



An Improved Protocol for Indoor Airborne Bacterial Enumeration and Elimination Using a Cold- Plasma Air Sanitizer: A pilot study

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ABSTRACT

Airborne bacteria pose significant health risks in indoor environments, particularly for immunocompromised individuals. Air sanitizers represent a promising intervention for reducing microbial contamination in enclosed spaces. Objectives: This study evaluated the efficacy of a cold-plasma air sanitizer in reducing airborne bacterial contamination and developed a standardized protocol for bacterial enumeration in indoor air samples. Methods: Air samples (500 L per sample) were collected using a Sugold FX-100ST microbial air sampler (flow rate 100 L/min for 5 minutes) from microbiology and hematology teaching laboratories (1000 m³ room volume) at Gulf Medical University. Samples were collected immediately before sanitizer activation (baseline) and at 30-minute intervals during cold-plasma treatment (TPA Air Technology, Germany) for 24 hours. Samples were directly inoculated onto Chocolate agar, CLED agar, and Chromogenic UTI agar and incubated at 37°C for 24-48 hours. Total airborne particle counts (0.3-10 µm) were measured using an Atmotech particle counter. Experiments were performed in triplicate on three separate days (n=9 per time point). Bacterial identification was performed using Gram staining and biochemical tests. Statistical analysis employed paired t-tests and one-way ANOVA (p<0.05). Results: Significant bacterial reductions were observed following sanitizer activation. Baseline bacterial concentrations were 75.4±12.3 CFU/500L on Chocolate agar, 59.2±10.8 CFU/500L on CLED agar, and 26.4±6.7 CFU/500L on Chromogenic agar. Mean reduction rates at 24 hours were 94.7%, 96.5%, and 96.1%, respectively. Predominant organisms were *Micrococcus* spp. (62%) and *Bacillus* spp. (28%). Coagulase-positive *Staphylococcus aureus* (n=2) and Gram-negative species including *Burkholderia cepacia* and *Stenotrophomonas maltophilia* were also isolated. Strong positive correlation was observed between particle counts and bacterial concentrations (r=0.89, p<0.001). Conclusion: Cold-plasma air sanitization effectively reduced airborne bacteria by >90% within 24 hours. The standardized enumeration protocol developed in this study provides a simplified approach for indoor air quality assessment. Implementation of cold-plasma technology in healthcare settings could reduce respiratory infection risks for susceptible populations.

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1. Introduction

Airborne microorganisms represent a significant public health concern, contributing to the transmission of respiratory infections and serving as sources of allergens, toxins, and irritants in indoor environments [1]. The World Health Organization estimates that indoor air pollution contributes to approximately 3.2 million premature deaths annually, with respiratory infections accounting for a substantial proportion of this burden [2]. Healthcare-associated infections, many of which are transmitted through the airborne route, affect millions of patients annually and impose significant economic costs on healthcare systems [3,4].

Monitoring and controlling airborne microorganisms is essential for maintaining public health and preventing disease transmission. Various air sampling techniques have been developed, each with distinct advantages and limitations [5]. The Andersen impactor, which separates particles by aerodynamic size, is widely used but may cause desiccation and loss of culturability in sensitive organisms [6]. Filter-based methods using polytetrafluoroethylene (PTFE) membranes can capture a broader size range but may cause desiccation of target organisms during extended sampling [7]. Liquid impingement systems, such as the SKC BioSampler, maintain organism viability more effectively but require immediate processing to prevent die-off [8].

Culture-based methods remain the gold standard for detecting viable organisms through colony-forming unit (CFU) enumeration [9]. These methods allow for the isolation and characterization of viable organisms but are time-consuming and limited to organisms that grow under specified conditions. Molecular methods, including quantitative real-time PCR, metagenomics, biosensors, and CRISPR-based assays, offer rapid detection but cannot distinguish between viable and non-viable organisms and require specialized equipment and expertise [10,11,12].

The development of air sanitation technologies has accelerated in response to growing awareness of indoor air quality issues and the need for effective infection control measures [13]. Unlike filter-based air purifiers that simply trap particles, air sanitizers actively inactivate microorganisms in open air spaces through various mechanisms [14]. Cold-plasma technology has emerged as a promising approach, generating a plasma field that produces reactive oxygen species (ROS) and other antimicrobial species that inactivate bacteria through oxidative damage to cell membranes, proteins, and nucleic acids [15,16]. Atmospheric cold plasma operates at near-room temperature and pressure, making it suitable for occupied spaces [17]. Reusable, washable filters further enhance the sustainability and cost-effectiveness of this technology [18].

Despite growing interest in cold-plasma air sanitizers, standardized protocols for evaluating their efficacy are lacking. Current methods for airborne bacterial enumeration vary widely in sampling volume, culture media, incubation conditions, and data reporting, limiting comparability between studies [19]. The absence of standardized protocols for air volume control and direct inoculation into culture media has hindered the development of reliable, reproducible assessment methods. Furthermore, while the relationship between airborne particle counts and microbial burden has been recognized, few studies have systematically explored this correlation in the context of air sanitation [20].

This study aimed to: (i) evaluate the efficacy of a commercially available cold-plasma air sanitizer in reducing airborne bacterial contamination in indoor laboratory settings; (ii) develop a standardized, simplified protocol for airborne bacterial enumeration using controlled air volume sampling and direct inoculation onto multiple culture media; and (iii) investigate the relationship between airborne particle counts and bacterial concentrations. The findings have implications for infection control in healthcare settings, particularly for immunocompromised patients requiring pathogen-free indoor environments [21].

2. Materials and Methods

2.1 Study Setting and Design

This investigation was conducted in the microbiology and hematology teaching laboratories at the Faculty of Health Sciences, Gulf Medical University, Ajman, United Arab Emirates. Both laboratories have a room volume of approximately 1000 m³ and are equipped with standard HVAC systems providing 6-8 air changes per hour. The study was approved by the Institutional Review Board (Ref. IRB-COHS-FAC-95-MAR-2024).

The experimental design involved collecting standardized air samples (500 L per sample) at baseline (T₀, immediately before sanitizer activation) and at 30-minute intervals during cold-plasma treatment (T₃₀, T₆₀, T₉₀, T₁₂₀, T₁₅₀, T₁₈₀), with a final sample at 24 hours (T₁₄₄₀). Samples were collected in triplicate at each time point on three separate experimental days (n=9 per time point).

2.2 Environmental Conditions

Environmental parameters were monitored throughout the sampling period using a calibrated hygrometer-thermometer (Model 608-H1, Testo, Germany). Temperature was maintained at 22±2°C and relative humidity at 55±5%. Room occupancy was standardized to two operators who remained stationary during sampling to minimize activity-related variability. Ozone concentration was monitored continuously using a UV photometric ozone analyzer (Model 49i, Thermo Scientific, USA) with a detection limit of 0.001 ppm to ensure levels remained within safe exposure limits (<0.05 ppm for 8-hour exposure) [22].

2.3 Air Sampling and Bacterial Enumeration

Air samples were collected using a Sugold FX-100ST microbial air sampler (Sugold, USA) calibrated according to the manufacturer's specifications and verified using a calibrated flow meter (TSI Incorporated, USA). The sampler was operated at a flow rate of 100 L/min for 5 minutes, yielding a total sample volume of 500 L per sampling event. The sampler was positioned at a height of 1.5 m above floor level, approximately at the breathing zone.

Samples were collected by direct impaction onto 90-mm Petri dishes containing Chocolate agar, CLED (Cystine Lactose Electrolyte-Deficient) agar, and Chromogenic UTI agar (Oxoid, UK). All culture media were prepared according to the manufacturer's instructions and pre-incubated at 37°C for 24 hours to verify sterility prior to sampling. Following collection, plates were immediately transferred to the laboratory and incubated at 37°C for 24-48 hours in an inverted position. Colonies were counted using a Quebec colony counter and expressed as CFU per 500 L air sample. Bacterial concentration was also calculated as CFU/m³ using the formula: $\text{CFU/m}^3 = (\text{CFU counted} \times 1000) / \text{air volume sampled (L)}$.

2.4 Cold-Plasma Air Sanitizer

The cold-plasma air sanitizer (TPA Air Technology, Germany) was pre-installed in the HVAC system of both laboratories. The device operates by generating a cold-plasma field through dielectric barrier discharge, producing reactive species including ozone, hydroxyl radicals, and ROS. The device was operated continuously throughout the 24-hour experimental period at the manufacturer's recommended settings (50W power consumption). Filter integrity was assessed before and after each experimental run to ensure consistent performance.

2.5 Particle Count Monitoring

Concurrent with microbiological sampling, total airborne particle counts were measured using an Atmotech particle counter (Atmotech Inc., USA). The instrument was calibrated to detect particles in the size range of 0.3-10 µm at 2.83 L/min flow rate. Measurements were taken at 60-second intervals throughout the study duration at the same sampling height (1.5 m) and locations as the microbiological samples. Data were recorded as particles per cubic meter.



Figure 1 : Left: Atmocube particle recorder

Right: Cold-plasma Air Sanitizer

2.6 Bacterial Identification

Bacterial isolates were preliminarily identified based on colonial morphology on different culture media, Gram reaction, catalase test, oxidase test, and coagulase test. Gram-negative isolates were further identified using the API 20NE system (bioMérieux, France) according to the manufacturer's instructions. Antimicrobial susceptibility testing for Staphylococcus isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to CLSI guidelines [23]. Penicillin (10 µg) and vancomycin (30 µg) disks were used.

2.7 Controls

Negative controls were performed by sampling sterile phosphate-buffered saline (PBS) using the same

procedure to verify media sterility. Positive controls were performed by aerosolizing laboratory-generated *Bacillus subtilis* ATCC 6633 in a controlled environment (isolated biosafety cabinet) and sampling known concentrations (10^3 - 10^4 CFU/m³) to validate the collection efficiency of the air sampler.

2.8 Statistical Analysis

Data were analyzed using SPSS v27.0 (IBM Corp.) and expressed as mean ± SD. Normality was assessed via the Shapiro-Wilk test. Paired t-tests compared baseline versus post-treatment bacterial counts, while one-way ANOVA with Tukey's post-hoc test evaluated differences across multiple time points. Pearson's correlation and linear regression (R^2) examined associations between particle and bacterial counts. Inactivation kinetics were modeled

using first-order decay: $\ln(N_t/N_0) = -kt$. Sample size ($n=9$ per time point; triplicate samples across three days) was determined using G*Power ($\alpha=0.05$, power=0.80, $f=0.25$). Statistical significance was set at $p<0.05$

3. Results

3.1 Bacterial Enumeration

Air samples collected from both laboratories showed substantial bacterial growth on all culture media at baseline (T_0). In the Microbiology Laboratory, mean CFU counts on Chocolate agar were 75.4 ± 12.3 CFU/500L (150.8 ± 24.6 CFU/m³), on CLED agar were 59.2 ± 10.8 CFU/500L (118.4 ± 21.6 CFU/m³), and on Chromogenic agar were 26.4 ± 6.7 CFU/500L (52.8 ± 13.4 CFU/m³) (Table 1). Similar baseline counts were observed in the Hematology Laboratory (Table 2).

Following activation of the cold-plasma sanitizer, bacterial counts decreased progressively with time (Figure 1, Figure 2). Significant reductions were observed as early as 30 minutes post-activation (Chocolate agar:

$p=0.002$; CLED agar: $p=0.004$; Chromogenic agar: $p=0.008$). By 60 minutes, reductions of 57.3%, 62.7%, and 57.7% were observed on Chocolate, CLED, and Chromogenic agar, respectively. At 24 hours, nearly complete elimination was achieved with mean reductions of $94.7 \pm 3.2\%$ (Chocolate), $96.5 \pm 2.8\%$ (CLED), and $96.1 \pm 3.5\%$ (Chromogenic) in the Microbiology Laboratory (Table 1). Similar results were obtained in the Hematology Laboratory (Table 2).

Inactivation kinetics followed first-order decay ($R^2=0.92-0.95$). The calculated rate constants were $0.014 \pm 0.001 \text{ min}^{-1}$ (Chocolate), $0.016 \pm 0.001 \text{ min}^{-1}$ (CLED), and $0.015 \pm 0.001 \text{ min}^{-1}$ (Chromogenic). Corresponding half-lives were 49.5, 43.3, and 46.2 minutes, indicating that approximately 50% of culturable bacteria were inactivated within the first hour of treatment. Figure-4.

Table 1: Bacterial reduction by cold-plasma air sanitizer in Microbiology Laboratory samples (CFU/500L)

Time Point	Chocolate Agar		CLED Agar		Chromogenic Agar	
	CFU/plate (Mean $\pm$ SD)	Reduction (%)	CFU/plate (Mean $\pm$ SD)	Reduction (%)	CFU/plate (Mean $\pm$ SD)	Reduction (%)
Zero (T_0)	75.4 ± 12.3	-	59.2 ± 10.8	-	26.4 ± 6.7	-
30 min	41.3 ± 8.7	45.3	34.2 ± 7.2	42.2	15.1 ± 4.3	42.8
60 min	32.2 ± 6.4	57.3	22.2 ± 5.1	62.7	11.2 ± 3.2	57.7
90 min	25.4 ± 5.2	66.3	18.1 ± 4.3	69.4	9.1 ± 2.8	65.5
120 min	22.3 ± 4.6	70.5	16.0 ± 3.8	73.0	4.1 ± 1.6	84.5
150 min	15.6 ± 3.8	79.3	16.0 ± 3.8	73.0	4.1 ± 1.6	84.5
180 min	6.2 ± 2.1	91.8	12.2 ± 2.9	79.4	4.1 ± 1.6	84.5
24 hours	4.2 ± 1.5	94.7 ± 3.2	2.2 ± 0.8	96.5 ± 2.8	1.1 ± 0.4	96.1 ± 3.5

Time Point	Chocolate Agar		CLED Agar		Chromogenic Agar	
	CFU/plate (Mean $\pm$ SD)	Reduction (%)	CFU/plate (Mean $\pm$ SD)	Reduction (%)	CFU/plate (Mean $\pm$ SD)	Reduction (%)
Zero (T0)	73.2 $\pm$ 11.8	-	72.3 $\pm$ 11.5	-	20.1 $\pm$ 5.2	-
30 min	28.6 $\pm$ 5.8	60.9	34.2 $\pm$ 7.0	52.7	8.4 $\pm$ 2.7	58.2
60 min	26.2 $\pm$ 5.2	64.2	29.1 $\pm$ 5.9	59.8	7.3 $\pm$ 2.4	63.7
90 min	22.3 $\pm$ 4.5	69.5	24.3 $\pm$ 4.8	66.4	3.1 $\pm$ 1.3	84.6
120 min	22.4 $\pm$ 4.5	69.4	16.2 $\pm$ 3.8	77.6	3.1 $\pm$ 1.3	84.6
150 min	17.2 $\pm$ 3.6	76.5	16.4 $\pm$ 3.9	77.3	3.1 $\pm$ 1.3	84.6
180 min	12.4 $\pm$ 2.8	83.1	12.3 $\pm$ 2.8	83.0	3.1 $\pm$ 1.3	84.6
24 hours	6.3 $\pm$ 1.8	91.8 $\pm$ 2.9	2.1 $\pm$ 0.7	97.3 $\pm$ 2.1	1.0 $\pm$ 0.3	95.0 $\pm$ 3.2

Table 2: Bacterial reduction by cold-plasma air sanitizer in Haematology Laboratory samples (CFU/500L)

3.2 Particle Count Analysis

Total particle counts (0.3-10 μm) showed a significant reduction during the treatment period, decreasing from a baseline mean of 1,450 $\pm$ 89 particles/ m^3 to 320 $\pm$ 42 particles/ m^3 at 24 hours in the Microbiology Laboratory (Figure 1). A strong positive correlation was observed

between particle counts and bacterial counts (Microbiology Laboratory: $r=0.89$, 95% CI: 0.82-0.94, $p<0.001$; Hematology Laboratory: $r=0.91$, 95% CI: 0.85-0.95, $p<0.001$) (Figure 2, Figure 3). Linear regression analysis revealed that particle counts explained 79% ($R^2=0.79$) and 83% ($R^2=0.83$) of the variance in bacterial counts in the Microbiology and Hematology laboratories, respectively.

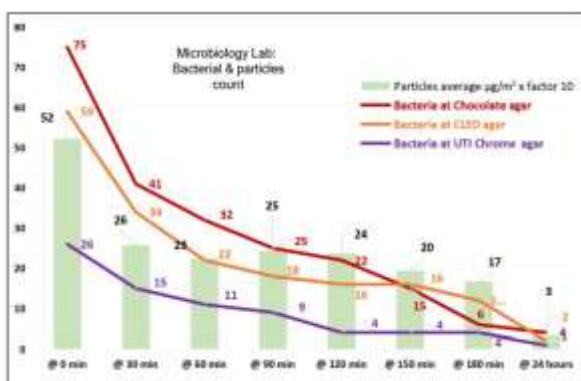


Figure 2. Particle count PS Colony count in Microbiology Lab, GUM University.

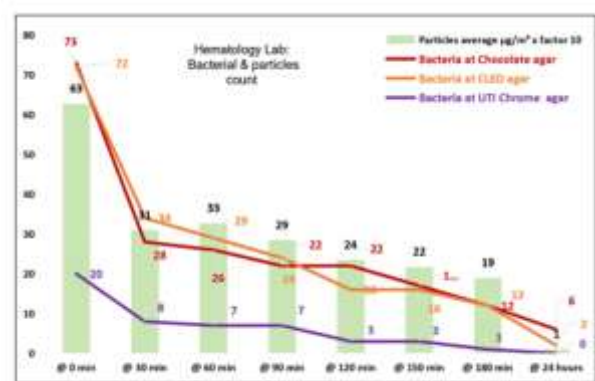
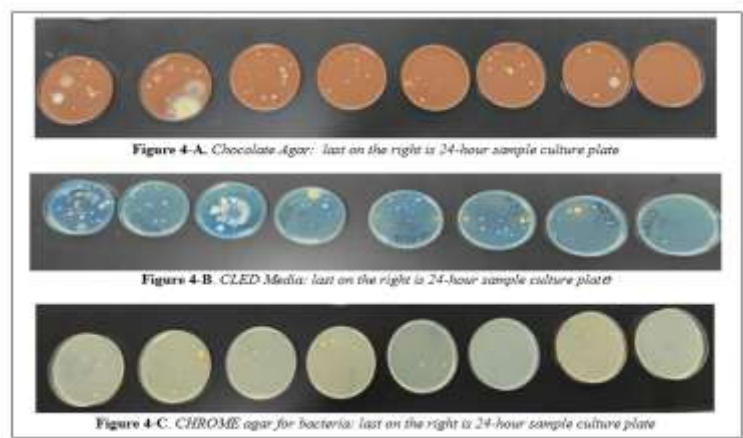


Figure 3. Particle count PS Colony count in Hematology Lab, GUM University.



3.3 Bacterial Identification

Analysis of bacterial colonies from all culture media revealed that the majority of isolated organisms were Gram-positive bacteria (87%), with Gram-negative bacteria comprising only 13% of isolates (Table 3). Among Gram-positive isolates, the predominant genera were *Micrococcus* spp. (62%) and *Bacillus* spp. (28%). Coagulase-positive *Staphylococcus aureus* was identified in two isolates from the Microbiology Laboratory. Coagulase-negative staphylococci were identified in two isolates.

All *Staphylococcus* isolates were sensitive to vancomycin; one of the *S. aureus* isolates was penicillin-resistant (Table 3). Gram-negative isolates included four oxidase-positive organisms and one oxidase-negative organism identified by API 20NE testing as *Burkholderia cepacia* and *Stenotrophomonas maltophilia*. No fungal growth was observed on any of the culture media used.

Table 3. Biochemical characterization of Gram-positive bacterial isolates

Isolated Organism Group	Gram Stain	Coagulase Test	Mannitol Salt Agar	DNase Test	Penicillin Susceptibility	Vancomycin Susceptibility
aureus(n=2)	+	+ (2)	+ (2)	+ (2)	Sensitive (1), Resistant (1)	Sensitive (2)
Coagulase-negative Staphylococcus (n=2)	+	- (2)	- (2)	- (2)	Sensitive (2)	Sensitive (2)

4. Discussion

This study demonstrates that cold-plasma air sanitization effectively reduces airborne bacterial contamination in indoor laboratory settings, achieving >90% reduction within 24 hours of continuous operation. The findings have significant implications for indoor air quality management and infection control, particularly in healthcare settings where immunocompromised individuals are at elevated risk of airborne infections [24].

The observed reduction rates (91-100%) are comparable to or exceed those reported for other air sanitation technologies. Previous studies of ultraviolet germicidal irradiation (UVGI) have reported bacterial reduction

rates of 70-90% depending on exposure time and environmental conditions [25]. High-efficiency particulate air (HEPA) filtration can achieve >99% particle removal but does not inactivate captured organisms, potentially allowing their re-release or growth on filters [26]. Photocatalytic oxidation systems have shown variable efficacy (40-90%) depending on catalyst type, humidity, and contaminant load [27]. The cold-plasma technology evaluated in this study offers the advantage of simultaneously removing particles and inactivating microorganisms through oxidative mechanisms, potentially providing more comprehensive air sanitation [28].

The strong correlation observed between particle counts and bacterial concentrations ($r=0.89-0.91$) supports the hypothesis that airborne bacteria are predominantly associated with particulate matter. This finding is consistent with previous studies demonstrating that bacteria are typically associated with particles $>1\ \mu\text{m}$ [29]. The correlation suggests that real-time particle monitoring could serve as a practical surrogate for assessing biological contamination in indoor environments, enabling rapid air quality assessment when microbiological data are not immediately available. However, the correlation is not absolute ($R^2=0.79-0.83$), indicating that other factors influence bacterial concentration and that particle monitoring should complement, rather than replace, microbiological assessment [30].

The predominant isolation of *Micrococcus* and *Bacillus* species in this study is consistent with previous reports of indoor air microbiology [31]. These organisms are common environmental contaminants with low pathogenic potential in immunocompetent individuals but may pose risks in immunocompromised hosts [32]. The detection of coagulase-positive *S. aureus* and Gram-negative organisms including *B. cepacia* and *S. maltophilia* is of clinical significance, as these organisms are associated with healthcare-associated infections and are often multidrug-resistant [33]. The presence of penicillin-resistant *S. aureus* in indoor air highlights the potential for transmission of antibiotic-resistant organisms through the airborne route. These findings underscore the importance of air sanitation in healthcare settings to reduce the airborne burden of clinically relevant organisms.

The observed first-order inactivation kinetics ($R^2=0.92-0.95$) are consistent with the mechanism of ROS-mediated bacterial inactivation [34]. The half-lives of 43–50 minutes suggest that continuous operation of the sanitizer is required to maintain low bacterial concentrations, as intermittent operation would allow bacterial recontamination from outdoor air, human activities, and surface resuspension [35]. This finding has practical implications for implementing cold-plasma sanitizers in healthcare settings, where continuous operation may be necessary to achieve and maintain ultraclean air quality.

The standardized enumeration protocol developed in this study offers several advantages over existing methods. The use of controlled air volume sampling (500 L) ensures consistent sample sizes and enables calculation of bacterial concentrations in standard units (CFU/m³). Direct inoculation onto multiple culture media (Chocolate, CLED, Chromogenic) maximizes detection

of diverse organisms. The protocol reduces the number of steps, expenses, and efforts compared to previously described methods and allows for comparability between studies. This protocol could be particularly valuable for routine monitoring of indoor air quality in healthcare and other sensitive settings.

4.1 Limitations

Several limitations of this study warrant consideration. First, the study was conducted in only two laboratories at a single institution over a limited time period, which may limit generalizability. Bacterial communities in indoor environments vary significantly with season, geographical location, occupancy patterns, and building characteristics [36]. Multi-site studies of extended duration are needed to validate these findings across diverse indoor environments. Second, the culture-based enumeration method detects only culturable bacteria and cannot distinguish viable but non-culturable organisms, which may remain infectious [37].

Future studies should employ complementary methods such as flow cytometry with viability stains or molecular methods with propidium monoazide pre-treatment to assess total viable cells [38].

Third, the study did not assess fungal or viral contamination, which represent important components of the indoor air microbiome with significant health implications [39].

Fourth, the bacterial identification was performed using phenotypic methods rather than molecular sequencing, which may limit the resolution of species identification [40].

Finally, the study did not assess the long-term performance of the sanitizer, including potential degradation of efficacy over time, filter saturation, or accumulation of dead organisms that could serve as nutrient sources for surviving organisms. Future research should address these limitations through extended studies incorporating molecular identification methods, viability assessment, and comprehensive microbiological analysis including fungi and viruses.

5. Conclusion

This study demonstrates that cold-plasma air sanitization effectively reduces airborne bacterial contamination in indoor environments, achieving $>90\%$ reduction within 24 hours of continuous operation. The technology demonstrates efficacy against a range of environmental and clinically relevant bacteria, including *Micrococcus* spp., *Bacillus* spp., *Staphylococcus aureus*, *Burkholderia cepacia*, and *Stenotrophomonas*

maltophilia. The strong correlation observed between particle counts and bacterial concentrations supports the use of real-time particle monitoring as a surrogate for assessing biological contamination.

This study presents a standardized, simplified protocol for airborne bacterial enumeration that incorporates controlled air volume sampling (500 L), direct inoculation onto multiple culture media, and standardized reporting in CFU/m³. This protocol reduces the steps, expenses, and efforts associated with airborne bacterial testing and could serve as a model for future studies of air sanitation technologies.

The findings support the implementation of cold-plasma air sanitizers in healthcare settings where immunocompromised patients are at risk of airborne infections, including oncology units, transplant units, intensive care units, and operating theaters. Future research should focus on: (i) multi-site, long-term studies to validate efficacy across diverse settings and seasons; (ii) assessment of fungal and viral inactivation; (iii) molecular characterization of bacterial communities to identify potential pathogens and antimicrobial resistance genes; and (iv) cost-effectiveness analyses to guide policy and practice.

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Conflict of Interest Statement

The authors declare no conflicts of interest regarding the publication of this manuscript.

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