systematic review, it's noticeable that mechanical air ventilation significantly impacts fallow time.

Ehtizazi et al. (2021) noted that particles between 0.05 and 0.236 μm remained at high concentrations in the macro-environment for longer than in the experimental period in the absence of aerosol-management measures. The total particle concentration may not return to baseline levels for at least 28 to 34 min following the termination of AGPs. Similar findings by Shahdad et al. (2021) and Fennelly et al. (2022) were observed. When they reported a fluctuating fallow time with non-ventilated clinic particle levels, they failed to return to baseline after one hour.

Conversely, once mechanical ventilation was employed, an enormous alteration in fallow time was recorded. Ehtizazi et al. (2021) and Fennelly et al. (2022) could return the particle concentration directly after the end of the procedures within the baseline range. That happened by utilising two different ventilation ACS and LEV, respectively. Thus, no

further fallow time was needed to be added. ACS has increased the ACH to 24, and LEV can reach 3000l/min. Even a simple increase in ventilation during dental procedures can affect fallow time, like what was found in the study by Shahdad et al. (2021). Fallow time can be lower to 10 min in a standard dental clinic with 6 ACH and an extraoral scavenger. That is the needed time for particles to return to the pre-procedure level.

Interestingly, there needs to be more studies and experiments in hospital settings to measure the fallow time. However, Phillips et al. (2022) conducted their study during endoscopy in a laminar flow theatre, which can reach 300 ACH. He concluded that the particle clearance was faster than the detecting machine. Moreover, Zhang et al. (2021) are the first to discuss the value of air change rate in the surgical microenvironment. The lowest contamination rate occurs while using a higher air change rate, such as 20 or 25 ACH, according to an analysis of several ventilation rates (10, 15, 20, and 25). Increasing ventilation parameters will aid in reducing the staff's exposure risk in various healthcare settings.

In conclusion, even with the limitation in this study, a limited number of experiments measured the fallow time with mechanical ventilation as an intervention. Also, all the dental studies have been done, not in actual patients. The significance of ventilation during AGPs is obvious to reduce contamination which could help reducing infection transmission in dental clinics. Despite the advantage of increasing ventilation, the primary mitigation strategy still appears to be meticulous four-handed dentistry with HVS and SE, along with the strict implementation of all infection prevention and control interventions. Importantly, all mechanical ventilations do not entirely prevent exposure during AGPs. Thus, dental professionals must employ proper respiratory safety equipment.

2.6. Aims and Objectives of the Primary Study

2.6.1. Aims

To identify the effect of different ventilation parameters (6ACH and 10ACH) on microbial contamination of air when AGPs are performed in open clinical areas at different dental clinical settings.

2.6.2. Objectives

1. Sampling air in an open clinical area with ventilation of 6ACH when AGPs are performed.
2. Sampling air in an open clinical area with ventilation of 10ACH when AGPs are performed.
3. Define microbial air contamination by standard microbiology (CFU/2500l).
4. Investigate all samples' air contamination with SARS-CoV-2 by reverse transcriptase (RT) PCR.
5. Compare different groups to identify the effect of ventilation on air contamination.

3. Material and Methods

3.1. Air Sampling

Air sampling was done using an MD8 air scan sampling device (Sartorius, Epsom, UK) first with sterile gelatine filters (80 mm diameter and three µm pores; Sartorius) to enable DNA and RNA extraction from bacteria and viruses. Each sample was done at a speed of 50 l/min for 20 min. The second sampling was done using BACTair culture media which is made of polystyrol with 400 impaction holes, each measuring 0.47mm in diameter (110 mm diameter; Sartorius, Epsom, UK) for standard culture in an aerobic environment to calculate total viable count (TVC). Each sample was done at a speed of 125 l/min for 20 min, as shown in (Figure 3.1). All samples were taken in the below time points, places, and ventilation parameters. At least three samples were taken for each variable. All gelatin samples were stored at -80 °C storage. However, all culture media was incubated in an aerobic incubator for 48h.

Figure 3.1. Air Sampling by Using MD8 air scan sampling device (Sartorius, Epsom, UK)

3.1.1. Timepoints

Sampling air in open clinical areas with ventilation of 6 ACH (with and without in-between bays separators) and 10 ACH when AGPs and non-AGPs are performed at the start of the day as a baseline reading (Monday morning) and during active dental treatments (Tuesday afternoon).

3.1.2. Air Change

Sampling was done at 6ACH and 10ACH in the Barts Health NHS Dental Hospital (BHDH) and Sir Ludwig Guttmann (SLG).

3.1.3. Dental Activity

Air sampling has been taken at different clinics and hospitals. Sampling took place in an open plan clinical area when (1) AGPs are performed using a high-speed motor (more than 160,000 rpm), (2) AGPs are performed using ultrasonic scaling, (3) non-AGPs are performed, and (4) orthodontic treatment is performed.

3.1.4. Site

Samples were taken in BHDH and SLG.

3.1.5. Position of The Air Sampler

All samples were taken when the air sampler was placed between four dental units in an open dental clinic area away from walls or any significant obstacles for 20 min. This ensures taking the samples from outside the dirty zone 2 m away from the dental chair.

3.2. Standard Microbiology

Standard microbiology sampling was performed to define the microbial air contamination. The number of CFU was counted using a polystyrol culture media plate measuring 116 x 24 mm (BACTair culture media, Sartorius, Germany). Each plate has 400 impaction holes with a diameter of 0.47 mm each and a high particle retention ability for particles measuring less than 0.65μm. After taking the air samples, the plates were placed in an aerobic incubator for two days (48h) at a mean temperature of 36+/1

°C. The CFU per plate was recorded by dividing the plate into four equal quadrants and counting the bacterial colonies. The results are expressed in CFU/plate, equivalent to CFU/2500L air (Figure 3.2.).

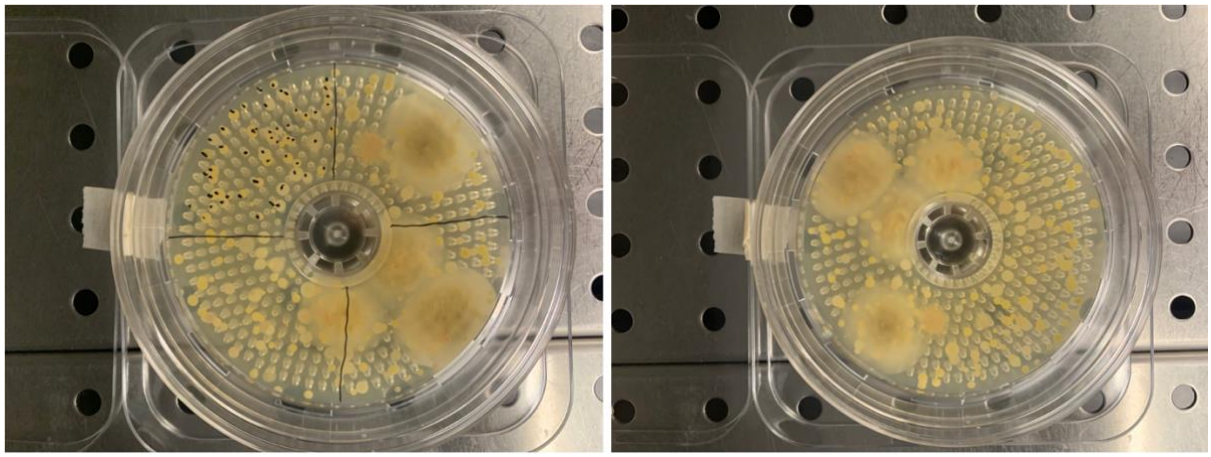


Figure 3.2. Standard Microbiology Reading

3.3. Statistics

The data was inputted into an Excel sheet and analysed with the advanced statistical software GraphPad Prism (GraphPad, USA). The Kolmogorov-Smirnov and Shapiro-Wilk normality test investigated the data's nature to determine the required test. Normally distributed data were described using mean and standard deviation, while non-parametric data were described by median and range. For normally distributed data, differences between groups were analysed by t-test and Bonferroni's test. In addition, ANOVA was used for more than two groups. For non-parametric data, differences between groups were identified by the Mann-Whitney U test for two groups and the Kruskal-Wallis test for more than two groups.

3.4. Project Registration and Approval

This project did not require ethics approval as no patient samples were collected. Furthermore, there was no interference with the clinical team or patients while delivering dental care. The dental director's permission was granted, and the project was registered (ID**:** 12514) (Appendice1).

4. Molecular Microbiology Training, Method Development, and Optimisation

4.1. Optimisation Stage

Before extracting DNA or RNA from air samples, an optimisation stage was conducted on sterile gelatine filters. To ensure accuracy, multiple optimisation experiments were conducted. Bacterial DNA and Centre d'Étude du Polymorphism Humain (CEPH) were inoculated on different sterile gelatine membranes at various concentrations (1.0, 0.1, 0.01, and 0.001 ng/ µl). The membrane was dissolved with 1.7 ml of 0.2% Tween 20 and RNase-free water in a falcon tube. An 80 mm Sartorius gelatine membrane was removed from the -80°C storage and allowed to equilibrate to room temperature. Using disposable tweezers, 1x Sartorius gelatine membrane was inserted into a clean 50mL falcon tube. Subsequently, 1.7 ml of 0.2% Tween 20 was added to the membrane filter inside a 50ml falcon tube and briefly centrifuged the tubes at 3,000 x g to dissolve the gelatine and allow extraction of RNA and DNA. Then, the 50ml falcon tube was placed into a preheated oven. The tube(s) was incubated for 10 min at 37 °C to dissolve the membrane. The dissolved membrane is ready for DNA and RNA extraction. Although there was no significant difference in the PCR outcome (Table 4.1.), 0.2%Tween 20 produced a more homogeneously dissolved product.

Table 4.1. PCR for Bacteria and CEPH

Sample Name	Ct Mean
Bacteria DNA 1.0	31.73
Bacteria DNA 0.1	34.61
Bacteria DNA 0.01	35.65
Bacteria DNA 0.001	34.99
CEPH 1.0	23.14
CEPH 0.1	24.14
CEPH 0.01	26.89
CEPH 0.001	29.85
Neg	37.85

4.2. Inculcation Gelatine Membrane with Artificial DNA

To observe the DNA amount obtained from two real samples and a blank gelatine membrane, Quantum bit (Qubit) and Nanodrop were used. The following protocol was used.

To begin with the Nanodrop test, first by wiping the upper and lower pedestals with a moistened kimwipe using Mili-q water. Then dry it with a kimwipe. Next, select the nucleic acid option on the screen. After that, load clean water to initialise the instrument and pipette 1-2µl of MilliQ water to the lowering pedestals. Lower the arm and click "Continue". Open the arm and wipe away the water from the upper and lower pedestals with a dry kimwipe. The software will then give instructions to blank the instrument. Followed by loading 1-2µl of suspension buffer onto the lower pedestals, lowering the arm and clicking "Continue". Then the cleaning step was repeated, followed by selecting sample load mode and type of ample (DNA). The next step was to load 1-2µl of sample onto each corresponding pedestal, lower the arm and click "Measure". Once results were shown, the pedestals were cleaned again and repeated these steps for all needed samples. At the end of measuring all samples, show report was ordered. Findings are illustrated in (Table 4.2.). The Nanodrop is illustrated in (Appendix 3).

Table 4.2. Results from the Two Real Samples and Blank gelatine membrane

Sample Name	Qubit (ng/ul)	Nanodrop (ng/ul)	260/280	260/230
Real sample at 9:00 am in the AGP clinics	<0.2	1.805	22.9	0.01
Real sample at 8:00 am in the AGP clinics	3.54	179.2	3.59	0.28
Blank membrane	<0.2	1.109	-2.19	0.02

To assess the quality of the extracted DNA, a second test using a Qubit fluorometer was conducted. Each kit contained a fluorescent assay reagent, dilution buffer, and two pre-diluted standards. The Qubit standards were prepared by diluting the fluorescent assay reagent 1:200 with the dilution buffer provided. A working solution of 200 µl was prepared per sample, plus three standards with a 10% excess. To each of the two 0.5 ml tubes labelled "S1" and "S2", 190 µl of the working solution was pipetted. To another 0.5 mL tube labelled "CT", 199 µl of the working solution was pipetted. Ten µl of Standard 1 was pipetted to the tube labelled "S1", while ten µl of Standard two was pipetted to the tube labelled "S2". Finally, one µl of 25ng/ µl pre-prepared calf thymus DNA was pipetted to the tube labelled "CT". The tubes were then vortexed for five seconds and spun briefly.

To measure the Qubit standards, these steps were followed: first, to select the sample type (DNA, RNA, or Protein) on the Qubit home screen. Then, to choose the reagent kit being used (dsDNA HS, dsDNA BR, etc.). When prompted to measure new standards, selecting "Yes" and then following the on-screen prompts to measure the two kit standards. The S2 was removed from the Qubit and the "CT" tube was inserted. To measure the "CT" control DNA, the "Sample" tab at the bottom of the screen was selected. Then selecting "Calculate stock conc." and the wheel to choose the volume of the DNA sample measured was used to determine the actual sample concentration. This value in the report was reported. Checking the raw fluorescent readings by selecting "Check Stds." on the screen and recording the values in the Qubit quantification report. Finally, it should be ensured to record the values of the standards and control sample in the Qubit quantification report sheet.

To complete the Qubit test, the final step was to measure the samples by taking 199 µl of the Qubit working solution, which was prepared previously, and putting it in a 0.5 ml tube for each sample. Then, one µl of the sample was added to each 0.5 ml tube. Afterwards, it was vortexed for five seconds and briefly spun. The CT sample was removed from the qubit and inserted into the first sample. The button "Read next sample" was pressed to take the measurement then choosing "Calculate stock conc." Adjusting the volume used as necessary, then recording the result. The steps were repeated for each sample. Findings are illustrated in (Table 4.2). Once the DNA quantitate and qualitative assessments were done, the amount of DNA from the samples could be expected. This led to anticipating a small quantity.

4.3. Dissolving Gelatine Membrane in Different Dissolvent

A series of tests to determine the samples' best PCR protocol for 16/18S rRNA were conducted. Different primer concentrations (0.5, 0.25, and 0.125 ng/ µl) for fungal and bacterial samples at varying temperatures (55, 60, 65, and 70 ºC) were tested. Additionally, the controls for these samples with different concentrations (1.0, 0.1, 0.01, 0.001, 0.0001 ng/µl) at the same temperatures were tested as well. Based on the results, it was found that using a concentration of 0.25 ng/µl for fungal primer and 0.125 ng/µl for bacterial primer at a temperature of 65 ºC was the most effective solution. For both controls, also a concentration of 0.0001 ng/µl primers worked best. The findings of PCR optimisation results are shown in (Tables 4.3., 4.4., and 4.5.).

Table 4.3. Ct Mean of Different Bacterial and Fungal DNA Concentration

Sample Name	Ct Mean for Bacteria primer	Ct Mean for fungal primer
E.Coli 1	15.412	34.057
E.Coli 0.1	19.001	34.274
E.Coli 0.01	20.370	34.120
E.Coli 0.001	23.810	33.943
E.Coli 0.0001	27.236	33.888
Yeast 1	30.023	9.620
Yeast 0.1	32.487	13.328
Yeast 0.01	33.080	16.512
Yeast 0.001	33.553	20.089
Yeast 0.0001	33.281	23.785

Table 4.4. Ct Mean of Bacterial and Fungal 0.0001 DNA Concentration at 70ºC with Different Primers Concentrations

Sample name	Ct Mean 70ºC					
	Bacteria 0.5	Fungal 0.5	Bacteria 0.25	Fungal 0.25	Bacteria 0.125	Fungal 0.125
Bacteria 0.0001	32.18	38.135	33.419	UD	35.131	38.236
Yeast 0.0001	37.753	27.3	37.028	21.159	UD	31.636
Neg	36.42	36.472	39.945	UD	UD	UD

Table 4.5. Ct Mean of Bacterial and Fungal 0.0001 DNA Concentration at 55ºC vs 60ºC vs 65ºC

Sample name	Ct Mean 55ºC		Ct Mean 60ºC		Ct Mean 65ºC	
	Bacteria	Fungal	Bacteria	Fungal	Bacteria	Fungal
Bacteria 0.0001	27.668	35.844	26.512	35.098	27.241	36.421
Yeast 0.0001	32.882	23.785	33.081	21.159	32.920	20.816
Neg	33.666	34.197	32.914	34.483	31.805	35.850

4.4. DNA and RNA Extraction

DNA extraction is done using (Qiagen DNA Blood and Tissue kit) following the manufacturer's protocol. After preparing the sample, the first step is to purify the viral nucleic acid by pipetting a 560 µl of prepared Buffer AVL containing carrier RNA into a 1.5 ml microfuge tube. Then an amount of 140µl of sample is added to Buffer AVL, mixed by pulse-vortexing for 15 seconds and briefly centrifuged. Subsequently, the solution is incubated for 10 min at room temperature. For washing, 560 µl of ethanol

(96–100%) is added to the sample and mixed by pulse-vortexing for 15 seconds and a short centrifuge. 630 µl of solution from the previous step is added to the QIAamp Mini Spin column on a clean 2 ml collection tube without wetting the rim. The tubes get centrifuged at 6000 x g (8000 rpm) for 1 min. Next, place the QIAamp Mini column into a clean 2 ml collection tube.

After repeating the last two steps until all lysate has been passed through the column, 500 µl Buffer AW1 is added to each spin tube containing the sample and centrifuge the tubes at 6000 x g (8000 rpm) for 1 min. After that, the QIAamp Mini column is placed in a new clean 2ml collection tube and added 500 µl Buffer AW2 is followed by centrifuging at full speed (14,000 rpm) for 3 min. The last two steps were to wash any contaminations from the DNA. Then repeat the centrifuging for only 1 min at full speed to ensure the DNA is washed properly.

The next step is to place the QIAamp Mini column in a clean 1.5 ml microcentrifuge tube and add 30 µl RNase-free water equilibrated to room temperature to leave it to be incubated for 1 min. Finally, centrifuge the 1.5 ml microcentrifuge at 6000 x g (8000 rpm) for 1 min, replace the eluent to the spin column membrane and centrifuge at the same speed and time. Samples are stored at -80°C until further analysis by Real-Time PCR.

4.5. Preparation for cDNA

RNA is transcribed to cDNA by using high-capacity cDNA Super Script Vilo (Superscript Vilo Master Mix, Invitrogen, France), according to the kit protocol. Following the reverse transcription procedure, a PCR is performed to convert

the mRNA to cDNA. The reaction is completed in one step. Starting by adding 4 µl Super Script Vilo Master Mix plus two µl Super Script Enzyme Mix for each sample in the 96-well PCR plate. After that, a 14 µl RNA template sample will be added to the PCR plate. Each sample will be mixed and incubated on the plate for 10 min at 25°C. The 96-well plate should be set at 42°C for 120 min. A more extended period of incubation was needed to increase the yields of cDNA. Terminate the reaction by increasing the incubation temperature to 85°C for only 5 min. Finally, the 96 well plates can be stored at -20°C, and the cDNA will be diluted with 40 µl of RNase-free water to be enough for the qPCR.

4.6. Real-time PCR for 16/18S rRNA

Broad-range primers targeting the 16/18S rRNA gene are used, according to Zucol et al. (2006). SYBR green PCR mix (Applied Biosystems, Cheshire, UK) is used according to the manufacturer's instructions.

After extracting the DNA and store at -20°C, samples are removed to equilibrate to room temperature. First, prepare the bacterial and fungal primers for the master mix, as shown in (Table 4.6.). Ten µl to be added from the forward and reverse primer (bacterial and fungal). Then to make it 100 µl in total, 80 µl RNase-free water will be added.

Subsequently, the master mix is prepared by adding 0.25 µl of fungal primer and 0.75 µl of RNase-free water to the reaction tube and 0.125 µl of bacterial primer and 0.875 µl of RNase-free water in a separate reaction tube. After that, five µl 5X SYBR green dye mix is added, and two µl RNase-free water is added, followed by vortex and

centrifuge. This master mix is enough only for one sample; thus, it will be multiplied by the sample running for PCR. From the master mix pipette, eight µl is added to the 384 well plates. Then, two µl extracted DNA is added to the 384 well plates as a final step. Three positive controls will be added to the 384 well plates as well. The first is bacterial DNA, the second is yeast DNA, and the last is human DNA. The final step is to add RNase-free water as a negative control. The exact amount will be used for the positive and negative controls, two µl from the DNA or RNase-free water and eight µl from the master mix. After completing the above steps, the plate is sealed and centrifuged for 1 min at 3000 rpm. From each solution, 10% should be added for pipetting errors.

After preparing the plate, it is put in the Quant Studio 7 RT-PCR machine for processing. The first stage is the "hold" stage, consisting of two steps: the first at 50ºC for 2 min, followed by the second at 95ºC for 10 min. This is followed by the PCR stage, which has two steps as well: the first at 95ºC for 15 seconds and the second at 65ºC for 1 min. Finally, the curve stage has three stages: the first at 95ºC for 15 seconds, the second at 65ºC for 1 min, and the third at 95ºC for 15 seconds. This process is repeated 40 times for qPCR amplification.

Table 4.6. Summary of primers for 16/18S rRNA real-time PCR

Primer name	Conc (uM)	Primer sequence (5'-3')	Target
CS1-16S-V1-V2-F	100	ACACTGACGACATGGTTCTACAAGAGTTTGATYMTGGCTCAG	Bacterial
CS2-16S-V1-V2-R	100	TACGGTAGCAGAGACTTGGTCTTGCTGCCTCCCGTAGRAGT	Bacterial
CS1-ITS3-for	100	TACGGTAGCAGAGACTTGGTCTTCCTCCGCTTATTGATATGC	Fungal
CS2-ITS4-1-rev	100	ACACTGACGACATGGTTCTACAGCATCGATGAAGAACGCAGC	Fungal

4.7. PCR for SARS-CoV-2

The PCR for SARS-CoV-2 is conducted by preparing the reaction mix as specified in (Table 4.6.). A research kit was purchased from Integrated DNA Technologies, Inc (IDT, U.S.A.) containing 2 Sars-CoV-2 sequences (N1 and N2), plus human RNase P acting like a positive and internal control. The reaction mix is summarised in (Table 4.7.). Each reaction mix is placed in a 1.5 ml microcentrifuge tube and carefully mixed using the pipette. An eight µl of the reaction mix is put into each well of 384 well plates then two µl of the cDNA is added to the plate. To proceed, prepare two wells. One contained two µl of RNase-free water (Blank Control), while the other had two µl of Positive Control, which is a human cDNA utilised as a control. Centrifuge the plate for 1 min at 3000 rpm. A 10% should be added to each solution for pipetting errors.

The amplification stage is performed by QuantStudioTM 5 Real-time PCR instrument (Applied Biosystems, Cheshire, UK). This step begins with modifying the thermal cycling conditions. In the “Holding stage”, step one at 50ºC, 2 min for one cycle. Step two at the same stage set at 95ºC, 10 min for another cycle. The second stage is the “PCR stage”. Step one is set at 95ºC for 15 seconds, and the second step is set at 55ºC for 1 min. The number of total cycles is 45 cycles. The QuantStadio TM and the kit used for SARS-CoV-2 PCR are in (Appendix 5).

Table 4.7 Reaction mix for SARS-CoV-2 PCR

Reagent		**Quantity**
Primers	2019-nCoV_N1 2019-nCoV_N2 Human RNAse P	1 µl
Master mix		5 µl
RNase-free water		2 µl

5. Results

5.1. Standard Microbiology

The CFU per plate was determined by incubating 2500 L of air samples collected using the impaction technique at a rate of 125 l/min for 20 min in an aerobic incubator for 48 h.

5.1.1. Air Contamination at 6 ACH Ventilation During the Performance of AGP and non-AGP in RLDH

Air contamination levels in dental clinics were compared using 6 ACH ventilation. During AGP in dental clinics, the mean and standard deviation (SD) of contamination level was 288.5 ± 108.6, while non-AGP clinics had a mean contamination level of 245.3 ± 93.01. Both were significantly higher than the baseline mean contamination level (68.67 ± 74.73). The results are summarised in (Tables 5.1.,5.2. and Figure 5.1.).

Table 5.1. Contamination Value During AGP vs non-AGP at 6 ACH in RLDH

	Baseline	AGP	Non-AGP
Number of values	9	15	6
Minimum	24.00	112.0	112.0
25% Percentile	28.00	208.0	160.0
Median	37.00	268.0	258.0
75% Percentile	87.50	400.0	327.0
Maximum	247.0	452.0	348.0
Mean	68.67	288.5	245.3
Std. Deviation	74.73	108.6	93.01
Std. Error	24.91	28.03	37.97
Lower 95% CI	11.23	228.3	147.7
Upper 95% CI	126.1	348.6	342.9

Table 5.2. Statistical Analysis Summary During AGP and non-AGP at 6 ACH in RLDH

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Baseline vs AGP	-219.8	5.386	Yes	****	-324.0 to -115.6
Baseline vs Non-AGP	-176.7	3.463	Yes	**	-306.9 to -46.46
AGP vs Non-AGP	43.13	0.9226	No	ns	-76.20 to 162.5

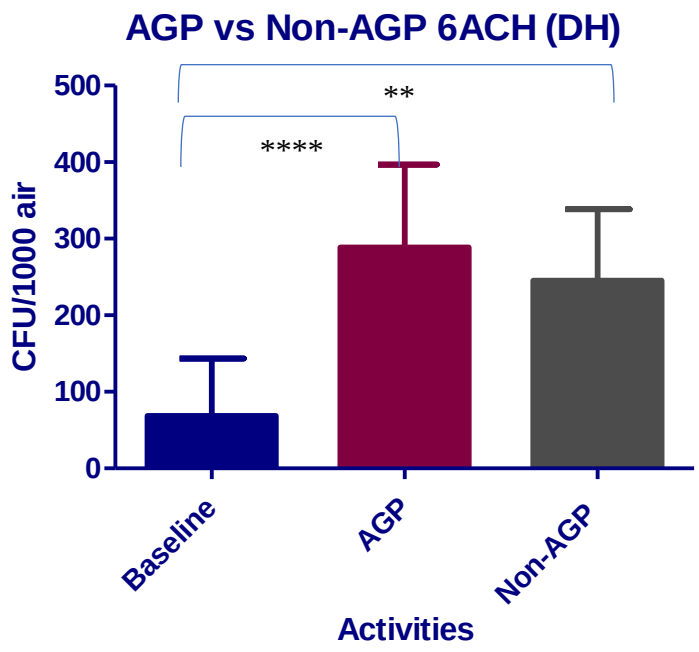


Figure 5.1. Contamination of AGP vs non-AGP at 6 ACH in RLDH, 9 samples for baseline, 15 of AGP and 6 of non-AGP, repeated 3 times

5.1.2. Air Contamination at 10 ACH Ventilation During the Performance of AGP and non-AGP in RLDH

The contamination levels at 10 ACH in RLH were measured and found to be as follows: AGP (177.3 ± 19.04), non-AGP (114.7 ± 23.69), and baseline (13.83 ± 5.4). Significant differences were observed between the contamination levels in AGP and non-AGP and between baseline and both groups, as shown in (Tables 5.3.,5.4. and Figure 5.2.).

Table 5.3. Contamination Value During AGP vs non-AGP at 10 ACH in RLDH

	Baseline	AGP	Non-AGP
Number of values	12	6	6
Minimum	5.000	152.0	96.00
25% Percentile	9.000	158.0	99.00
Median	14.50	180.0	106.0
75% Percentile	16.75	194.0	130.0
Maximum	24.00	200.0	160.0
Mean	13.83	177.3	114.7
Std. Deviation	5.408	19.04	23.69
Std. Error	1.561	7.775	9.670
Lower 95% CI	10.40	157.3	89.81
Upper 95% CI	17.27	197.3	139.5

Table 5.4. Statistical Analysis Summary During AGP and non-AGP at 10 ACH in RLDH

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Baseline vs AGP	-163.5	21.32	Yes	****	-183.4 to -143.6
Baseline vs Non-AGP	-100.8	13.15	Yes	****	-120.8 to -80.88
AGP vs Non-AGP	62.67	7.077	Yes	****	39.63 to 85.70

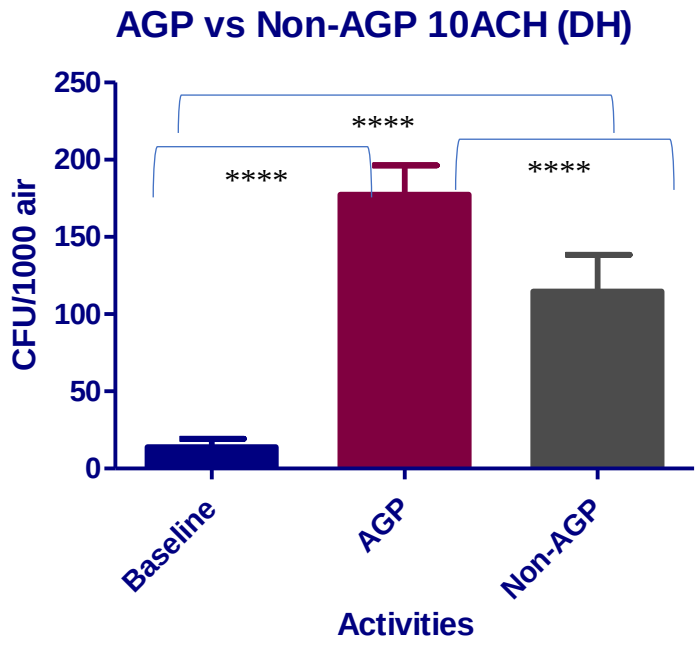


Figure 5.2. Contamination of AGP vs non-AGP at 10 ACH in RLDH, 12 samples of baseline, 6 of AGP and 6 of non-AGP, repeated 3 times

5.1.3. Baseline at 6 ACH vs 10 ACH in RLDH

The average contamination level at 6 ACH was (68.67 ± 74.73) and at 10 ACH (13.83 ± 5.4). The P value indicates a significant difference in ventilation between the two groups, illustrated in (Tables 5.5., 5.6. and Figure 5.3.)

Table 5.5. Baseline Contamination Value at 6 ACH vs 10 ACH in RLDH

	6ACH	10ACH
Number of values	9	12
Minimum	24.00	5.000
25% Percentile	28.00	9.000
Median	37.00	14.50
75% Percentile	87.50	16.75
Maximum	247.0	24.00
Mean	68.67	13.83

Std. Deviation	74.73	5.408
Std. Error	24.91	1.561
Lower 95% CI	11.23	10.40
Upper 95% CI	126.1	17.27

Table 5.6. Statistical Analysis Summary of Baseline at 6 ACH vs 10 ACH in RLDH

Unpaired t test	
P value	0.0193
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.555 df=19
How big is the difference?	
Mean ± SEM of column A	68.67 ± 24.91 N=9
Mean ± SEM of column B	13.83 ± 1.561 N=12
Difference between means	54.83 ± 21.46
95% confidence interval	9.921 to 99.75
R square	0.2558
F test to compare variances	
F,DFn, Dfd	191.0, 8, 11
P value	< 0.0001
P value summary	****
Are variances significantly different?	Yes

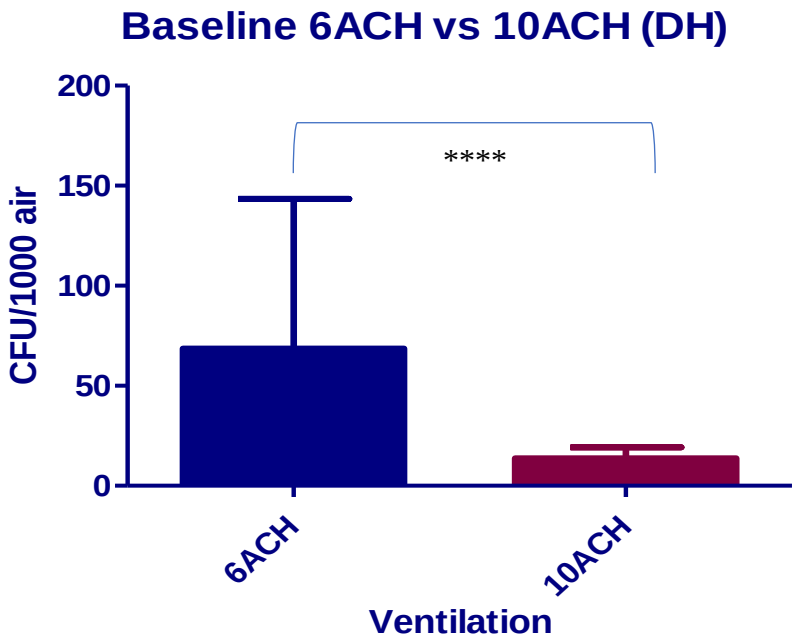


Figure 5.3. Contamination at Baseline 6 ACH vs 10 ACH in RLDH. 9 samples of 6 ACH and 12 of 10 ACH, repeated 3 times

5.1.4. Air contamination at 6 ACH vs 10 ACH during AGP in RLDH

In dental clinics, the AGP procedure showed a significant difference in contamination levels between 6 ACH (with a mean value of 288.5 ± 108.6) and 10 ACH (with a mean value of 177.3 ± 19.04). The summary is in (Tables 5.7, 5.8. Figure 5.4.).

Table 5.7. Contamination Value During AGP at 6 ACH vs 10 ACH in RLDH

	6ACH	10ACH
Number of values	15	6
Minimum	112.0	152.0
25% Percentile	208.0	158.0
Median	268.0	180.0
75% Percentile	400.0	194.0
Maximum	452.0	200.0
Mean	288.5	177.3
Std. Deviation	108.6	19.04
Std. Error	28.03	7.775
Lower 95% CI	228.3	157.3
Upper 95% CI	348.6	197.3

Table 5.8. Statistical Analysis Summary During AGP at 6 ACH vs 10 ACH in RDLH

Unpaired t test	
P value	0.0239
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.455 df=19
How big is the difference?	
Mean ± SEM of column A	288.5 ± 28.03 N=15
Mean ± SEM of column B	177.3 ± 7.775 N=6
Difference between means	111.1 ± 45.26
95% confidence interval	16.40 to 205.9
R square	0.2409
F test to compare variances	
F,DFn, Dfd	32.50, 14, 5
P value	0.0012
P value summary	**
Are variances significantly different?	Yes

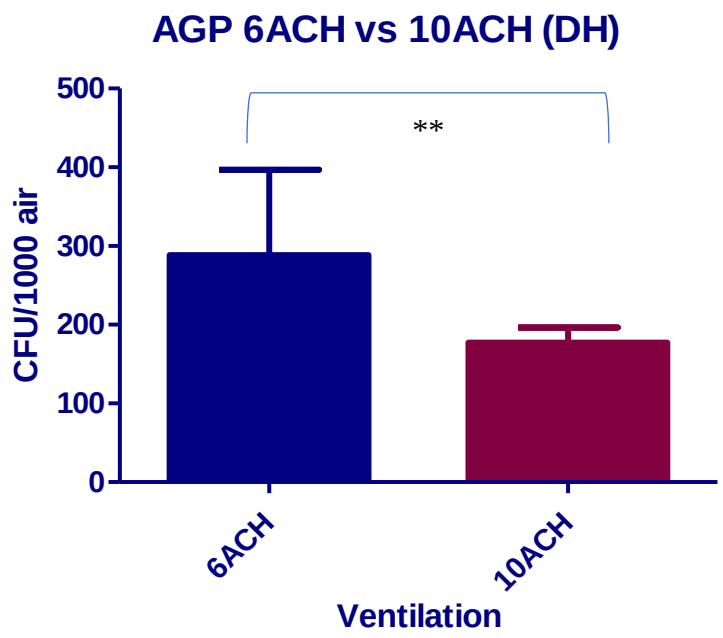


Figure 5.4. Contamination of AGP at 6 ACH vs 10 ACH in RLDH. 15 samples of 6 ACH and 6 of 10 ACH, repeated 3 times

5.1.5. Air Contamination at 6 ACH vs 10 ACH during non-AGP in RLDH

During non-AGP in dental clinics, the mean air contamination value was (245.3 ± 93.01) at 6 ACH and (114.7 ± 23.69) at 10 ACH, with a significant difference of P=0.0093. The statistical summary can be found in (Tables 5.9. ,5.10 and Figure 5.5.).

Table 5.9. Contamination Value During non-AGP at 6 ACH vs 10 ACH in RLDH

	6ACH	10ACH
Number of values	6	6
Minimum	112.0	96.00
25% Percentile	160.0	99.00
Median	258.0	106.0
75% Percentile	327.0	130.0
Maximum	348.0	160.0
Mean	245.3	114.7
Std. Deviation	93.01	23.69
Std. Error	37.97	9.670
Lower 95% CI	147.7	89.81
Upper 95% CI	342.9	139.5

Table 5.10. Statistical Analysis Summary During non-AGP at 6 ACH vs 10 ACH in RLDH

Unpaired t test	
P value	0.0076
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.335 df=10
How big is the difference?	
Mean ± SEM of column A	245.3 ± 37.97 N=6
Mean ± SEM of column B	114.7 ± 9.670 N=6
Difference between means	130.7 ± 39.18
95% confidence interval	43.37 to 218.0
R square	0.5265
F test to compare variances	
F,DFn, Dfd	15.42, 5, 5
P value	0.0093
P value summary	**
Are variances significantly different?	Yes

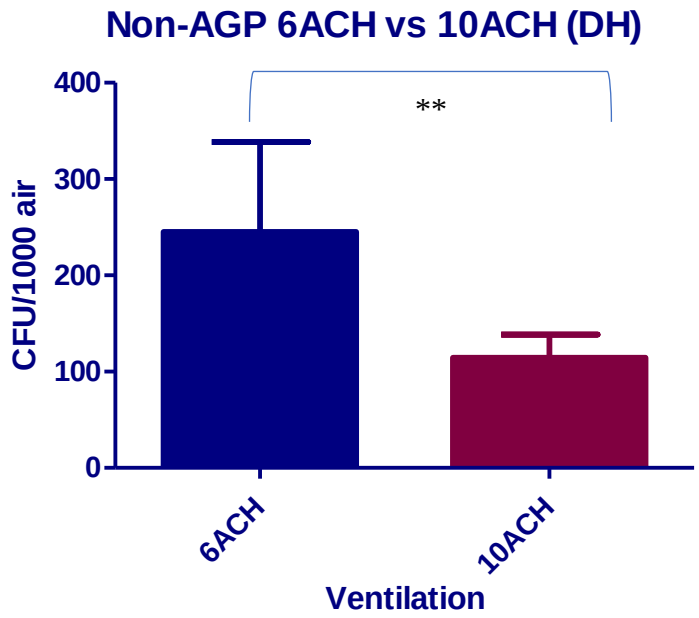


Figure 5.5. Contamination of non-AGP at 6 ACH vs 10 ACH in RLH. 6 samples of each, repeated 3 times

5.1.6. Air Contamination at 6 ACH vs 10 ACH During Dental Procedures in SLG

At SLG dental clinics, during AGP, the average air contamination level in 10 ACH clinics was (18.33 ± 11.85). This is lower than the contamination levels during non-

AGP in 10 ACH clinics (30.33 ± 26.73) and 6 ACH clinics (192.0 ± 34.64). There is a significant difference between the contamination levels comparing 10 ACH to 6 ACH. The mean value and summary of the statistics are shown in (Tables 5.11.,5.12. and Figure 5.6.).

Table 5.11. Contamination Value During AGP and non-AGP at 6 ACH vs 10 ACH in SLG

	NonAGP- 10ACH	AGP- 10ACH	NonAGP- 6ACH
Number of values	3	3	3
Minimum	12.00	11.00	172.0
25% Percentile	12.00	11.00	172.0
Median	18.00	12.00	172.0
75% Percentile	61.00	32.00	232.0
Maximum	61.00	32.00	232.0
Mean	30.33	18.33	192.0
Std. Deviation	26.73	11.85	34.64
Std. Error	15.43	6.839	20.00
Lower 95% CI	-36.06	-11.09	105.9
Upper 95% CI	96.73	47.76	278.1

Table 5.12. Statistical Analysis Summary During AGP and non-AGP at 6 and 10 ACH in SLG

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
NonAGP- 10ACH vs AGP- 10ACH	12.00	0.5616	No	ns	-58.25 to 82.25
NonAGP- 10ACH vs NonAGP- 6ACH	-161.7	7.566	Yes	***	-231.9 to -91.42
AGP- 10ACH vs NonAGP- 6ACH	-173.7	8.127	Yes	***	-243.9 to -103.4

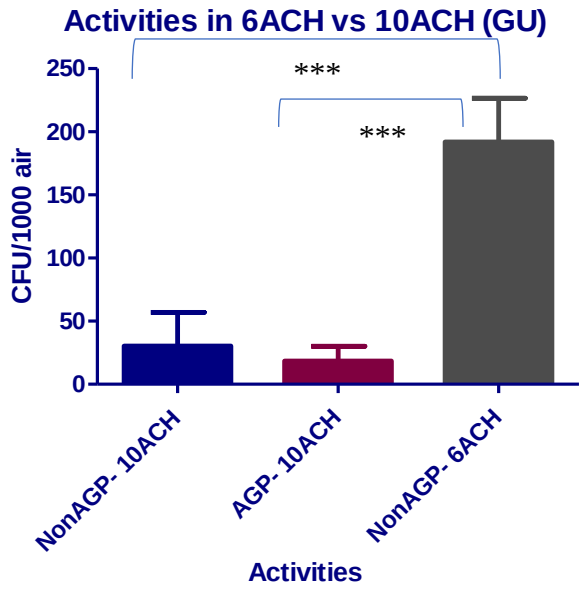


Figure 5.6. Contamination During Activities 6 ACH 10 ACH in SLG. 3 samples of each one, repeated 3 times

5.1.7. Air contamination at 6 ACH During non-AGP in RLDH vs SLG

The contamination levels at non-AGP dental clinics in RLH and SLG, at 6 ACH, were (245.3 ± 93.01) and (192 ± 34.64) respectively. Although there was a slight increase in contamination levels in RLH, the difference was not statistically significant, with a p-value of 0.381. For a detailed analysis, refer to (Tables 5.13., 5.15 and Figure 5.7.).

Table 5.13. Contamination at 6 ACH During non-AGP in RLDH vs SLG

	RLH	SLG
Number of values	6	3
Minimum	112.0	172.0
25% Percentile	160.0	172.0
Median	258.0	172.0
75% Percentile	327.0	232.0
Maximum	348.0	232.0
Mean	245.3	192.0
Std. Deviation	93.01	34.64
Std. Error	37.97	20.00
Lower 95% CI	147.7	105.9
Upper 95% CI	342.9	278.1

Table 5.14. Statistical Analysis Summary During non-AGP at 6 ACH in RLDH vs SLG

Unpaired t test	
P value	0.3814
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.9340 df=7
How big is the difference?	
Mean ± SEM of column A	245.3 ± 37.97 N=6
Mean ± SEM of column B	192.0 ± 20.00 N=3
Difference between means	53.33 ± 57.10
95% confidence interval	-81.72 to 188.4
R square	0.1108
F test to compare variances	
F,DFn, Dfd	7.209, 5, 2
P value	0.2526
P value summary	ns
Are variances significantly different?	No

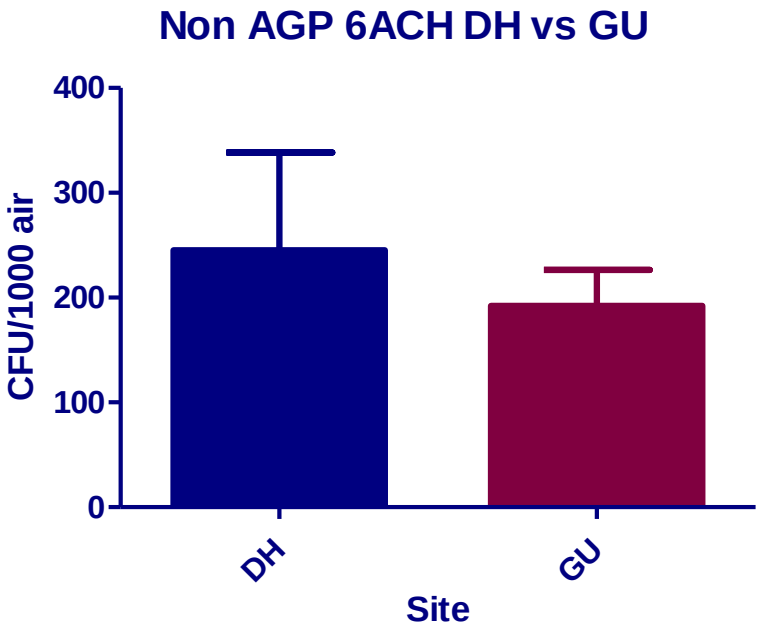


Figure 5.7. Contamination of non-AGP at 6 ACH in RLDH vs SLG. 6 samples at DH and 3 at GU, repeated 3 times

5.1.8. Air contamination at 10 ACH During non-AGP in RLDH vs SLG

The non-AGP dental clinics in RLH and SLG, at 10 ACH, had contamination levels of (114.7± 23.69) and (30.33 ± 26.73) respectively. The contamination levels in RLDH significantly increased, and the difference was statistically significant with a p-value of 0.0019. For more information, refer to (Tables 5.15.,5.16 and Figure 5.8.).

Table 5.15. Contamination at 10 ACH During non-AGP in RLDH vs SLG

	RLH	SLG
Number of values	6	3
Minimum	96.00	12.00
25% Percentile	99.00	12.00
Median	106.0	18.00
75% Percentile	130.0	61.00
Maximum	160.0	61.00
Mean	114.7	30.33
Std. Deviation	23.69	26.73
Std. Error	9.670	15.43
Lower 95% CI	89.81	-36.06
Upper 95% CI	139.5	96.73

Table 5.16. Statistical Analysis Summary During non-AGP at 10 ACH in RLDH vs SLG

Unpaired t test	
P value	0.0019
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=4.849 df=7
How big is the difference?	
Mean ± SEM of column A	114.7 ± 9.670 N=6
Mean ± SEM of column B	30.33 ± 15.43 N=3
Difference between means	84.33 ± 17.39
95% confidence interval	43.20 to 125.5
R square	0.7706
F test to compare variances	
F,DFn, Dfd	1.273, 2, 5
P value	0.7147
P value summary	ns
Are variances significantly different?	No

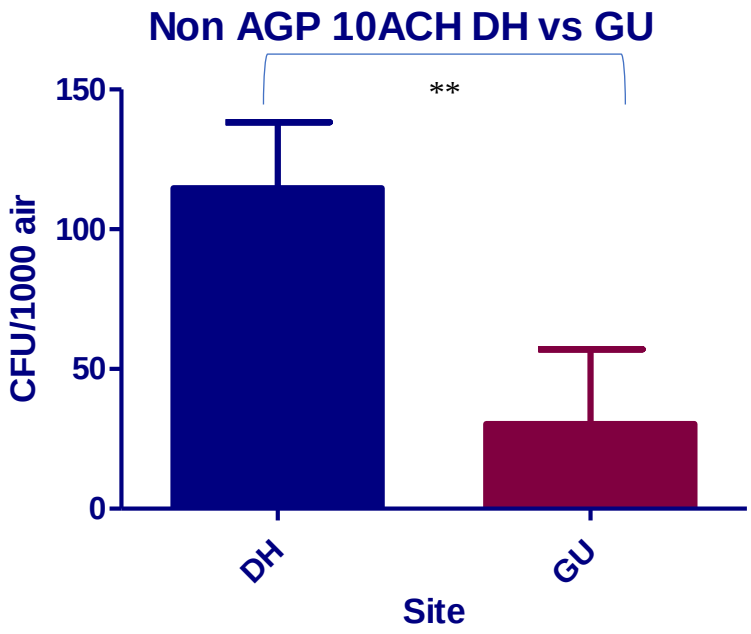


Figure 5.8. Contamination of non-AGP at 10 ACH in RLDH vs SLG. 6 samples at DH and 3 at GU, repeated 3 times

5.1.9. Air contamination at 10 ACH During AGP in RLDH vs SLG

The AGP dental clinics in RLH and SLG, at 10 ACH, had contamination levels of (177.3± 19.04) and (18.33 ± 11.85) respectively. The contamination levels in RLDH

significantly increased, and the difference was statistically significant with a p-value of 0.0019. For more information, refer to (Table 5.17.,5.18. and Figure 5.9.).

Table 5.17. Contamination at 10 ACH During AGP in RLDH vs SLG

	RLH	SLG
Number of values	6	3
Minimum	152.0	11.00
25% Percentile	158.0	11.00
Median	180.0	12.00
75% Percentile	194.0	32.00
Maximum	200.0	32.00
Mean	177.3	18.33
Std. Deviation	19.04	11.85
Std. Error	7.775	6.839
Lower 95% CI	157.3	-11.09
Upper 95% CI	197.3	47.76

Table 5.18. Statistical Analysis Summary During AGP at 10 ACH in RLDH vs SLG

Unpaired t test	
P value	< 0.0001
P value summary	****
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=13.00 df=7
How big is the difference?	
Mean ± SEM of column A	177.3 ± 7.775 N=6
Mean ± SEM of column B	18.33 ± 6.839 N=3
Difference between means	159.0 ± 12.23
95% confidence interval	130.1 to 187.9
R square	0.9602
F test to compare variances	
F,DFn, Dfd	2.584, 5, 2
P value	0.6043
P value summary	ns
Are variances significantly different?	No

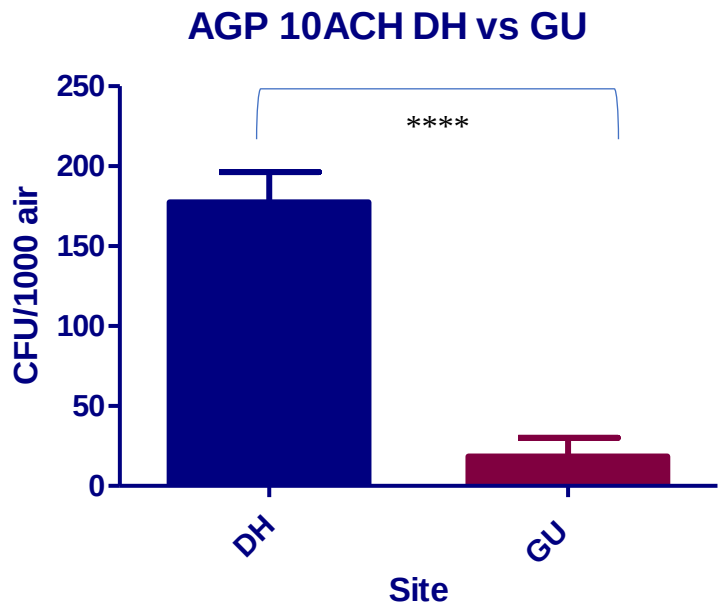


Figure 5.9. Contamination of AGP at 10 ACH in RLDH vs SLG. 6 samples at DH and 3 at GU, repeated 3 times

5.1.10. Air Contamination at 6 ACH During Different Dental Activates in RLDH

The mean value during the different procedures were as follows, orthodontic treatment (324.0 ± 22.27), ultrasonic treatment (198.6 ± 83.72), and cavity preparation (433.0 ± 23.64) (Table 4.19.). Air was significantly contaminated when using a high-speed motor for cavity preparation or debonding in the orthodontic clinic compared to AGPs using an ultrasonic scaler (Table 5.19., 5.20. and Figure 5.10.).

Table 5.19. Air Contamination During Dental Procedures at 6 ACH in RLDH

	Cavity preparation	Ultrasonic/Endo	Ortho
Number of values	4	5	3
Minimum	400.0	112.0	304.0
25% Percentile	408.0	130.0	304.0
Median	440.0	168.0	320.0
75% Percentile	451.0	282.5	348.0
Maximum	452.0	321.0	348.0
Mean	433.0	198.6	324.0
Std. Deviation	23.64	83.72	22.27
Std. Error	11.82	37.44	12.86
Lower 95% CI	395.4	94.64	268.7
Upper 95% CI	470.6	302.6	379.3

Table 5.20. P Value and 95% CI for Dental procedures at 6 ACH

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Cavity preparation vs Ultrasonic/Endo	234.4	5.982	Yes	***	119.5 to 349.3
Cavity preparation vs Ortho	109.0	2.443	No	ns	-21.86 to 239.9
Ultrasonic/Endo vs Ortho	-125.4	2.940	Yes	*	-250.5 to -0.2711

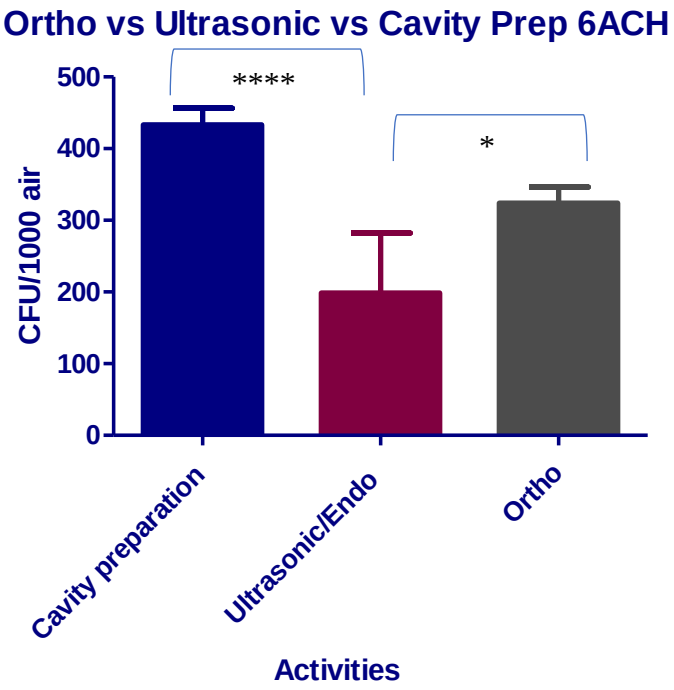


Figure 5.10. Contamination During Different Dental Activities at 6 ACH in RLDH. 4 samples of cavity preparation, 5 of ultrasonic, and 3 of ortho, repeated 3 times

5.1.11. Air Contamination at 6 ACH During Different Dental Activates in RLH with Separator in RLDH

The mean value during the different procedures with separators between the dental units were as follows, orthodontic treatment (114.7 ± 23.69), ultrasonic and endodontic treatment (156.0 ± 5.65), and cavity preparation (188.0 ± 11.78) (Table 4.21.). No significant differences between the ultrasonic scaler with endodontic and cavity preparation or orthodontic treatment. There is only a significant difference between

cavity preparation and orthodontic treatment with a P value < 0.05 (Table 5.21., 5.22. and Figure 5.11.).

Table 5.21. Air Contamination During Dental Procedures at 6 ACH with The Separators in RLDH

	Cavity preparation	Ultrasonic/Endo	Ortho
Number of values	4	2	6
Minimum	172.0	152.0	96.00
25% Percentile	176.0	152.0	99.00
Median	190.0	156.0	106.0
75% Percentile	198.0	160.0	130.0
Maximum	200.0	160.0	160.0
Mean	188.0	156.0	114.7
Std. Deviation	11.78	5.657	23.69
Std. Error	5.888	4.000	9.670
Lower 95% CI	169.3	105.2	89.81
Upper 95% CI	206.7	206.8	139.5

Table 5.22. P Value and 95% CI for Dental procedures at 6 ACH with The Separators in RLDH

Bonferroni's Multiple Comparison Test	Mean Diff.	t	Significant? P < 0.05?	Summary	95% CI of diff
Cavity preparation vs Ultrasonic/Endo	32.00	1.943	No	ns	-16.30 to 80.30
Cavity preparation vs Ortho	73.33	5.975	Yes	***	37.33 to 109.3
Ultrasonic/Endo vs Ortho	41.33	2.663	No	ns	-4.203 to 86.87

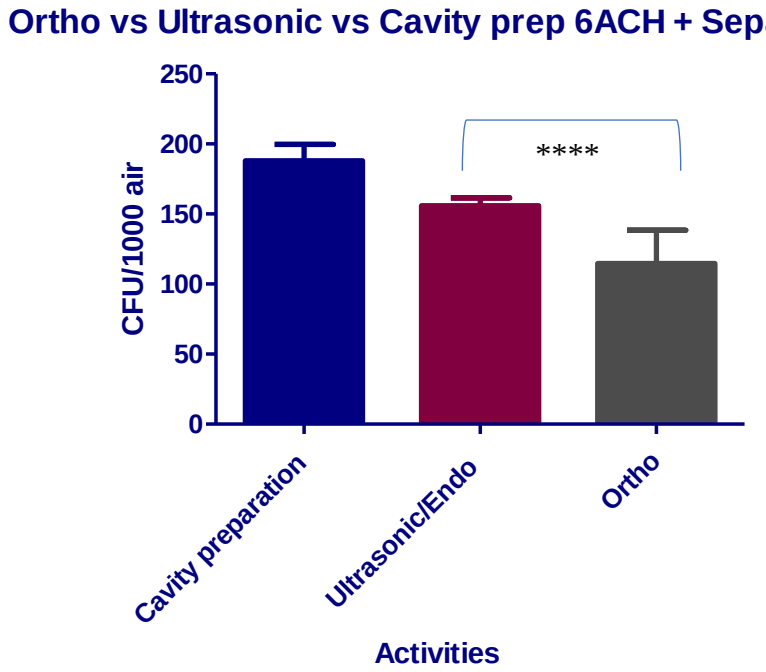


Figure 5.11. Contamination During Different Dental Activates at 6 ACH in RLDH with Separator. 4 samples of cavity preparation, 2 for ultrasonic and 6 for ortho repeated 3 times

5.1.12. Air Contamination at 6 ACH During Cavity Preparation in RLDH with and without Separator

Before installing the separators, the average air contamination at 6 ACH during cavity preparation was (433.0 ± 23.64). However, after the separators were installed, the contamination reduced to (188 ± 11.78). This shows a significant difference, with a P value of less than 0.0001. The summary of the statistics is in (Tables 5.23., 5.24. and Figure 5.12.).

Table 5.23. Air Contamination Cavity Preparation at 6 ACH with and without The Separators in RLDHL

	Without Separator	With Separator
Number of values	4	4
Minimum	400.0	172.0
25% Percentile	408.0	176.0
Median	440.0	190.0
75% Percentile	451.0	198.0
Maximum	452.0	200.0
Mean	433.0	188.0
Std. Deviation	23.64	11.78
Std. Error	11.82	5.888
Lower 95% CI	395.4	169.3
Upper 95% CI	470.6	206.7

Table 5.24. Statistical Analysis Summary During Cavity Preparation at 6 ACH in RLDH with and without The Separators

Unpaired t test	
P value	< 0.0001
P value summary	****
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=18.56 df=6
How big is the difference?	
Mean ± SEM of column A	433.0 ± 11.82 N=4
Mean ± SEM of column B	188.0 ± 5.888 N=4
Difference between means	245.0 ± 13.20
95% confidence interval	212.7 to 277.3
R square	0.9829
F test to compare variances	
F,DFn, Dfd	4.029, 3, 3
P value	0.2824

P value summary	ns
Are variances significantly different?	No

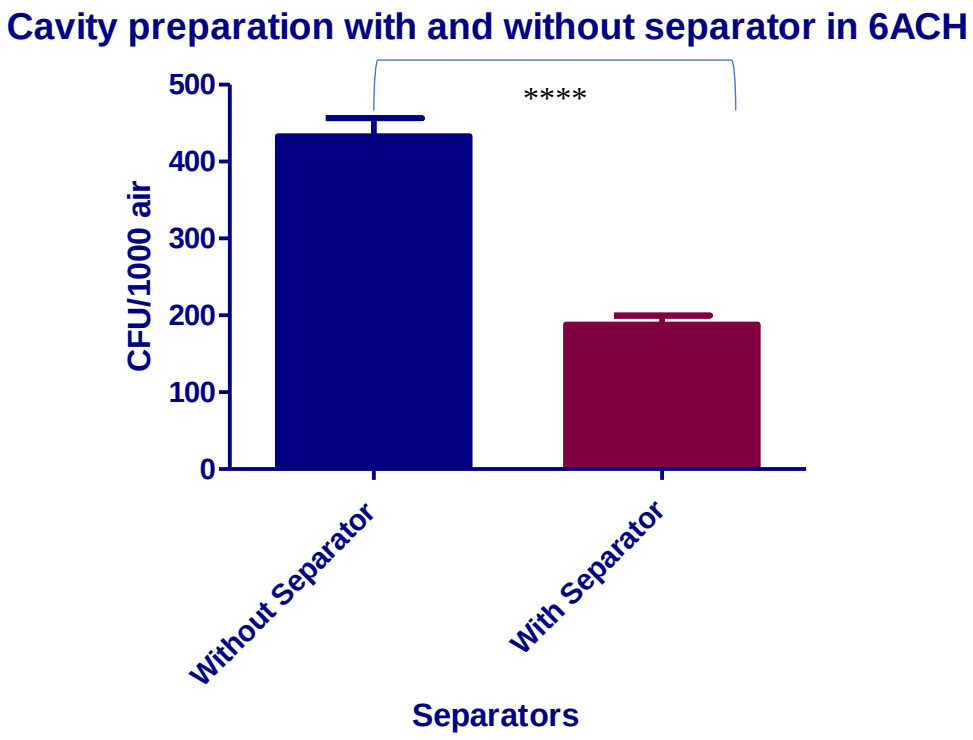


Figure 5.12. Contamination During Cavity Preparation at 6 ACH in RLDH with Separator. 4 samples of each, repeated 3 times

5.1.13. Air Contamination at 6 ACH During Ultrasonic Treatment in RLDH with and without Separator

The level of air contamination at 6 ACH during the ultrasonic scaler averaged (198.6 ± 83.72) before the installation of the separators. However, after the installation of the separators, the level of contamination decreased to (156 ± 5.65). This shows a significant difference, with a P value of less than 0.0001. The summary of the statistics is in (Tables 5.25.,5.26. and Figure 5.13.).

Table 5.25. Air Contamination During Ultrasonic Scaler at 6 ACH with and without The Separators in RLDH

	Without Separator	Separator
Number of values	5	2
Minimum	112.0	152.0
25% Percentile	130.0	152.0
Median	168.0	156.0
75% Percentile	282.5	160.0
Maximum	321.0	160.0
Mean	198.6	156.0
Std. Deviation	83.72	5.657
Std. Error	37.44	4.000
Lower 95% CI	94.64	105.2
Upper 95% CI	302.6	206.8

Table 5. 26. Statistical Analysis Summary During Ultrasonic Scaler at 6 ACH in RDLH with and without The Separators

Unpaired t test	
P value	0.5270
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.6795 df=5
How big is the difference?	
Mean ± SEM of column A	198.6 ± 37.44 N=5
Mean ± SEM of column B	156.0 ± 4.000 N=2
Difference between means	42.60 ± 62.69
95% confidence interval	-118.6 to 203.8
R square	0.08455
F test to compare variances	
F,DFn, Dfd	219.1, 4, 1
P value	0.1013
P value summary	ns
Are variances significantly different?	No

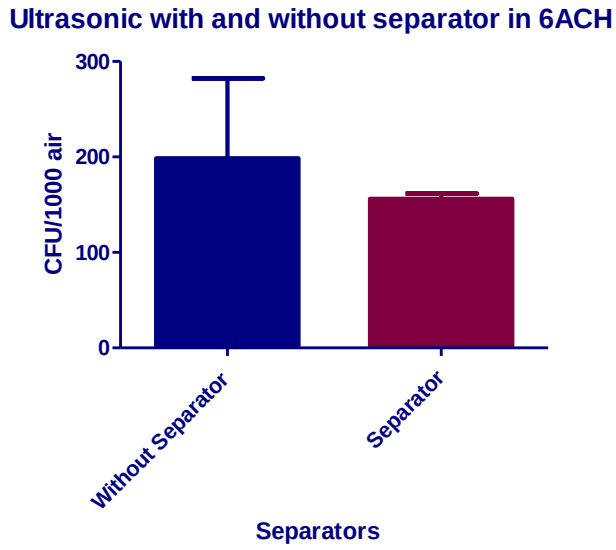


Figure 5.13. Contamination During Ultrasonic Treatment at 6 ACH in RLDH with Separator. 5 samples without a separator and 2 with, repeated 3 times

5.1.14. Air Contamination at 6 ACH During Orthodontic Treatment in RLDH with and without Separator

Before the separators were installed, the air contamination level at 6 ACH during orthodontic treatment was averaged at (324 ±22.27). But after the installation of the separators, the contamination level decreased significantly to (114.7 ± 23.69), with a P value of less than 0.0001. The summary of the statistics is in (Tables 5.27., 5.28. and Figure 5.14.). The summary of all activities is in (Figure 5.15.).

Table 5.27. Air Contamination During Orthodontic Treatment at 6 ACH with and without The Separators in RLDHL

	Without Separator	Separator
Number of values	3	6
Minimum	304.0	96.00
25% Percentile	304.0	99.00
Median	320.0	106.0
75% Percentile	348.0	130.0
Maximum	348.0	160.0
Mean	324.0	114.7
Std. Deviation	22.27	23.69
Std. Error	12.86	9.670
Lower 95% CI	268.7	89.81
Upper 95% CI	379.3	139.5

Table 5.28. Statistical Analysis Summary During Orthodontic Treatment at 6 ACH in RLDH with and without The Separators

Unpaired t test	
P value	< 0.0001
P value summary	****
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=12.71 df=7
How big is the difference?	
Mean ± SEM of column A	324.0 ± 12.86 N=3
Mean ± SEM of column B	114.7 ± 9.670 N=6
Difference between means	209.3 ± 16.47
95% confidence interval	170.4 to 248.3
R square	0.9585
F test to compare variances	
F,DFn, Dfd	1.131, 5, 2
P value	1.0000
P value summary	ns
Are variances significantly different?	No

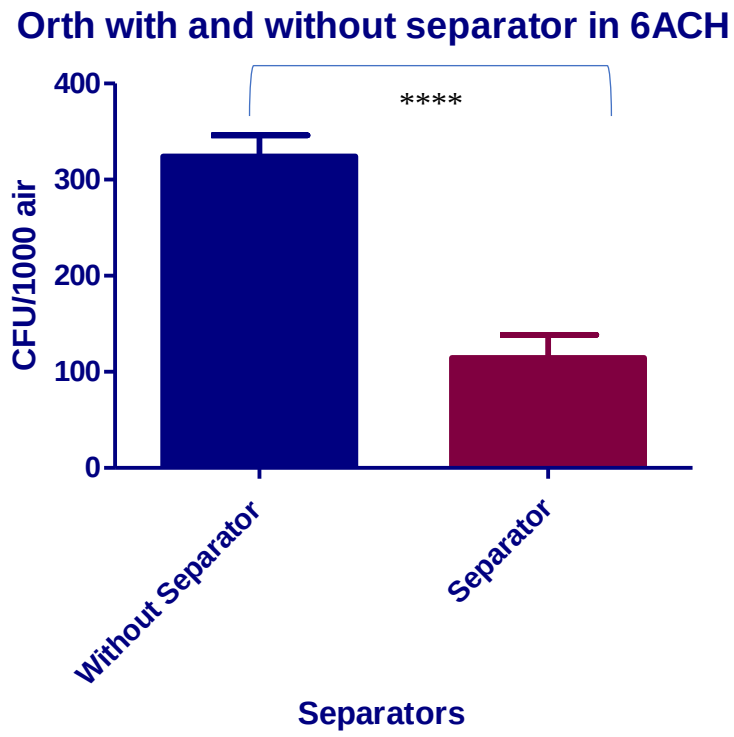


Figure 5.14. Contamination During Orthodontic Treatment at 6 ACH in RLDH with Separator. 3 samples without a separator and 6 with, repeated 3 times

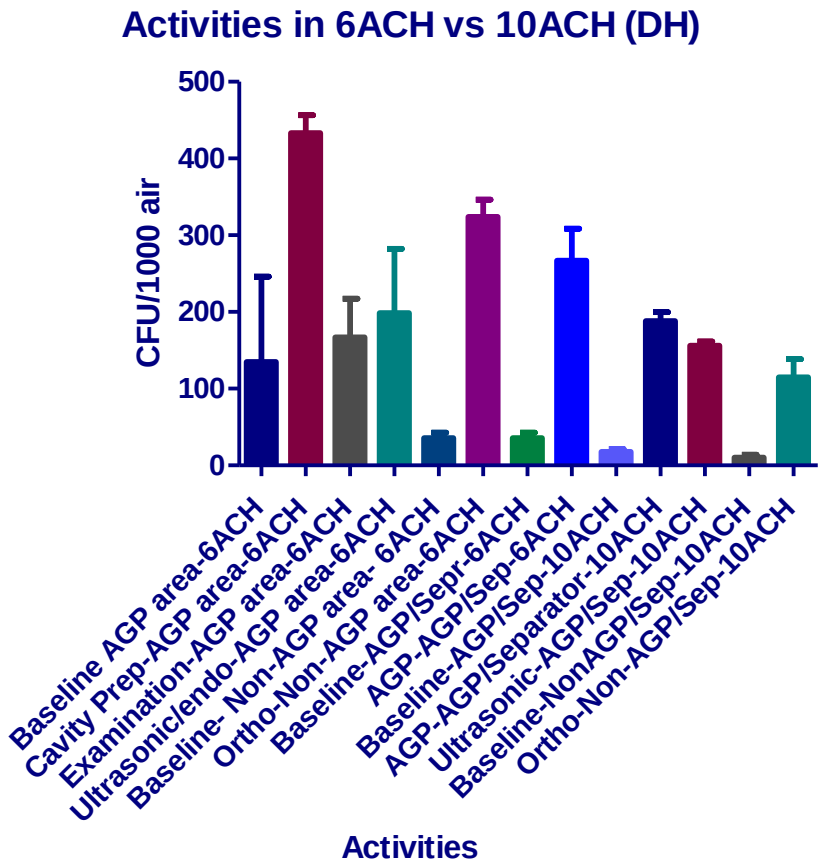


Figure 5.15. Summary of All Activates

5.2. PCR for SARS-CoV-2

The results of all the SARS-CoV-2 PCR tests were negative. This includes the 12 samples taken from 6 ACH and 10 ACH for baseline testing, and six samples from both AGP and non-AGP at 6 and 10 ACH. All of these tests came back negative, indicating no presence of the virus. PCR results are shown in (Tables 5.29. and 5.30.).

Table 5.29. PCR Results for COVID-19 at 6 ACH

Covid (6ACH)		
	Positive	**Negative**
Baseline	0	12
AGP	0	6
Non-AGP	0	6

Table 5.30. PCR Results for COVID-19 at 10 ACH

Covid (10ACH)		
	Positive	**Negative**
Baseline	0	12
AGP	0	6
Non-AGP	0	6

6. Discussion

Ensuring a safe working environment is crucial for providing dental healthcare. During dental procedures, microorganisms present in patients' mouths and respiratory tracts can become airborne as bioaerosols, posing a potential infection risk within the clinic. The microorganisms responsible for tuberculosis, pneumonia, and influenza are particularly concerning, especially when treating immunocompromised patients (Hallier et al., 2010).

Various methods have been developed to minimise the effects of bioaerosols in dental environments. High-volume aspiration and pre-procedural mouth rinse with antiseptic solutions have been proven effective in reducing the spread of airborne infections. Aspiration can reduce airborne bacterial contamination by over 90% (Harrel and Molinari, 2004).

To avoid contamination from splatter droplets or direct contact, wearing a mask, eye protection, and gloves is essential. Keep in mind that even high-quality masks only filter between 60-99% of airborne microorganisms and may not fit properly, leading to leakage (Hallier et al., 2010; Robinson et al., 2022). It's important to note that once the mask is removed after a procedure, any protection from airborne particles is no longer adequate. This is concerning because bioaerosols may remain in the air for up to 30 min after the procedure has ended (Gund et al., 2021). Using a rubber dam during dental procedures has been proven to remove almost all microorganisms in the air from blood or saliva in the mouth. The leading cause of airborne contamination when using a rubber dam is the water supply from dental instruments (Hallier et al., 2010).

Numerous techniques have been used to reduce air contamination during dental procedures, including LEV, HVE, PAC, and ACD. The aerosol-containing air

evacuated by HVE equipped with HEPA filters and UV light reduced bioaerosols by up to 98% (Hallier et al., 2010; Lee et al., 2004). Moreover, using hands-free HVE has been proven to reduce operation site contamination by more than 90%, according to Fennelly et al. (2022).

According to recent studies, the ventilation system is crucial in removing aerosol particles from dental outpatient clinics. Rooms with insufficient mechanical ventilation led to accumulating aerosol particles, while those with adequate ventilation systems prevented it. The air contamination levels in dental clinics heavily depend on the mechanical ventilation rates (Ehtizazi et al., 2021; Phillips et al., 2022).

A study by Mauraist et al. (2021) demonstrated a significant difference in air contamination levels between dental clinics that use AGP and those with non-AGP at 6 ACH. The AGP contamination level was measured at 2034 PM_{10} $\mu g/m^3$, while the non-AGP contamination level was 1704 PM_{10} $\mu g/m^3$. The baseline contamination level was only 1134 PM_{10} $\mu g/m^3$. This study discovered that AGP and non-AGP activities had higher contamination levels than the baseline contamination at 6 ACH. The contamination level in AGP was (288.5 ± 108.6) cfu/2500l, while non-AGP clinics had a mean contamination level of (245.3 ± 93.01) cfu/2500l. Both were significantly higher than the baseline mean contamination level (68.67 ± 74.73) cfu/2500l.

According to a recent experiment by Mauraist et al. (2021), higher ventilation systems resulted in air contamination closer to the background level in both AGP and non-AGP procedures. During dental procedures, the use of ACD led to an AGP contamination level of 1206 PM_{10} $\mu g/m^3$, slightly higher than the background level of 1134 PM_{10} $\mu g/m^3$. However, the level of contamination for non-AGP procedures was lower than the background, at 925 PM_{10} $\mu g/m^3$. This study shows a similar finding to this study.

The contamination level at 10 ACH was significantly higher in the AGP group (177.3 ± 19.04) cfu/2500l compared to the non-AGP group (114.7 ± 23.69) cfu/2500l. This contamination level was also statistically significant compared to the baseline level (13.83 ± 5.4) cfu/2500l.

A study conducted by Hallier et al. (2010) found that increasing air ventilation can effectively reduce bioaerosol levels. The baseline level of bioaerosol without ACS was 23.9 cfu/m^3. However, with ACS, it significantly decreased to 5.6 cfu/m^3. The bacterial count also decreased significantly during both AGP and non-AGP. The level of contamination decreased from 105.1 cfu/m^3 to 38.4 cfu/m^3 in the AGP clinic and from 66.1 cfu/m^3 to 38.5 cfu/m^3 in the non-AGP clinic. This aligns with this study's results, showing that using 10 ACH during dental procedures can be more effective in reducing air contamination levels than using 6 ACH. The baseline has significantly reduced from 68.67 ± 74.73 to 13.83 ± 5.4 cfu/2500l, while the AGP has experienced a notable decrease from 288.5 ± 108.6 to 177.3 ± 19.04 cfu/2500l. Additionally, the non-AGP has significantly declined from 245.3 ± 93.01 to 114.7 ± 23.69 cfu/2500l.

The study was carried out in two locations: a hospital called RLDH, and a clinical outreach service named SLG. At SLG, the contamination level of bioaerosols is lower due to several factors. These include less activity by staff and patients, as well as the design of the clinic which features separators between the dental units and no dental unit on the opposing side. A significant difference was observed when comparing the contamination levels in 10 ACH during AGP and non-AGP activities. During AGP activities, the contamination level at RLH was 177.3 ± 19.04 cfu/2500l, while at SLG, it was 18.33 ± 11.85 cfu/2500l. During non-AGP activities, the contamination level at RLH was 114.7 ± 23.69 cfu/2500l, and at SLG, it was 30.33 ± 26.73 cfu/2500l. It's

possible that the clinic with the 10 ACH in SLG had a lower number of dental units in operation, only two out of a total of four. Additionally, there were separators between the units but no opposite dental units, which could explain the observed difference. Even in clinics with 6 ACH, when a non-AGP activity occurs, the contamination is significantly less in SLG, with a count of 192 ± 34.64 cfu/2500l, compared to RLH, with a count of 245.3 ± 93.01 cfu/2500l. This could be due to the reasons mentioned above.

The level of contamination produced during dental treatment varies depending on the activity being performed. According to research, there is a significant difference in contamination levels between cavity preparation and ultrasonic scaling at 6 ACH. Cavity preparation produced 433.0 ± 23.64 cfu/2500l, while ultrasonic scaling had a level of 198.6 ± 83.72 cfu/2500l. Similar findings were reported in studies conducted by Hailler et al. (2010) and Shahdad et al. (2020). A study by Choi et al. in 2023 found that using a high-speed handpiece for drilling and an ultrasonic scaler created aerosols at a volume of 4.73(±0.774) ×108 µm3/m3 and 4.18(±1.22) ×108 $\mu m^3/m^3$, respectively. However, this contradicts the findings of previous studies conducted by Hailler et al. in 2010 and Shahdad et al. in 2020 and this study.

This study has shown that ultrasonic scalers produce less air contamination than air turbines. Additionally, utilising the SE and HVE during ultrasonic treatment can significantly reduce air contamination. The WHO recognises that dental procedures using ultrasonic scalers and contra-angle handpieces with speeds exceeding 60,000 rpm can create varying levels of aerosol contamination, classified as an AGP. A similar finding was observed by Choi et al. (2023) when they compared the ultrasonic scaler contamination with HVE and without suction. Using an ultrasonic scaler with HVE

reduces mean particle volume from $8.06 \pm 1.21 \times 10^4$ $\mu m^3/m^3$ to 4.18(±1.22) ×108 $\mu m^3/m^3$.

In contrast, the contamination level from orthodontic treatment was similar to that from cavity preparation, with levels measuring 324 ±22.27 cfu/2500l and 433.0 ± 23.64 cfu/2500l, respectively. The reason for this could be the absence of measures to control air contamination, like using an HVE system while removing braces with a slow handpiece. Additionally, the patient's airflow rate could also be a contributing factor. According to a study by Llandro et al. (2021), when orthodontic debonding is done in a clinical setting with good ventilation (at least 6.5 air changes per hour), a slow speed handpiece, and medium volume dental suction, any spatter is limited to the area around the dental chair. The risk of aerosol generation is low.

In Jun 2022, the RLDH's open bay clinics installed a glass separator that has dramatically reduced the bioaerosol levels in the clinic. As a result, there has been a significant decrease in air contamination during cavity preparation from 433.0 cfu/m^3 to 188.0 cfu/m^3, orthodontic treatment from 324.0 cfu/m^3 to 114.7 cfu/m^3, and ultrasonic scaler from 198.0 cfu/m^3 to 156.0 cfu/^{3} at 6 ACH.

Zhang et al. (2021) introduced the effectiveness of air change rate in the surgery microenvironment. Different ventilation rates (10, 15, 20, and 25 ACH) were assessed, showing that the lowest contamination rate happens when applying a higher air change rate, such as 20 or 25 ACH. Increasing ventilation parameters will help mitigate the exposure risk for the staff in different clinical settings. This also applies to dental open bay clinics, as shown by the above-mentioned results.

Ren et al. (2021) have concluded that the higher the mechanical ventilation, the faster aerosol removal from dental clinics. Also, a ventilation system with six or less ACH cannot reach 100% aerosol removal, unlike higher ventilation. That is similar to this study's results illustrating that 10 ACH is efficient at maintaining contamination levels at baseline during the different dental procedures, including AGPs. Nonetheless, it is crucial to acknowledge the presence of contamination and take the necessary steps to guarantee the proper implementation of all IPC interventions.

Another two studies, one in a dental clinic conducted by Chen et al. (2010) and the other study conducted by Rodríguez et al. (2021) in a hospital room with COVID-19-infected individuals, showed a great reduction and elimination of air contamination by using air cleaner devices. This is in line with this study illustrating that using enhanced parameters of air cleaning such as 10 ACH control air contamination during the different dental procedures.

It was proven that a higher ventilation system and portable air cleaners could help decrease fallow time in dental practice (Ren et al.,2021, Rodríguez et al., 2021, Chen et al., 2010). This is in line with this study's results showing that 10 ACH maintain the baseline air contamination level throughout the performance of the different dental procedures.

As part of the NHS's efforts to minimise the risk of transmission and provide safe care, healthcare practices were advised to screen patients before treatment. Dental offices were explicitly instructed to use the UK IPC screening tool for COVID-19, which has proven highly effective in reducing infections among patients and staff. Along with the vaccination program, these measures have successfully prevented any cases of COVID-19 from being detected in dental clinics using PCR in both ACH parameters.

In February 2022, samples were taken from SLG to compare air contamination levels during dental treatment in both AGP and non-AGP clinics with 6 and 10 ACH. The study showed a significant reduction of air contamination in non-AGP clinics at 10 ACH clinics (30.33 ± 26.73 cfu/2500l) compared to the 6 ACH clinics (192.0 ± 34.64 cfu/2500l). Meanwhile, the AGP clinic at the 10 ACH had much lower contamination levels (18.33 ± 11.85 cfu/2500l). These findings led to the decision to implement the same ventilation parameters at RLDH.

The results of this study should serve as a wake-up call to dental practices and medical institutions alike. They must prioritise improving their ventilation systems, particularly those with high air contamination levels or patients with low immunity or who are susceptible to infection. By taking action, these facilities can create safer and healthier environments for everyone who enters their doors.

6.1. Limitations of The Study

It was the first time to conduct a DNA and RNA extraction from a Sartorius gelatine filter at the Blizard Institute. Due to that, optimisation and method development was followed before the experiment. This gives an area for further study to confirm the results of this study in the future. Furthermore, it was impossible to separate the procedures from each other while taking the samples, as in AGP clinics, both high-speed handpieces and ultrasonic scalers were used simultaneously in some situations. The same happened in non-AGP clinics, as surgery clinics and orthodontic treatment were happening simultaneously.

7. Conclusion

Recent research suggests that the ventilation system is crucial in eliminating aerosol particles from dental clinics. Rooms without adequate mechanical ventilation tend to accumulate these particles, whereas those with good ventilation systems do not. The degree of air pollution in dental clinics is directly linked to the mechanical ventilation rates. The study's conclusions are summarised below:

- The separator insulation between the open dental clinic bay has greatly reduced air cross-contamination in both AGPs and non-AGP clinics.
- Increasing the ACH from six to ten has greatly improved air quality and significantly reduced air contamination during both AGPs and non-AGPs dental procedures.
- Although orthodontic treatment is classified as non-AGP, not using a SE and HVE during the procedure can still create air contamination similar to that of an AGP. On the other hand, the use of HVE during an ultrasonic scaler treatment, which is considered an AGP, has been found to significantly reduce air contamination. Therefore, further investigation is necessary.
- It has been beneficial to follow NHS guidelines for patient screening and risk assessment before admitting them or beginning dental procedures. This was demonstrated by all PCR tests showing negative results in dental clinics with both types of ventilation (6 and 10 ACH).
- In order to prevent the transmission of infections in healthcare settings, it is important to use a high-quality ventilation system.

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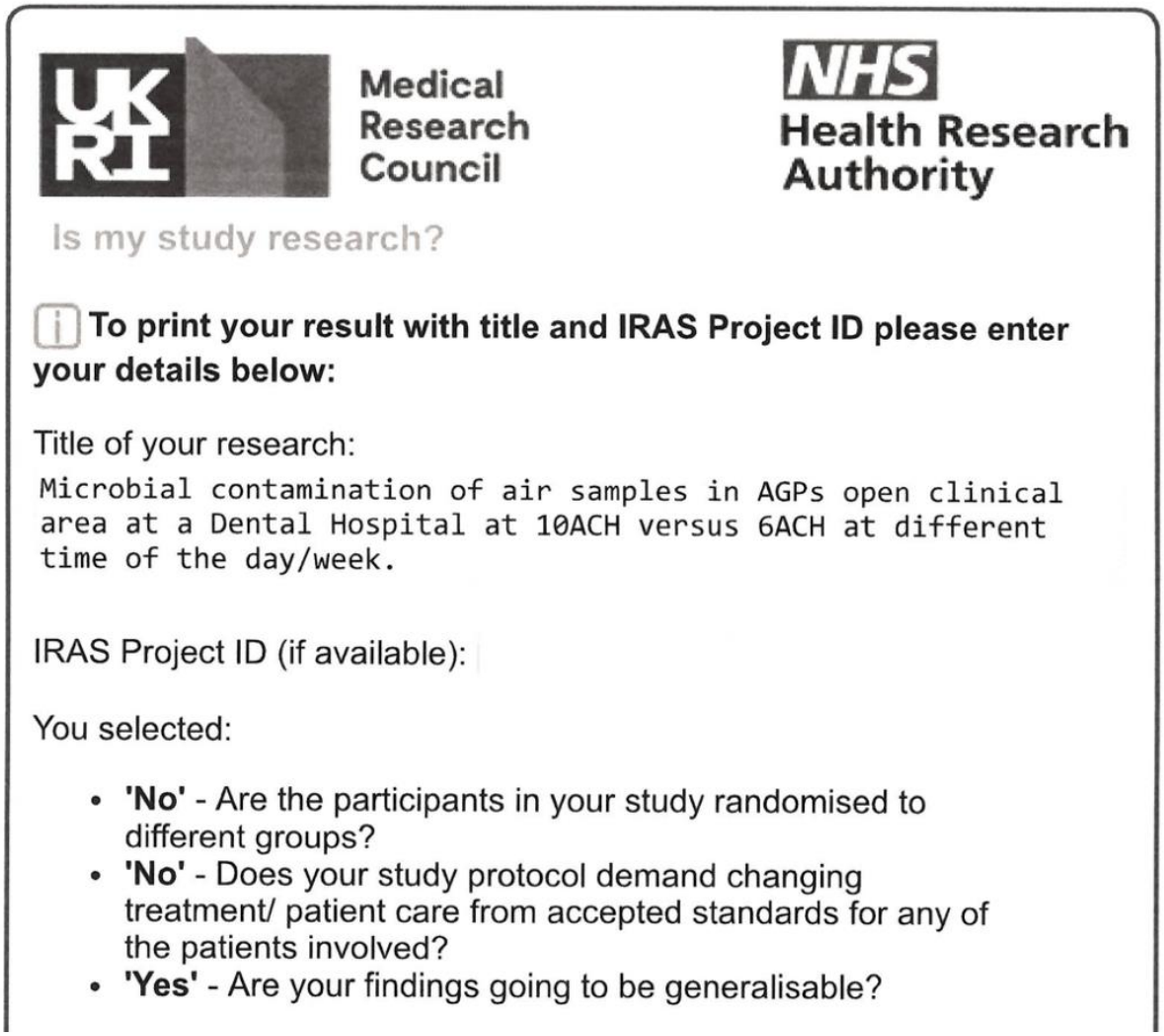
9. Appendix 1 The Research Registration



Medical Research Council

NHS Health Research Authority

Is my study research?

To print your result with title and IRAS Project ID please enter your details below:

Title of your research:

Microbial contamination of air samples in AGPs open clinical area at a Dental Hospital at 10ACH versus 6ACH at different time of the day/week.

IRAS Project ID (if available):

You selected:

- **'No'** - Are the participants in your study randomised to different groups?
- **'No'** - Does your study protocol demand changing treatment/ patient care from accepted standards for any of the patients involved?
- **'Yes'** - Are your findings going to be generalisable?

IRAS Project ID (if available):

Your answers to the following questions indicate that **you do not need NHS REC review for sites in England.**

This tool only considers whether NHS REC review is required, it does not consider whether other approvals are needed. You should check what other approvals are required for your research.

You have answered **'YES'** to: Is your study research?

You answered **'NO'** to all of these questions:

Question Set 1

- Is your study a clinical trial of an investigational medicinal product?
- Is your study one or more of the following: A non-CE marked medical device, or a device which has been modified or is being used outside of its CE mark intended purpose, and the study is conducted by or with the support of the manufacturer or another commercial company (including university spin-out company) to provide data for CE marking purposes?
- Does your study involve exposure to any ionising radiation?
- Does your study involve the processing of disclosable protected information on the Register of the Human Fertilisation and Embryology Authority by researchers, without consent?

Question Set 2

- Will your study involve potential research participants identified in the context of, or in connection with, their past or present use of services (NHS and adult social care), including participants recruited through these services as healthy controls?
- Will your research involve prospective collection of tissue (i.e. any material consisting of or including human cells)

from any past or present users of these services (NHS and adult social care)?
- Will your research involve prospective collection of information from any past or present users of these services (NHS and adult social care)?
- Will your research involve the use of previously collected tissue and/or information from which individual past or present users of these services (NHS and adult social care), are likely to be identified by the researchers either directly from that tissue or information, or from its combination with other tissue or information likely to come into their possession?
- Will your research involve potential research participants identified because of their status as relatives or carers of past or present users of these services (NHS and adult social care)?

Question Set 3

- Will your research involve the storage of relevant material from the living or the deceased on premises in England, Wales or Northern Ireland without a storage licence from the Human Tissue Authority (HTA)?
- Will your research involve storage or use of relevant material from the living, collected on or after 1st September 2006, and the research is not within the terms of consent for research from the donors?
- Will your research involve the analysis of human DNA in cellular material (relevant material), collected on or after 1st September 2006, and this analysis is not within the terms of consent for research from the donor? And/or: Will your research involve the analysis of human DNA from materials that do not contain cells (for example: serum or processed bodily fluids such as plasma and semen) and this analysis is not within the terms of consent for research from the donor?

Question Set 4

- Will your research involve at any stage procedures (including use of identifiable tissue samples or personal information) involving adults who lack capacity to consent for themselves, including participants retained in study following the loss of capacity?
- Is your research health-related and involving offenders?
- Does your research involve xenotransplantation?
- Is your research a social care project funded by the Department of Health and Social Care (England)?
- Will the research involve processing confidential information of patients or service users outside of the care team without consent? And/ or: Does your research have Section 251 Support or will you be making an application to the Confidentiality Advisory Committee (CAG) for Section 251 Support?

10. Appendix 2 JBI Critical Appraisal Checklist for Quasi-Experimental Studies

JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies)

Reviewer_____Date_____

Author _____Year_____Record Number_____

		Yes	No	Unclear	Not applicable
1.	Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?	☐	☐	☐	☐
2.	Were the participants included in any comparisons similar?	☐	☐	☐	☐
3.	Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?	☐	☐	☐	☐
4.	Was there a control group?	☐	☐	☐	☐
5.	Were there multiple measurements of the outcome both pre and post the intervention/exposure?	☐	☐	☐	☐
6.	Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	☐	☐	☐	☐
7.	Were the outcomes of participants included in any comparisons measured in the same way?	☐	☐	☐	☐
8.	Were outcomes measured in a reliable way?	☐	☐	☐	☐
9.	Was appropriate statistical analysis used?	☐	☐	☐	☐

Overall appraisal: Include ☐ Exclude ☐ Seek further info ☐

Comments (Including reason for exclusion)

11. Appendix 3 The Nanodrop

12. Appendix 4 The QuantStadio TM and the kit used for SARS-CoV-2 PCR