

# Managing Saliva and Surface Contamination in Dental Clinics: A Hypochlorous acid -Based Approach to Sustainable Infection Control

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## ABSTRACT

Effective microbial control in dental clinics is crucial to preventing healthcare-associated infections, particularly during aerosol-generating procedures. This study evaluates the efficacy of vaporized hypochlorous acid (HOCl) in reducing microbial contamination from saliva on surfaces under various conditions. This study aligns with the Sustainable Development Goals (SDGs) by contributing to SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production). This novel disinfection technique promotes sustainable, eco-friendly disinfectant solutions by offering an alternative to harsher disinfectants.

## Objectives

This study aimed to evaluate the antimicrobial efficacy of a vaporized HOCl based dental unit water line (DUWL) disinfectant in reducing microbial growth from whole saliva under both wet and dry conditions, across different challenge media. Secondary objectives included assessing the influence of time and medium composition on its effectiveness.

## Materials and Methods

Experiments were conducted in a controlled laboratory environment. Inoculated 96-well microtiter plates were exposed to vaporized HOCl (0.005%) for two different time points, 2 hours and 24 hours. The surfaces of the microtiter plates were treated with the vaporized disinfectant under both dry and wet conditions. Microbial inhibition was assessed by measuring optical density (OD) values, which provided an indication of microbial growth and the disinfectant's efficacy at each time interval.

## Results

Vaporized HOCl significantly reduced microbial contamination, especially in dry conditions, with lower OD values indicating greater efficacy. Low-nutrient media showed better disinfectant performance, while nutrient-rich media supported higher microbial growth. Longer

exposure times in nutrient-rich media resulted in decreased disinfectant efficacy, emphasizing the importance of pre-cleaning.

## Conclusion

Vaporized HOCl is an effective, eco-friendly disinfectant for dental clinics, with its performance influenced by medium composition and exposure time.

## Keywords

Hypochlorous acid, Infection control, Sustainable disinfectants, Surface contamination, Eco-friendly disinfectants, SDG 3 (Good Health and Well-Being), SDG 12 (Responsible Consumption and Production).

## INTRODUCTION

Dental clinics present unique challenges in infection control, primarily due to the frequent generation of bioaerosols during AGPs. These bioaerosols, produced from saliva and other bodily fluids, can settle on various surfaces and equipment, leading to surface contamination and increased cross-transmission risks.<sup>1-3</sup> Conventional methods, such as spray-and-wipe techniques and the use of disposable barriers, help reduce contamination, but there is growing interest in more sustainable solutions that can ensure both safety and environmental responsibility. HOCl emerges as a promising, eco-friendly disinfectant with broad-spectrum antimicrobial efficacy, offering a viable solution to minimize contamination risks effectively.<sup>4</sup>

The effectiveness of infection control strategies in dental settings relies heavily on staff adherence to stringent hygiene protocols, including regular handwashing and surface decontamination.<sup>5,6</sup> However, bioaerosols from saliva can linger in the clinic environment and settle on multiple surfaces, often escaping thorough cleaning between patient appointments. The high-risk occupational exposure to saliva makes dental staff particularly vulnerable to pathogens carried in saliva. Factors such as patient health, type of procedure, infection control practices, and clinic ventilation can significantly affect the potential for pathogen dissemination.<sup>7</sup>

Saliva droplets serve as vectors for a broad range of infectious agents, including bacteria, viruses, and fungi, that can survive for extended periods on surfaces depending on environmental conditions.<sup>8,9</sup> Microorganisms within saliva, such as *Streptococcus* and *Candida* species, can form biofilms on clinic surfaces, which not only make disinfection challenging but also act as reservoirs that perpetuate cross-infection risks.<sup>10,11</sup>

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The microbial composition within saliva varies widely across individuals and is influenced by diet, hygiene, and systemic health.<sup>12-14</sup> Saliva, while playing a key role in immune defence, can also facilitate biofilm formation on surfaces, complicating disinfection protocols and heightening the importance of effective surface management strategies.

The recent COVID-19 pandemic underscored the importance of mitigating saliva contamination, given SARS-CoV-2's potential to spread through salivary droplets and aerosols generated during dental procedures.<sup>15</sup> COVID-19 demonstrated how bioaerosols could carry viral particles over distances and allow them to linger in clinical environments, potentially compromising the safety of dental staff and patients alike. The sustainability of infection control practices became a focal point, with HOCl gaining attention as an effective, sustainable option for managing both surface and aerosolized contaminants.<sup>16,17</sup>

HOCl, with its ability to penetrate biofilms and eliminate both bacterial and fungal pathogens, offers a promising alternative to conventional chemical disinfectants, effectively disrupting biofilm formation while maintaining a safer profile for clinic environments. In addition to HOCl's potential benefits, the oral cavity's diverse microbiome, which includes bacteria, viruses, and fungi, presents ongoing challenges in maintaining a balanced environment within the clinic.

HOCl's efficacy in deactivating pathogens on surfaces, coupled with its ability to degrade quickly into environmentally safe byproducts, highlights its potential as a mainstay in dental clinic disinfection protocols. In the context of bioaerosol management, preprocedural mouth rinses can reduce salivary microbial load but cannot fully eliminate contaminants, underscoring the need for a complementary surface disinfectant capable of comprehensive microbial elimination.<sup>18,19</sup> By integrating HOCl into routine cleaning practices, dental clinics can advance towards a more sustainable approach to infection control, reducing reliance on single-use plastics and harsh chemicals while maintaining a safe clinical environment for both patients and staff. Incorporating a vaporizer to dispense HOCl offers an efficient method for achieving comprehensive surface disinfection in dental clinics.

The vaporized HOCl can reach hard-to-clean areas and delicate equipment that may not be adequately sanitized through traditional methods, reducing contamination from bioaerosols and saliva particles generated during dental procedures. Using a vaporizer to disperse HOCl as a fine mist ensures even, thorough coverage across all surfaces, including high-touch areas, and helps break down biofilms that harbor infectious agents. This approach not only enhances the efficacy of HOCl in maintaining clinic hygiene but also supports sustainable infection control by minimizing the need for excessive manual cleaning and harsh chemicals.

This study hypothesizes that vaporized HOCl can effectively reduce saliva contamination in dental clinics across both wet and dry conditions. Given the role of saliva as a major vector for microbial transmission, particularly during aerosol-generating procedures, we aim to evaluate HOCl's efficacy in managing microbial contamination on surfaces exposed to saliva. The study will assess HOCl's disinfectant performance against a range of challenge media, representing various microbial loads and environmental conditions typical of dental settings. By comparing its effectiveness across

different mediums and surface states, this research seeks to establish HOCl vapor as a viable, sustainable solution for infection control in dental clinics.

In addition, this study aligns with the United Nations Sustainable Development Goals, particularly SDG 3 (Good Health and Well-Being) and SDG 12 (Responsible Consumption and Production). By evaluating the efficacy of vaporized HOCl as an eco-friendly disinfectant, the research supports the promotion of safe, sustainable healthcare practices that minimize environmental impact while enhancing infection control in dental clinics. The adoption of HOCl as a disinfectant helps reduce reliance on harsh chemicals and single-use plastics, contributing to more sustainable infection control solutions and improving the health and safety of both dental professionals and patients.

## MATERIALS AND METHODS

### Disinfectant and delivery

A novel approach was used in this study, where the Ultra-Low Volume (ULV) vaporizer dispensed IvoCLEAN DUWL disinfectant in vaporized form for a 1-minute exposure, followed by a 2-minute dwell time. The spray volume settings on the ULV cold fogging sprayer were set to the maximum level to ensure the widest coverage and fine particles of approximately 20 µm, as recommended by the manufacturer. This controlled method optimized the conversion of liquid into fine particles.

IvoCLEAN DUWL disinfectant, containing 0.05% anolyte HOCl at a pH of 5-7, was used. It is designed to decontaminate and remove biofilm from dental unit waterlines (DUWL) and is eco-friendly, non-toxic, and free of alcohol, formaldehyde, and bleach. For this study, the product was diluted to a 0.005% HOCl solution (1-part IvoCLEAN to 9 parts water), making it an ideal choice for surface decontamination in addition to its intended use in DUWL maintenance.

The study utilized a Neptune Portable Electric ULV cold vaporizer/fogger, which is known for efficiently converting liquid solutions into a fine mist of consistent particle size (20-50 microns). This ULV technology maximizes surface coverage, reduces disinfectant usage, and minimizes environmental impact. The vaporizer's 4-liter tank capacity prevents spills and minimizes the need for frequent refills, offering convenience and efficiency in a compact, portable design.

During exposure, 96-well microtiter plates were positioned within a fume cupboard to contain aerosols. The plates were opened at the beginning of exposure and closed after the 2-minute dwell time to ensure controlled exposure to the disinfectant. IvoCLEAN DUWL disinfectant is widely used in dental clinics for DUWL decontamination, making it an ideal product for evaluating its potential for dual use—both for cleaning DUWLs and for surface decontamination in dental environments.

### Preparation of whole saliva

Whole saliva samples from two investigators, both dentists that voluntarily provided samples replicate a real-life culture scenario. The participants refrained from brushing their teeth for 24 hours prior to sample collection, which occurred in the morning after chewing paraffin wax for 5 minutes. All saliva was collected in sterile vials following infection control protocols, with any excess disposed of after the experiment. Genetic microbial identification was not performed, and only

agar plate inoculation was conducted. Bacterial samples were suspended in 100ml phosphate-buffered saline, and a mixed colony was used to create a McFarland standard of 1 in phosphate-buffered saline (PBS). Standardization of the McFarland standard was achieved using a DensiCheck plus instrument.

#### Challenge mediums used in the study.

In this study, a range of growth mediums was utilized to evaluate pathogen behaviour and growth under controlled conditions. The challenge media used in the study were prepared according to standard protocols to replicate the variety of environments encountered in dental clinics. Distilled Water (DW), a purified, sterile medium free from salts and ions, served as a baseline control for pathogen growth without external nutrients. Phosphate-Buffered Saline (PBS), a stable salt solution, provided a pH-neutral environment that supports cell suspension without altering physiological conditions. Nutrient-rich mediums such as Yeast Peptone Dextrose Agar (YPD), Brain Heart Infusion Broth (BHI), and Tryptone Soy Broth (TSB) were included to foster microbial growth under nutrient-enhanced conditions. YPD, containing yeast extract, peptone, and dextrose, supports microbial proliferation, while BHI, derived from beef heart and brain infusions, supplies essential nutrients to sustain bacterial cultures. TSB, comprising soybean meal and casein digest, is versatile in supporting a broad spectrum of microbial species.<sup>20</sup> The challenge mediums that were selected to assist in understanding the basic cultivation and growth of pathogens. They don't necessarily emulate the oral cavity in its entirety, but they do allow for a controlled environment allowing for research into the characteristics of microorganisms and their response to a chemical application such as a disinfectant.

#### 96-well microtiter plates inoculation

24-hour whole saliva isolates were standardized in phosphate-buffered saline to 1.0 McFarland. The McFarland Standards are turbidity standards used to approximate the number of microorganisms present within a liquid suspension. The cell density was adjusted to 1 McFarland standard ( $3 \times 10^6$  CFU/mL) by measuring absorbance in a spectrophotometer at a wavelength of 530 nm in PBS. This was followed by mixing 10ml of the 1 McFarland standard with 10ml of a challenge medium to achieve a 0.5 McFarland ( $1.5 \times 10^6$  CFU/mL). The challenge mediums are: 1.) DW, 2.) PBS, 3.) YPD medium, 4.) BHI medium, and 5.) TSB in flat-bottom 96-well microtiter plates. Each microorganism was placed in its individual well plate that were subdivided into the various mediums used. 10 µl of this 0.5 McFarland standard/challenge medium was placed in each well.



Image 1: Inoculation of 96- well microtiter plates

**Part A: Dry challenge via dried inoculum:** 96- well microtiter plates were inoculated by applying 10 µL of the challenge suspension of micro-organisms to the wells, which was then kept in a sterile fume cupboard at ambient temperature overnight to allow drying of the inoculum in the wells. The inoculated wells remained closed to avoid contamination. Drying occurred in these 96-well microtiter plates at ambient temperatures. This stage of the experiment aimed to simulate scenarios of post AGP contamination, where bioaerosols settle on surfaces and dry either due to inadequate disinfection or the challenges of cleaning hard-to-reach places

**Part B: Wet challenge via wet inoculum:** 96-well microtiter plates were inoculated by applying 10 µL of the challenge suspension of micro-organisms in the 96 well plates. The inoculated wells remained closed to avoid contamination. This part of the experiment was aimed to simulate the surface contamination that occurs immediately after AGP.

#### Experimental design – fume cupboard

All experiments were conducted in a climate-controlled laboratory, maintaining an ambient temperature of  $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and relative humidity between 40–60% to minimize external factors that could affect microbial growth or the stability of the disinfectant. To ensure consistency, all substrates were of the same type and size, and control groups were included to assess natural microbial growth without disinfectant exposure. These controls were subjected to the same conditions as experimental samples but without HOCl application. Additionally, the experimental setup was standardized for both wet and dry conditions to ensure comparability across groups.

The ventilated fume cupboard at the University of the Western Cape dental research laboratory was used to safely conduct the study. The fume cupboard has a volume of 900 litres and approximate dimensions of 1 meter in depth, 1 meter in width, and 0.9 meters in height. It was used to provide a controlled environment to prevent any exposure and contamination. Within the fume cupboard, the 96-well microtiter plates containing the challenge mediums were positioned securely.



Image 2: Ventilated fume cupboard in the dental research laboratory

After exposing the 96-well microtiter plates to HOCl vapor in the fume cupboard, cell viability and proliferation were assessed using the XTT assay. This colorimetric assay measures cellular enzyme activity by converting XTT to an orange formazan product, indicating metabolically active

cells. Each well contained 100 µL of challenge medium and 50 µL of XTT solution from the Cell Proliferation Kit II (Roche Diagnostics GmbH, Mannheim, Germany). The plates were incubated for two hours, and absorbance was measured at 450 nm using a Smart Microplate Reader (Model SMR16.1, USCN Life Science Kit Inc., Wahan, China).



Image 3: Portable electric ULV cold vaporiser in ventilated fume cupboard

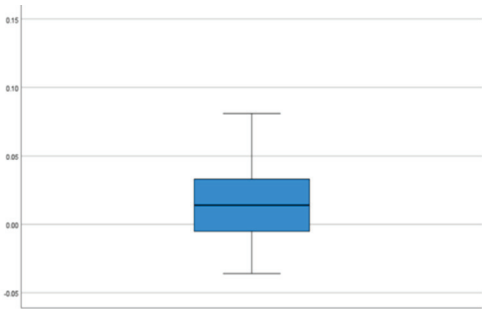
Statistical analysis was performed to assess the effects of HOCl on whole saliva across five challenge mediums by analysing optical density (OD). At least three biological replicates were tested, with triplicates for each, ensuring robust results. A Two-Way ANOVA with repeated measures was used to analyse the effects of time, medium, and their interaction on pathogen levels. Post-hoc tests, including Tukey's Honestly Significant Difference (HSD) and Scheffe tests, identified significant differences between mediums. Statistical assumptions, such as normality and sphericity, were validated using the Shapiro-Wilk and Mauchly's Test of Sphericity.

RESULTS

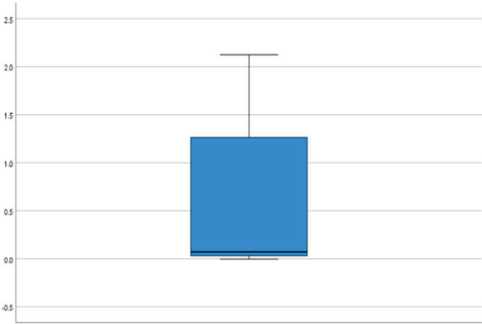
The graph illustrates pathogen growth in whole saliva under wet and dry conditions, showing significant growth from 2 hours to 24 hours. YPD medium consistently exhibited

the highest proliferation in both conditions, with OD values increasing from 0.050 to 0.060 in dry conditions and from 0.085 to 0.227 in wet conditions. DW medium showed minimal growth, from 0.010 to 0.001 in dry conditions and from 0.01 to 0.025 in wet conditions. Overall, the wet study exhibited higher OD values at both 2 and 24 hours, suggesting greater microbial growth compared to the dry study, where HOCl was more effective in inhibiting growth.

The results of the whole saliva growth study reveal significant differences in OD across dry and wet conditions, as well as between time intervals and mediums as resulted by the following graphs:

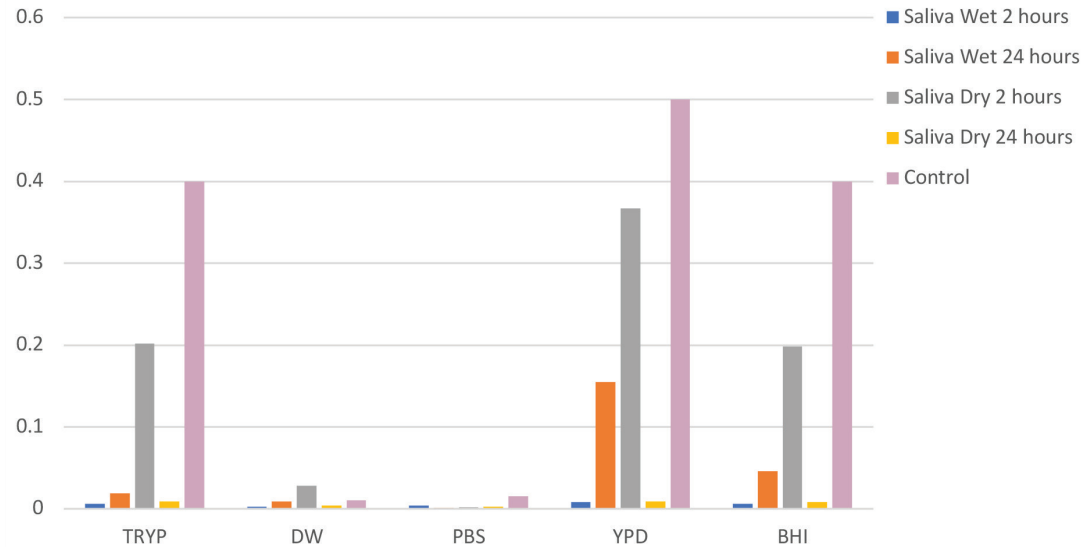


Graph 2: Effect of HOCl vapor on the whole saliva OD values after 2 hours in dry conditions.



Graph 3: Effect of HOCl vapor on the whole saliva OD values after 24 hours in dry conditions

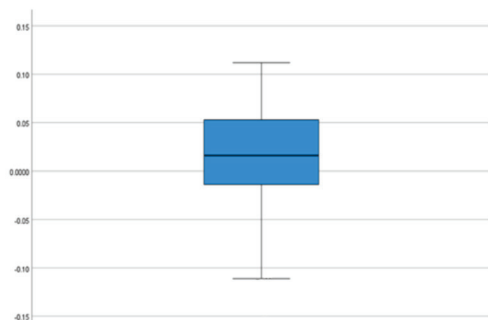
Saliva Growth in Different Mediums



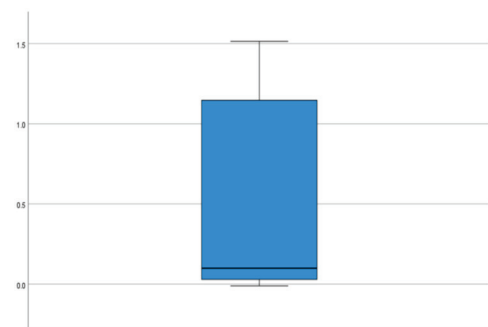
Graph 1: The graph illustrates unidentified pathogen growth in whole saliva under wet and dry conditions, showing a significant increase from 2 hours to 24 hours.



In the dry condition (Part A), there was a significant effect of time ( $p < 0.001$ ), with OD increasing markedly from 2 hours (mean = 0.038) to 24 hours (mean = 0.863), indicating higher cell proliferation over time. The effect of medium was also significant ( $p = 0.010$ ), with YPD exhibiting the highest OD at 2 hours, while TSB medium showed the highest OD at 24 hours, suggesting variation in medium-specific support for cell growth over time. Notably, YPD displayed consistently high OD values at both 2 and 24 hours, indicating substantial cell density.



**Graph 4:** Effect of HOCl vapor on the whole saliva OD values after 2 hours in wet conditions



**Graph 5:** Effect of HOCl vapor on the whole saliva OD values after 24 hours in wet conditions

#### Impact of Duration (Time-Dependent Changes)

In the wet condition (Part B), time had a significant effect ( $p < 0.05$ ), with OD increasing from 2 hours (mean = 0.006) to 24 hours (mean = 0.815). The effect of medium was also significant ( $p < 0.05$ ), with YPD yielding the highest OD at 24 hours, followed by BHI, suggesting that nutrient-rich mediums enhance cell growth. The interaction effect further demonstrated that YPD consistently produced the highest OD across both 2 and 24 hours, indicating optimal conditions for cell proliferation.

#### Influence of Medium on Growth and Inhibition

In the wet study, the disinfectant had minimal effect at 2 hours in DW and PBS, with OD values similar to controls, suggesting limited growth inhibition. However, in YPD, BHI, and TSB, OD values were higher than controls, indicating reduced efficacy. By 24 hours, the disinfectant showed stronger antimicrobial effects, with OD values in all mediums lower than controls, particularly in YPD (0.227) and BHI (0.091), suggesting potent inhibition over time.

#### Effect of Moisture Content (Dry vs Wet Conditions)

In the dry study, the disinfectant showed minimal effect in DW and PBS at 2 hours, with OD values close to controls. However, in YPD, BHI, and TSB, slightly higher OD values were observed, indicating moderate effectiveness. After 24 hours, the disinfectant demonstrated more pronounced

antimicrobial activity, with OD values higher than controls in most mediums, particularly in YPD (0.060) and BHI (0.059), indicating significant inhibition.

#### Comparison of Efficacy in Wet vs Dry Conditions

Comparing wet and dry conditions, HOCl was more effective in the dry study, with lower average OD values at both 2 hours (0.024) and 24 hours (0.0356). In contrast, the wet study showed higher OD values at both time points (2 hours: 0.0398, 24 hours: 0.0974). The log kill values in the dry study ranged from 0.695 to 0.954 across different mediums, indicating variable effectiveness of HOCl, with stronger inhibition in YPD, BHI, and TSB. These findings highlight the differential efficacy of IvoCLEAN DUWL disinfectant under varying environmental conditions and its potential for use in diverse disinfecting contexts.

The results of the IvoCLEAN study demonstrate that vaporized HOCl significantly reduced microbial growth, particularly in whole saliva samples, under varying experimental conditions. However, to fully understand the factors influencing this antimicrobial activity, it is crucial to explore how different challenge media and exposure times impacted the disinfectant's efficacy. Notably, the effects of time and medium composition on microbial reduction were found to vary significantly, providing insights into how the presence of organic matter and nutritional content in the medium can influence pathogen survival. These findings are essential for optimizing disinfection protocols in clinical settings, where variations in surface contamination and environmental factors are common.

#### Discussion

This study hypothesized that vaporized HOCl could effectively reduce saliva contamination in dental clinics under both wet and dry conditions, given the role of saliva in microbial transmission. The findings confirm this hypothesis, demonstrating that HOCl effectively reduces microbial contamination on well plates contaminated with saliva, which is a simulation of surfaces exposed to saliva in dental clinics. This result is crucial for minimizing infection risks, particularly in dental settings where aerosol-generating procedures are common. The study's experimental conditions, using various challenge media, replicate typical environmental factors in dental clinics, supporting the potential for HOCl vapor as a viable, eco-friendly solution for infection control.

Beyond its clinical benefits, this study aligns with the United Nations Sustainable Development Goals, particularly Goal 3: Good Health and Well-being and Goal 6: Clean Water and Sanitation. Ensuring effective infection control in dental clinics is essential for preventing healthcare-associated infections, directly supporting Goal 3 by safeguarding patient health and improving overall quality of care. Furthermore, by exploring environmentally friendly disinfection agents like HOCl, our research promotes sustainable practices in healthcare. HOCl represents a safer, non-toxic alternative to traditional disinfectants, reducing the risk of chemical exposure for patients and healthcare workers while contributing to the responsible management of resources in healthcare settings, as emphasized in Goal 6.

#### Efficacy of HOCl Concentrations in Dental Disinfection

HOCl is widely used for dental disinfection, with concentrations ranging from 0.01% to 0.1% for routine cleaning and up to 0.5% for blood contamination. Lower

concentrations, such as 0.005% to 0.02%, are effective in dental settings for surface disinfection and reducing cross-infection risks. Aerosolized HOCl concentrations of 0.002% to 0.01% show strong antimicrobial activity. The IvoCLEAN study uses 0.005% HOCl in vaporized form, which aligns with recommendations for effectively eliminating biofilm-forming microorganisms in DUWL. This concentration offers a potent, safe approach to dental surface disinfection.

#### Efficacy of HOCl in Reducing Microbial Growth on Whole Saliva

The results of the IvoCLEAN study on whole saliva demonstrated that HOCl was effective in reducing microbial growth. Lower OD values were observed in dry conditions indicating greater efficacy compared to wet conditions. Over a 24-hour period, samples treated with HOCl maintained lower OD values, suggesting a lower presence of microbial contaminants and better disinfection efficacy. The effectiveness of HOCl in whole saliva is supported by literature highlighting its ability to eliminate pathogens through disruption of cellular walls, resulting in cell inactivation.<sup>12,21-23</sup>

#### Impact of Time and Medium on HOCl Efficacy in Dental Disinfection

The results concerning the impact of time and various challenge mediums are crucial for evaluating the disinfectant's efficacy. The significant effect of time provides insight into understanding what influences pathogen proliferation. The study strategically chose different mediums with varying levels of nutritional content to simulate clinical scenarios involving splatter or large bioaerosol contaminated with microorganisms, either with organic matter (from the oral cavity) or smaller aerosolized particles without organic matter. The IvoCLEAN study used a range of mediums to mimic the organic matter and biological fluids typically found in the oral cavity. DSW served as a sterile control medium, and PBS provided a stable environment for studying the behaviour of the microorganism without altering the physiological environment. YPD, TSB, and BHI were nutrient-rich mediums supporting microbial growth, by providing nutritional support, that allowed for the proliferation of the microorganism. Through the use of these mediums, realistic conditions were simulated to obtain comprehensive results into bio-decontamination processes within dental clinics.

When interpreting the results of the effect of time and medium on the efficacy of the IvoCLEAN DUWL disinfectant, the following conclusions were made. In terms of efficacy, the results indicate that low-nutrient media (like distilled water and phosphate-buffered saline) were more effective at reducing microorganism concentrations and inhibiting cellular proliferation when exposed to HOCl. This is reflected in low OD values, which are indicative of fewer viable cells. The reduced presence of nutrients likely limits the growth of microorganisms, enabling HOCl to more effectively target and deactivate them.

On the other hand, high-nutrient media (such as TSB, BHI, and YPD) supported moderate efficacy of HOCl. The slightly higher OD values suggest that these media, which are rich in nutrients, allowed for more microbial growth, despite exposure to HOCl. In wet conditions, HOCl was still effective, but the presence of additional nutrients (from these media) provided more favourable conditions for the survival and proliferation of microorganisms.

The key takeaway here is that the efficacy of HOCl as a disinfectant is influenced by the nutritional content of the medium. Media with fewer nutrients appear to be more conducive to effective microbial deactivation, while richer media can potentially support microbial growth, even in the presence of HOCl. This highlights the importance of considering the nutrient composition of environments when evaluating the effectiveness of disinfectants.<sup>24,25</sup>

This aligns with other studies that show how specific conditions can influence pathogen growth patterns.<sup>26,27</sup> Although the mediums used in this study do not directly represent the human oral cavity, they provide a controlled environment to study microbial behaviour and the impact of disinfectants.<sup>11</sup> In the case of YPD, the nature of the medium can serve as a surrogate for organic matter that could be present in the oral cavity, similarly with the nutrient-rich BHI. The clinical significance of these findings reiterates the need to clean effectively and remove any residual matter that may remain on surfaces in the dental clinic.<sup>1</sup> This will support the studies that suggest pre-cleaning and removal of visible organic matter aids in complete decontamination.<sup>4</sup>

The significant interaction effect between time and medium groups further highlights the dynamic relationship between environmental factors and microbial growth dynamics.<sup>25</sup>

The statistical findings suggest that the influence of medium groups on pathogen levels varies over time in the whole saliva experiments, since the pathogen levels differed between the 2-hour and 24-hour time points. This can be attributed to the response of the microorganism to their environment over time. The time factor result reinforces the importance of effective disinfection protocols that prevent pathogens from establishing and proliferating over time, forming biofilm.<sup>1</sup>

Using a vaporized form of HOCl based infection ensures comprehensive coverage of surfaces and rapid action, especially in hard-to-reach areas, which is essential for maintaining a pathogen-free environment between patients. The efficacy of HOCl in inhibiting microbial growth shows a moderate decline from its initial effectiveness at 2 hours to 24 hours across various mediums.

The efficacy of HOCl was shown to be influenced by both medium composition and moisture content. Low-nutrient mediums such as DW and PBS demonstrated consistent effectiveness, with sustained low OD values even after 24 hours, suggesting they maintain microbial inhibition over extended periods. In contrast, nutrient-rich mediums like YPD, BHI, and TSB saw a significant increase in OD values, indicating decreased disinfectant efficacy and higher microbial proliferation over time.<sup>28,29</sup> This highlights the crucial role of medium composition in determining the effectiveness of disinfectants, as nutrient-rich environments can support microbial growth even in the presence of disinfectants. These findings have significant implications for future research in surface contamination management.<sup>30</sup>

The clinical implications of these findings are significant, especially in dental settings where splatter and biofilm formation can occur on various surfaces during procedures. The study's assessment of both wet and dry conditions is critical because dental splatter can vary in moisture content, influencing disinfectant efficacy.<sup>9,24,28</sup> Effective disinfectants like HOCl may be particularly vital in controlling microbial

growth in low-moisture environments where pathogens may otherwise proliferate. Therefore, understanding the role of medium composition and moisture in disinfection can aid in optimizing infection control strategies in dental clinics, improving patient safety and reducing the risk of cross-contamination.<sup>29,30</sup>

In conclusion, the findings of this study underscore the importance of both time and medium composition in influencing the efficacy of HOCl as a disinfectant in dental clinics. The time-dependent changes observed across various mediums, particularly in wet and dry conditions, highlight the dynamic relationship between disinfectant effectiveness and environmental factors. Nutrient-rich mediums such as YPD and BHI supported microbial growth, underscoring the need for comprehensive cleaning practices to address organic matter in clinical settings. On the other hand, low-nutrient mediums like DW and PBS consistently supported HOCl's antimicrobial efficacy, suggesting that surface cleanliness plays a pivotal role in ensuring successful disinfection. The interaction of time and medium further emphasizes the necessity for timely and effective disinfection protocols to prevent microbial proliferation over extended periods, ultimately contributing to safer clinical environments.

## CONCLUSION

This study demonstrates that vaporized HOCl is an effective disinfectant for reducing microbial contamination, including saliva-based pathogens, in dental clinics. Its effectiveness was influenced by the moisture content of surfaces and the type of medium, with dry conditions generally showing better results in terms of microbial inhibition. The use of HOCl in vaporized form offers an eco-friendly and efficient solution for infection control in dental settings, ensuring comprehensive surface decontamination, especially in hard-to-reach areas. The findings support its integration into infection control protocols, aligning with sustainable healthcare practices, and highlighting its potential for enhancing patient safety and reducing the risk of cross-contamination in dental clinics.

## REFERENCES

1. Van der Weijden F. (2023). Aerosol in the oral health-care setting: a misty topic. *Clinical oral investigations*, 27(Suppl 1), 23–32. <https://doi.org/10.1007/s00784-023-05034-x>
2. Lahdentausta, L., Sanmark, E., Lauretsalo, S., Korkee, V., Nyman, S., Atanasova, N., Oksanen, L., Zhao, J., Hussein, T., Hyvärinen, A., & Paju, S. (2022). Aerosol concentrations and size distributions during clinical dental procedures. *Heliyon*, 8(10), <https://doi.org/10.1016/j.heliyon.2022.E11074>
3. Malmgren, R., Välimaa, H., Oksanen, L., Sanmark, E., Nikuri, P., Heikkilä, P., Hakala, J., Ahola, A., Yli-Urpo, S., Palomäki, V., Asmi, E., Sofieva, S., Rostedt, A., Laitinen, S., Romantschuk, M., Sironen, T., Atanasova, N., Paju, S., & Lahdentausta-Suomalainen, L. (2023). High-volume evacuation mitigates viral aerosol spread in dental procedures. *Scientific Reports* 2023 13:1, 13(1), 1–8. <https://doi.org/10.1038/s41598-023-46430-3>
4. World Health Organisation. (2021). Hypochlorous acid: Expert review for the 2021 Essential Medicines List. World Health Organisation.
5. Cerghizan, D., János, K. M., Ciurea, C. N., Popelea, O., Balos, M. D., Craciun, A. E., Hantoiu, L. G., & Albu, A. I. (2023). The efficacy of three types of disinfectants on the microbial flora from the surface of impression materials used in dentistry—An in vitro study. *Applied Sciences*, 13(2), 1097. <https://doi.org/10.3390/app13021097>
6. Matys, J., Gedrange, T., Dominiak, M., & Grzech-Leśniak, K. (2023). Quantitative Evaluation of Aerosols Produced in the Dental Office during Caries Treatment: A Randomized Clinical Trial. *Journal of Clinical Medicine*, 12(14). <https://doi.org/10.3390/JCM12144597>
7. Centers for Disease Control and Prevention (CDC). (2024). Chemical disinfectants: Disinfection & sterilization guidelines. Retrieved from <https://www.cdc.gov>
8. World Health Organisation (WHO). (2024). Cleaning and disinfection of environmental surfaces in the context of COVID-19. Retrieved from
9. Mupparapu, M., & Kothari, K. R. M. (2019). Review of surface disinfection protocols in dentistry: A 2019 update. *Quintessence International*, 50(1), 58–65. <https://doi.org/10.3290/j.qi.a41337>
10. Allison, J. R., Currie, C. C., Edwards, D. C., Bowes, C., Coulter, J., Pickering, K., Kozhevnikova, E., Durham, J., Nile, C. J., Jakubovics, N., Rostami, N., & Holliday, R. (2021). Evaluating aerosol and splatter following dental procedures: Addressing new challenges for oral health care and rehabilitation. *Journal of Oral Rehabilitation*, 48(1), 61–72. <https://doi.org/10.1111/joor.13098>
11. Fujinami, W., Nishikawa, K., Ozawa, S., Hasegawa, Y., & Takebe, J. (2021). Correlation between the relative abundance of oral bacteria and *Candida albicans* in denture and dental plaques. *Journal of Oral Biosciences*, 63(2), 175–183. <https://doi.org/10.1016/j.job.2021.02.003>
12. Cerghizan, D., János, K. M., Ciurea, C. N., Popelea, O., Balos, M. D., Craciun, A. E., Hantoiu, L. G., & Albu, A. I. (2023). The efficacy of three types of disinfectants on the microbial flora from the surface of impression materials used in dentistry—An in vitro study. *Applied Sciences*, 13(2), 1097. <https://doi.org/10.3390/app13021097>
13. Sinha, D., Kumar, C., Gupta, A., Nayak, L., Subhash, S., & Kumari, R. (2020). Knowledge and practices about sterilization and disinfection. *Journal of Family Medicine and Primary Care*, 9(2), 793–798. [https://doi.org/10.4103/jfmpc.jfmpc\\_1069\\_19](https://doi.org/10.4103/jfmpc.jfmpc_1069_19)
14. Adachi, T., Kawanishi, N., Ichigaya, N., Sugimoto, M., Hoshi, N., & Kimoto, K. (2022). A preliminary pilot study: Metabolomic analysis of saliva in oral candidiasis. *Metabolites*, 12(12), 1250. <https://doi.org/10.3390/metabo12121294>
15. Maillard, J. Y., & Centeleghe, I. (2023). How biofilm changes our understanding of cleaning and disinfection. *Antimicrobial Resistance & Infection Control*, 12, 95. <https://doi.org/10.1186/s13756-023-01290-4>
16. Oliveira, I. M., Gomes, I. B., Simões, L. C., & Simões, M. (2024). A review of research advances on disinfection strategies for biofilm control in drinking water distribution systems. *Water Research*, 253, 121273. <https://doi.org/10.1016/j.watres.2024.121273>
17. Atukorallaya, D. S., & Ratnayake, R. K. (2021). Oral Mucosa, Saliva, and COVID-19 Infection in Oral Health Care. *Frontiers in medicine*, 8, 656926. <https://doi.org/10.3389/fmed.2021.656926>
18. Lauritano, D., Moreo, G., Limongelli, L., Nardone, M., & Carinci, F. (2020). Environmental disinfection strategies to prevent indirect transmission of SARS-CoV-2 in healthcare settings. *Applied Sciences*, 10(18), 6291. <https://doi.org/10.3390/app10186291>
19. Jasim, Z. M., & Abass, S. M. (2022). The Effect of Hypochlorous Acid Disinfectant on the Reproduction of Details and Surface Hardness of Type III Dental Stone. *Cureus*, 14(11), e32061. <https://doi.org/10.7759/cureus.32061>
20. Cheng-Feng, T., Jia-Jia, C., Shinn-Jyh, D., & Chun-Cheng, C. (2024). *In vitro* cytotoxicity and antibacterial activity of hypochlorous acid antimicrobial agent. *Journal of Dental Sciences*, 19(1), 345–356. <https://doi.org/10.1016/j.jds.2023.07.007>
21. Gumru, B., Tarcin, B., & Idman, E. (2021). Cross-contamination and infection control in intraoral digital imaging: a comprehensive review. *Oral Radiology*, 37(2), 180–188. <https://doi.org/10.1007/S11282-020-00452-Z>
22. Holliday, R., Allison, J. R., Currie, C. C., Edwards, D. C., Bowes, C., Pickering, K., Reay, S., Durham, J., Lumb, J., Rostami, N., Coulter, J., Nile, C., & Jakubovics, N. (2021). Evaluating contaminated dental aerosol and splatter in an open plan clinic environment: Implications for the COVID-19 pandemic. *Journal of Dentistry*, 105. <https://doi.org/10.1016/j.jdent.2020.103565>
23. Kameda, T., Oka, S., Igawa, J. I., Sakamoto, M., & Terada, K. (2022). Can hypochlorous acid be a powerful sanitizer to replace alcohol for disinfection? -Its bactericidal, degradation of the solutions under various storage condition, and steel rust effects. *Dental materials journal*, 41(1), 167–183. <https://doi.org/10.4012/dmj.2021-146>
24. Kundra, P., Goswami, S., & Parameswari, A. (2020). Advances in vaporisation: A narrative review. *Indian Journal of Anaesthesia*, 64(3), 171. [https://doi.org/10.4103/IJA.IJA\\_850\\_19](https://doi.org/10.4103/IJA.IJA_850_19)
25. Mehendale, F. V., Clayton, G., Homyer, K. M., & Reynolds, D. M. (2023). HOCl vs OCl<sup>-</sup>: clarification on chlorine-based disinfectants used within clinical settings. *Journal of Global Health Reports*, 7, e2023052. <https://doi.org/10.29392/001C.84488>
26. Merk (2024) Merk XTT Available at: <https://www.sigmaaldrich.com/ZA/en/product/roche/11465015001> (Accessed 5 December 2024)
27. Motwani, N., Ikhar, A., Chandak, M., & Gondivkar, S. (2021). Disinfection Measures during COVID-19 for Dental Operatories. *The Open Dentistry Journal*, 14(1), 305–309. <https://doi.org/10.2174/1874210602115010305>
28. Mupparapu, M., & Kothari, K. R. M. (2019). Review of surface disinfection protocols in dentistry: a 2019 update. *Quintessence International* (Berlin, Germany : 1985), 50(1), 58. <https://doi.org/10.3290/J.QI.A41337>
29. Nguyen, K., Bui, D., Hashemi, M., Hocking, D. M., Mendis, P., Strugnelli, R. A., & Dharmage, S. C. (2021). The Potential Use of Hypochlorous Acid and a Smart Prefabricated Sanitising Chamber to Reduce Occupation-Related COVID-19 Exposure. *Risk Management and Healthcare Policy*, 14, 247. <https://doi.org/10.2147/RMHP.S284897>
30. WHO. (2020). Cleaning and disinfection of environmental surfaces in the context of COVID-19: interim guidance, 15 May 2020. Accessed in 15.5.2024. <https://iris.who.int/handle/10665/332096>