



Sterilization of Clinical Spaces, High-Tech Instruments and Laser Optics: Initial Findings on Fast Chemical Sterilization System Based on Reducing Free Radicals

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Abstract

Objectives: To verify the effectiveness of a spray-based sanitization protocol based on the release of free radicals in reducing microbial contamination on dental operative surfaces. Materials and Methods: Twenty surface samples were collected in a dental hospital setting (SCDU Odontostomatology Unit, Sant'Andrea Hospital, Vercelli, Italy). Sampling points included the following: Lamp handle (dental unit); Dental unit control panel; Spittoon; Protective screen; Operator chair (seat); Operator chair (armrest); Work surface; Computer Keyboards; X-ray tubes; Lamp accessory. Microbial contamination was measured in CFU/cm² before and after disinfection with the experimental disinfectant (EC Ster Spray, ICM srl. ITALY), following UNI EN ISO 4833-1:2022 standards. Statistical analysis was performed using the Chi-square test to assess significant differences between pre- and post-treatment results. Results: Before sanitization, bacterial loads ranged from 2 to 8 CFU/cm², with a maximum of 360 CFU detected on a lamp accessory. After disinfection, all samples showed <1 CFU/cm², indicating almost complete microbial reduction. Statistical analysis confirmed a significant difference between pre- and post-treatment data, demonstrating the high effectiveness of the free-radical-based spray protocol ($\chi^2 = 54.55$; $df = 18$; $p = 0.000015$). Conclusions: The fast chemical sterilization system based on reducing free radicals is capable of achieving a substantial decrease in surface bacterial contamination in dental settings. Although the initial data are promising, the limited sample size restricts statistical strength. Further multicenter studies are required to confirm these results and to evaluate the long-term reliability and rapid action of this system. Overall, the study supports the use of free-radical-releasing sprays as a fast, safe, and reliable method to enhance biosafety and prevent cross-contamination in dental and clinical environments.

Introduction

A crucial element of contemporary medical practice relates to the prevention of infections linked to healthcare settings. Reusable instruments utilized in medical and dental treatment necessitate highly rigorous decontamination and sterilization procedures in order to reduce the likelihood of cross-contamination between patients and healthcare personnel [1].

To date, steam sterilization with autoclave represents the gold standard for sterilizing medical instruments due to its demonstrated microbicidal efficacy and broad regulatory acceptance [2]. Nevertheless, this method cannot be used for all instruments, as high temperatures, pressure, and humidity may affect the components of more heat-sensitive materials like lasers, handpieces, and optical fibers over time [3]. To address this issue, recent studies have explored low-temperature sterilization and disinfection systems that significantly lower the microbial load while maintaining the inherent properties of the material being treated [4].

Over the years, investigations have centered on several low-temperature antimicrobial techniques, including systems utilizing plasma, peracetic acid, ethylene oxide, and free radicals [5]. The controlled production of free radicals has shown to be particularly effective against microorganisms surviving against conventional disinfection techniques. Free radicals induce oxidative and reductive stress on the cellular structures of microorganisms, leading to their inactivation without generating toxic residues that pose risks to the patient [6]. These aspects make their application potentially useful for decreasing the microbial load of thermo-sensitive or non-autoclavable medical tools. However, while research has offered promising evidence regarding their potential use, investigations into their clinical application as rapid chemical sterilization systems are not yet comprehensive.

The aim of the present study is to evaluate the efficacy of a low-temperature sterilization technology based on free radicals as a valid approach combining effective infection control with the preservation of advanced medical devices.

Materials and Methods

EC STER sterilizing solution (ICM srl. ITALY, Italy) (Figure 1) was prepared at a concentration of 0.45% (4.5 g/L; batch 03/2025; internal code ICM/01/25) using municipal tap water as a diluent (Figure 2). The

solution was stored at room temperature and used 30 minutes after preparation. The pH was checked before use.

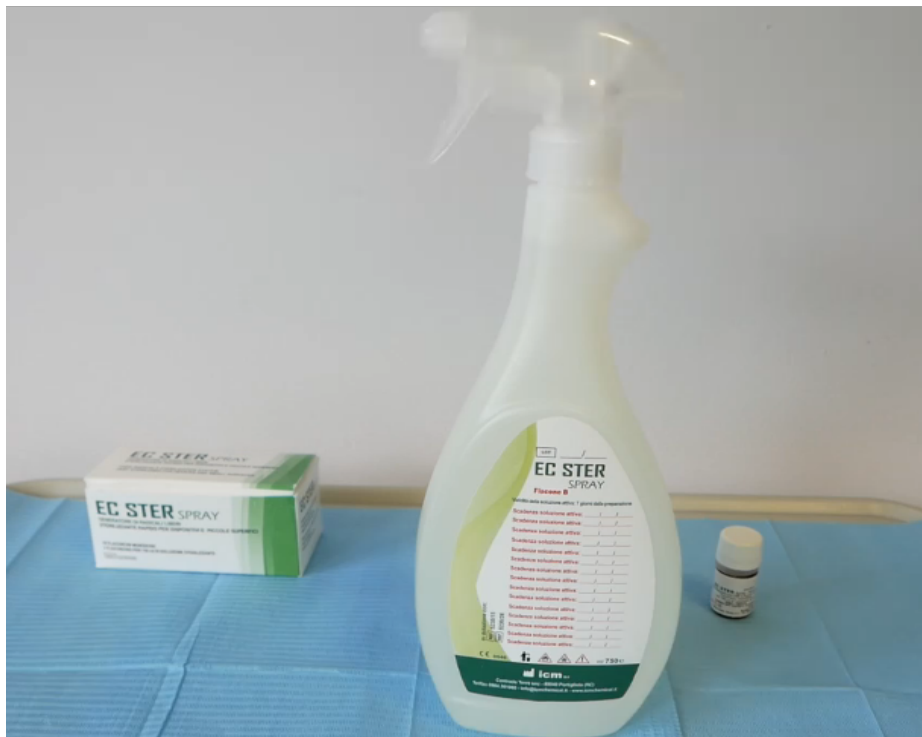


Figure 1. *EC Ster Spray. Powder box and dispenser. A label allows to register the expiration day of sterilizing activity.*

EC STER SPRAY Spray procedure

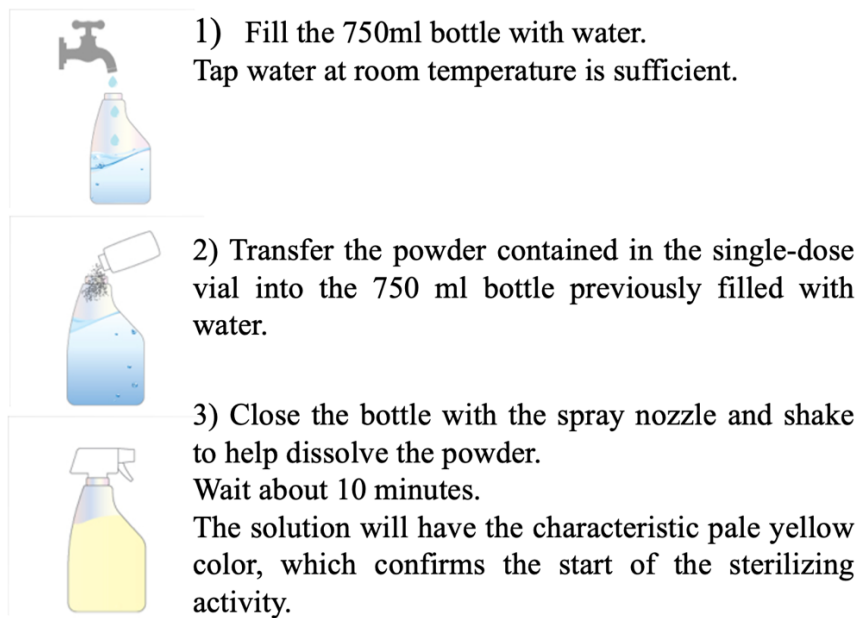


Figure 2 Preparation of SPRAY solution

Twenty surfaces were considered in a dental hospital setting (SCDU Odontostomatology Unit, Sant'Andrea Hospital, Vercelli, Italy) and sampled using sterile swabs before and after sanitization. Sampling points included the following:

- Lamp handle (dental unit);
- Dental unit control panel;
- Spittoon;
- Protective screen;
- Operator chair (seat);
- Operator chair (armrest);
- Work surface;
- Computer Keyboards;

- X-ray tubes;
- Lamp accessory.

Microbial contamination was measured in CFU/cm² before and after disinfection with the experimental free radicals releasing product. The microbial count was conducted following UNI EN ISO 4833-1:2022 standards.

Statistical analysis was performed using the Chi-square test to assess the significance of differences between pre- and post-treatment results. Statistical significance was set at $p < 0.05$.



Figure 3 APPLICATION OF FREE RADICALS STERILIZING SOLUTION ; SURFACE SAMPLING with sterile swab.

Results

Before sanitization, all sampled surfaces showed detectable microbial contamination, with values ranging from 2 to 8 CFU/cm² on most operative surfaces. A significantly higher level of contamination was observed on a lamp accessory, which exhibited a microbial load of 360 CFU. These findings confirm the presence of heterogeneous but clinically relevant surface contamination in routine dental clinical activity.

Following application of the free-radical-based sanitization protocol, microbial counts were reduced under the detection limit (<1 CFU/cm² or <1 CFU/sample) on the vast majority of tested surfaces as shown in Table 1. A complete absence of detectable growth was observed on dental unit control panels, spittoons, protective screens, operator chairs, work surfaces, computer keyboards, X-ray tubes, and lamp accessories. A minimal residual contamination (4 CFU/cm²) was detected after the sanitization protocol on one dental unit lamp handle.

Table 1. Surface microbial load before and after free-radical-based sanitization protocol.

Surface sampled	Condition	Microbial load (CFU/cm ² or CFU/sample)
Lamp handle (dental unit)	Before sanitization	5 CFU/cm ²
Lamp handle (dental unit)	After sanitization	4 CFU/cm ²
Dental unit control panel	Before sanitization	7 CFU/cm ²
Dental unit control panel	After sanitization	<1 CFU/cm ² (no growth)
Spittoon	Before sanitization	4 CFU/cm ²
Spittoon	After sanitization	<1 CFU/cm ² (no growth)
Protective screen	Before sanitization	2 CFU/cm ²
Protective screen	After sanitization	<1 CFU/cm ² (no growth)
Operator chair (seat)	Before sanitization	6 CFU/cm ²
Operator chair (seat)	After sanitization	<1 CFU/cm ² (no growth)

Surface sampled	Condition	Microbial load (CFU/cm ² or CFU/sample)
Operator chair (armrest)	Before sanitization	3 CFU/cm ²
Operator chair (armrest)	After sanitization	<1 CFU/cm ² (no growth)
Work surface	Before sanitization	5 CFU/cm ²
Work surface	After sanitization	<1 CFU/cm ² (no growth)
Computer keyboard	Before sanitization	8 CFU/cm ²
Computer keyboard	After sanitization	<1 CFU/cm ² (no growth)
X-ray tube	Before sanitization	3 CFU/cm ²
X-ray tube	After sanitization	<1 CFU/cm ² (no growth)
Lamp accessory	Before sanitization	360 CFU/sample
Lamp accessory	After sanitization	<1 CFU/sample (no growth)

Results are expressed as colony-forming units (CFU) per square centimeter or per sample, according to surface type. Post-sanitization values below the detection limit are reported as <1 CFU.

Overall, comparison between pre- and post-sanitization measurements demonstrated a pronounced reduction in surface microbial contamination across all sampled sites. Statistical analysis confirmed a significant decrease in microbial load following treatment ($\chi^2 = 54.55$; degrees of freedom = 18; $p = 0.000015$), indicating a robust effect of the applied sanitization protocol. These results demonstrate that the free-radical-based system provides rapid and effective microbial abatement on high-contact clinical surfaces under real-world hospital conditions.

Discussion

Medical device sterilization is an essential procedure to guarantee patient safety. However, heat-sensitive materials might not be a good fit for conventional steam autoclaves. The goal of the current study was to assess a novel cold sterilization method based on the controlled production of free radicals with reducing capabilities. Overall, comparison between pre- and post-sanitization measurements demonstrated a pronounced reduction in surface microbial contamination across all sampled sites, including lamp handle (dental unit), dental unit control panel, spittoon, protective screen, operator chair (seat), operator chair

(armrest), work surface, computer keyboards, X-ray tubes and Lamp accessory.

According to the results, the cold sterilization system based on reducing free radicals can achieve microbial reduction levels that are comparable to those of an autoclave cycle. Because of this, it is especially appealing for use on temperature-sensitive devices where applying heat would be dangerous or impractical [7-9]. These findings are consistent with earlier research, which shows that low-temperature systems based on plasma or oxidizing agents have reduced bacterial spores and viruses by at least 6 log₁₀; however, these systems occasionally need more complicated operating conditions or longer contact times [10-12]. One notable feature of the EC STER system is its extremely brief treatment duration (1 minute), which makes it ideal for clinical settings with high instrument turnover.

Regarding clinical application, the literature reports pilot studies on the use of similar systems in dentistry and outpatient surgery, where lowering free radical sterilization was linked to better preservation of the instruments' physical properties and the absence of post-treatment contamination when compared to steam [12-14]. Large-scale controlled clinical studies are still hard to come by, though, which indicates that more research is necessary to validate its efficacy in everyday use. The comprehensive microbial eradication observed in this study suggests that the EC STER system exhibits an efficacy comparable to that of conventional autoclave cycles while offering the significant advantage of markedly reduced treatment durations. The active principle underlying this system involves the generation of free radicals in an aqueous solution with a basic pH, leveraging electron transfer mechanisms to interact directly with microbial protein structures. This interaction results in irreversible chemical damage that effectively inactivates microorganisms. Unlike traditional oxidative agents, the mechanism employed by EC STER avoids inducing surface oxidative phenomena due to the reducing nature of the radicals and the basic pH environment. Consequently, the system maintains the structural integrity and functional properties of medical instruments, which is critical for their long-term performance. When benchmarked against findings reported in literature, other cold sterilization techniques, including plasma systems and oxidizing agents, have demonstrated comparable microbial reduction capabilities at levels of ≥ 6 log. However, these methods typically necessitate either extended exposure times or complex operational conditions [10,11]. The ability of EC STER to achieve similar levels of antimicrobial efficacy within significantly shorter timeframes renders it particularly advantageous in clinical environments characterized by high turnover rates for medical instruments, where rapid sterilization is essential. Mechanistically, the reducing radicals act effectively against intricate microbial formations, encompassing mature biofilms and resilient spores. This

confers efficacy even against particularly challenging organisms such as *Clostridioides difficile* [15].

As already reported in previous research conducted by our group [16], the absence of thermal stress offers a distinct advantage over autoclaving by enabling the safe treatment of delicate medical devices, including electronic handpieces, laser optics, handpieces and optical fibers, without compromising their operational integrity, a consideration of paramount importance in routine clinical applications where both sterility and instrument functionality are imperative. Cold sterilization utilizing reducing radicals thus represents a novel, efficient, and reliable approach. The substantial reductions in microbial counts documented in this investigation position EC STER as a potentially transformative solution for managing heat-sensitive medical instruments, with far-reaching implications for infection control and the optimization of clinical workflows. Nevertheless, certain limitations must be acknowledged. The current findings necessitate validation through real-world clinical studies incorporating a broader spectrum of instruments and conditions. Furthermore, evaluation of the long-term material compatibility following numerous treatment cycles is essential. Future research should aim to include controlled clinical trials and large-scale applications to elucidate additional dimensions related to safety, cost-effectiveness, and environmental sustainability. Particularly, comparative studies focusing on operational costs, maintenance requirements, and treatment duration will play a pivotal role in encouraging its adoption across healthcare and industrial domains.

Conclusions

Cold sterilization powered by reducing radicals emerges as a promising modality that combines rapid and effective antimicrobial activity with preservation of sensitive instrumentation. While preliminary findings are compelling, further empirical investigations remain crucial before generalized implementation can be confidently recommended.

AUTHOR CONTRIBUTION

Emanuele Ruga, Anna Maria Agnone and Simone Gallo designed the study and collected the data and wrote the paper. Andrea Melle and Simone Gallo were involved statistical analysis .Emanuele Ruga revised the paper. The authors contributed equally to conception, data analysis, writing and critical appraisal.

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CONFLICT OF INTEREST STATEMENT

All the authors have stated explicitly that there are no conflicts of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the first author, second author and reviewer, upon request.

AI NOTES: NO AI technology/software was used to write this article.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This investigation did not involve patients or animal subjects. No ethical approval was required.

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