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Comparative Evaluation Of Efficacy Of Four Methods Used For Sterilization Of Pediatric Endodontic Files - An In Vitro Study

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Abstract
Background:
Sterilization of instruments is essential for successful endodontic therapy. Conventional methods, though reliable, are time-consuming and less suitable for chairside use. This study evaluated and compared four sterilization techniques for H-files: autoclave, glass bead, ultraviolet (UV), and diode laser.
Methods:
Eighty pre-sterilized H-files (size 15, length 21 mm) were contaminated with Streptococcus mutans and divided into five groups (n=16). Each group underwent one sterilization method. Sterility was assessed by turbidity checks at 24, 48, 72, and 120 hours.
Results:
The chi-square test showed a statistically significant difference between methods ($\chi^2 = 41.057$, $p < 0.001$). Autoclave sterilization achieved 100% sterility in all samples. Glass bead sterilization had a 12.5% contamination rate, UV showed 25–37.5%, and diode laser was least effective with 37.5–56.3% contamination. Control samples remained fully contaminated.
Conclusion:
Autoclave sterilization proved superior and remains the gold standard. Glass bead showed moderate effectiveness, while UV and diode laser were less reliable.
Clinical Significance
Chairside methods are faster and more convenient but less effective than autoclaving. Evaluating practical alternatives is necessary, though autoclave remains indispensable in ensuring patient safety.
Keywords: *Autoclave, Glass bead sterilization, UV sterilization, Diode laser, Streptocccus mutans, H files,*

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I. Introduction

The oral microbiome is essential for maintaining oral and systemic health, with *Streptococcus mutans* recognized for both its cariogenic potential and its role in endodontic infections. Studies report its prevalence in about 60% of asymptomatic necrotic teeth and up to 70% of symptomatic cases with acute apical abscesses.[1] the goal of endodontic therapy is to eliminate infection and prevent microbial re-entry into the root canal and peri radicular tissues. Classic research by Kakehashi et al.[2] demonstrated that microorganisms are the primary etiological factor in pulpal and periradicular disease, emphasizing the need for meticulous infection control. Cleaning and shaping procedures remain critical, but the complex design of endodontic files often makes

sterilization difficult.

In pediatric endodontics, ensuring sterilization of instruments is essential because the primary dentition requires the use of smaller and more delicate instruments. Endodontic files, particularly Hedström and K-files, are characterized by flutes and spiral cutting edges that tend to trap organic and microbial debris. Even after reprocessing, up to 94% of files have been shown to retain residual debris, which poses a risk for cross-contamination.3 Infection control guidelines classify instruments into critical, semi-critical, and non-critical categories, with endodontic files and other intraoral instruments designated as critical items that must be sterilized before reuse.4 Professional organizations such as the American Dental Association and the Centers for Disease Control and Prevention have outlined strict protocols to minimize cross-infection and safeguard patient health.

Hedström files, which are particularly useful in pediatric endodontics for canal shaping, present significant sterilization challenges due to their intricate structure. An in vitro study by Almehamadi et al. (2022) reported microbial persistence on these files even after sterilization, highlighting limitations in standard methods and the potential need for alternative approaches.5

Various sterilization techniques are employed in dental practice. The steam autoclave is the most widely used and considered the gold standard, operating at 121 °C and 15 psi for 15 minutes to reliably eliminate microorganisms, including spores. Dry heat sterilization6 is reserved for materials that cannot tolerate moisture, such as glassware and powders, while cold sterilization using chemical agents like glutaraldehyde and sodium hypochlorite is applied to heat-sensitive instruments. Recently, laser sterilization, which achieves rapid microbial destruction with minimal time7, and ultraviolet radiation, which exerts antibacterial effects by damaging microbial DNA at wavelengths near 254 nm are being employed as means of sterilization8. Glass bead sterilization is another method, employing dry heat at approximately 225 °C, although it is typically restricted to sterilizing the tips of small instruments.

Despite their effectiveness, conventional sterilization methods are often time-consuming and may vary in reliability depending on the material or design of the instrument. Autoclaving, though indispensable, may contribute to metal fatigue over repeated cycles. Cold sterilization may leave chemical residues, and dry heat is impractical for many dental devices. Newer approaches such as lasers and ultraviolet light offer rapid, efficient sterilization, though their clinical application remains limited and requires further validation.

Sterilization remains a cornerstone of successful pediatric endodontic practice. The persistence of microbial contamination on files underscores the necessity of strict adherence to sterilization protocols and continuous evaluation of new technologies. While conventional methods continue to play an indispensable role, the incorporation of advanced approaches may enhance reliability, reduce chairside time, and further ensure patient safety. Preventing cross-contamination is not only a clinical requirement but also a professional responsibility that directly impacts the success of pediatric endodontic treatment.

II. Methodology

This in vitro study was conducted in the Departments of Pediatric and Preventive Dentistry and Periodontology at PSM College of Dental Science and Research, with microbiological analyses performed at CARe Keralam Ltd, KINFRA Park, Koratty.

Eighty size 15, 21 mm H-files were shortened to 18 mm and pre-sterilized. To prepare a uniform *Streptococcus mutans* suspension (0.5 McFarland standard), BHI broth was inoculated and incubated at 37 °C for 24 h. The files were contaminated by immersing them in this suspension for 5 min under Bio Safety Level-2 conditions, then incubated at 37 °C for 1 h followed by a 24 h incubation.

Contaminated files were randomized into 5 groups (16 samples in each group)
A – Control (no sterilization)
B – Autoclave (121 °C, 15 psi, 15 mins)
C – Glass bead (240 °C, 45 secs)
D – Diode laser (980nm,10W, 3 secs)
E - UV Chamber (254 nm,5 mins)

Post-sterilization, each file was placed in BHI and incubated at 37 °C. Turbidity was assessed at 24, 48, 72, and 120 h to detect bacterial survival.

Figure 1: H FILES

Figure 2: GLASS BEAD STERILIZER

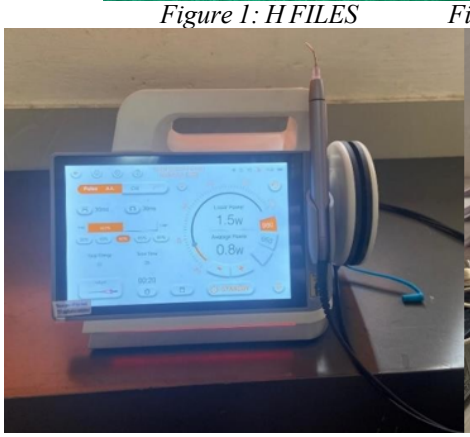


Figure 3: Diode laser

Figure 4: Autoclave,

Figure 5: UV Sterilizer

Figure 6: Contamination Of Files With Streptococcus Mutans

Figure 7: Turbidity Check After Autoclave Sterilization -After 1 Day, 2 Days, 3 Days And 5 Days

Figure 8: Turbidity Check After Glass Bead Sterilization -After 1 Day, 2 Days, 3days And 5 Days

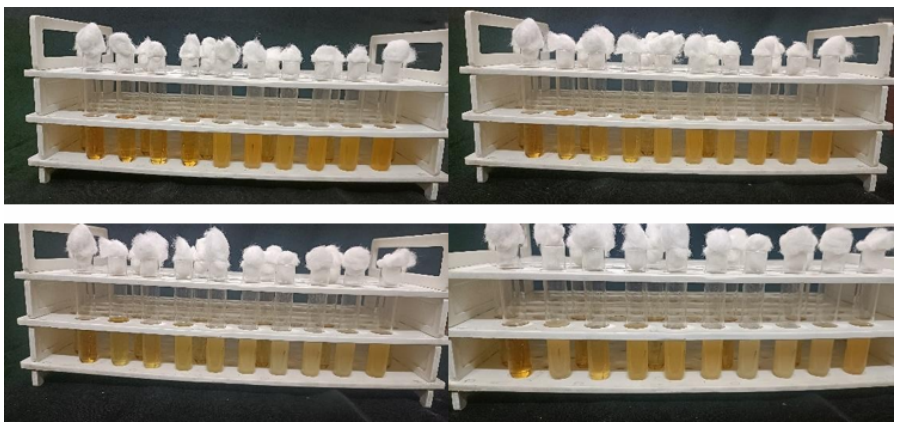


Figure 9: Turbidity Check After Diode Laser Sterilization- After 1 Day, 2 Days,3 Days And 5 Days

Figure 10: Turbidity Check After Uv Sterilization- After 1 Day, 2 Days,3 Days And 5 Days

III. Results

On Day 1

chi-square analysis showed significant difference among sterilization methods ($\chi^2 = 42.637$, $p < 0.001$). Autoclave sterilization achieved 100% absence of contamination (16/16), confirming it as the most effective method. Control group showed universal contamination (16/16). Glass bead sterilization had a contamination rate of 12.5% (2/16), ultraviolet 25% (4/16), and laser 37.5% (6/16) (Table 1, Graph 1).

On Day 2,

Results remained significant ($\chi^2 = 43.397$, $p < 0.001$). Autoclave maintained complete efficacy (16/16), while the control group showed 100% contamination. Glass bead sterilization showed 12.5% contamination (2/16), ultraviolet 25% (4/16), and laser decreased in effectiveness with 56.3% contamination (9/16) (Table 2, Graph 2).

On Day 3

Chi-square test confirmed significant differences ($\chi^2 = 41.057$, $p < 0.001$). Autoclave continued to show 100% sterility (16/16). The control remained fully contaminated, glass bead sterilization showed 12.5% contamination (2/16), ultraviolet 37.5% (6/16), and laser 56.3% (9/16) (Table 3, Graph 3).

On Day 5

Results showed statistical significance ($\chi^2 = 41.057$, $p < 0.001$). Autoclave remained fully effective (16/16), control fully contaminated, glass bead 12.5% (2/16), ultraviolet 37.5% (6/16), and laser the least effective with 56.3% contamination (9/16) (Table 4, Graph 4).

Overall, autoclave sterilization consistently achieved 100% sterility. Glass bead sterilization was the second most effective, maintaining a 12.5% contamination rate. Ultraviolet sterilization showed moderate efficacy with contamination increasing over time (25%–37.5%). Laser sterilization was the least reliable, with contamination ranging from 37.5% to 56.3%. Control samples consistently showed 100% contamination.
Tables 5–9 and Graphs 5–9 illustrate turbidity comparisons at 24, 48, 72, and 120 hours

Table I: Comparison of turbidity at different intervals with different sterilization methods at Day 1

Sterilization Methods	Day 1				Chi- Square	p-value
	Absent		Present			
	Number	N %	Number	%		
Autoclave	16	100.0%	0	0.0%		
Control	0	0.0%	16	100.0%		
Glass bead	14	87.5%	2	12.5%		
Laser	10	62.5%	6	37.5%		
Ultraviolet	12	75.0%	4	25.0%		
					42.637	**0.00**

Graph I: Comparison of turbidity at different intervals with different sterilization methods at Day 1

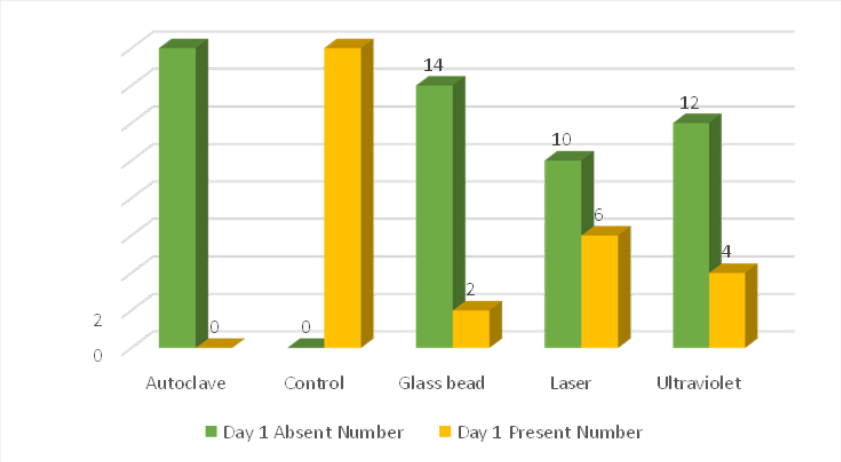


Table ii: Comparison of turbidity at different intervals with different sterilization methods at Day 2

Sterilization Methods	Day 2				Chi- Square	p-value
	Absent		Present			
	Number	N %	Number	%		
Autoclave	16	100.0%	0	0.0%	43.397	**0.00**
Control	0	0.0%	16	100.0%		
Glass bead	14	87.5%	2	12.5%		
Laser	7	43.8%	9	56.3%		
Ultraviolet	12	75.0%	4	25.0%		

Graph ii: Comparison of turbidity at different intervals with the different sterilization methods at Day 2

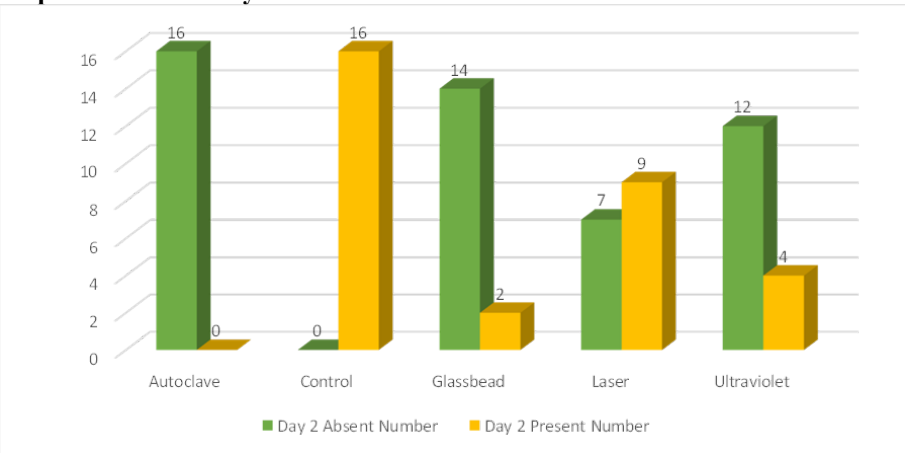


Table iii: Comparison of turbidity at different intervals with different sterilization methods at Day 3

Sterilization Methods	Day 3				Chi-Square	p-value
	Absent		Present			
	Number	N %	Number	%		
Autoclave	16	100.0%	0	0.0%		
Control	0	0.0%	16	100.0%		
Glass bead	14	87.5%	2	12.5%		
Laser	7	43.8%	9	56.3%		
Ultraviolet	10	62.5%	6	37.5%		
					41.057	**0.00**

Graph iii: Comparison of turbidity at different intervals with different sterilization methods at Day 3

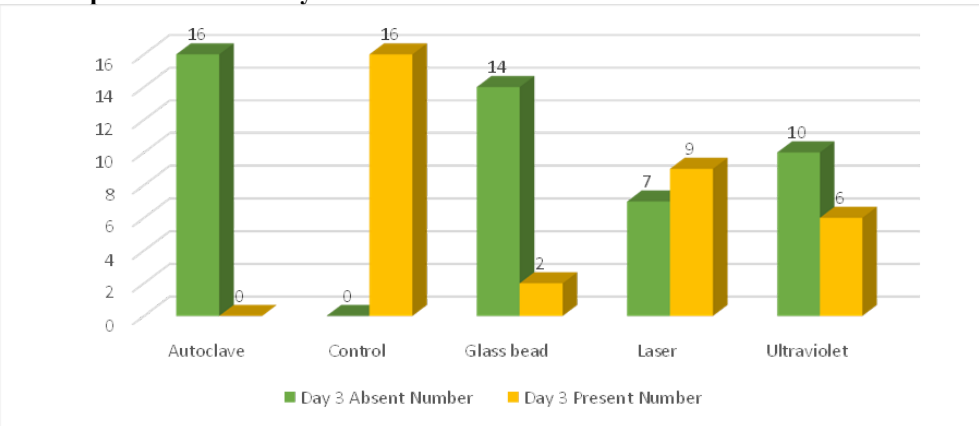


Table IV: Comparison of turbidity at different intervals with different sterilization methods at Day 5

Sterilization Methods	Day 5				Chi- Square	p-value
	Absent		Present			
	Number	N %	Number	%		
Autoclave	16	100.0%	0	0.0%		
Control	0	0.0%	16	100.0%		
Glass bead	14	87.5%	2	12.5%		
Laser	7	43.8%	9	56.3%		
Ultraviolet	10	62.5%	6	37.5%		
					41.057	**0.00**

Graph IV: Comparison of turbidity at different intervals with different sterilization methods at Day 5

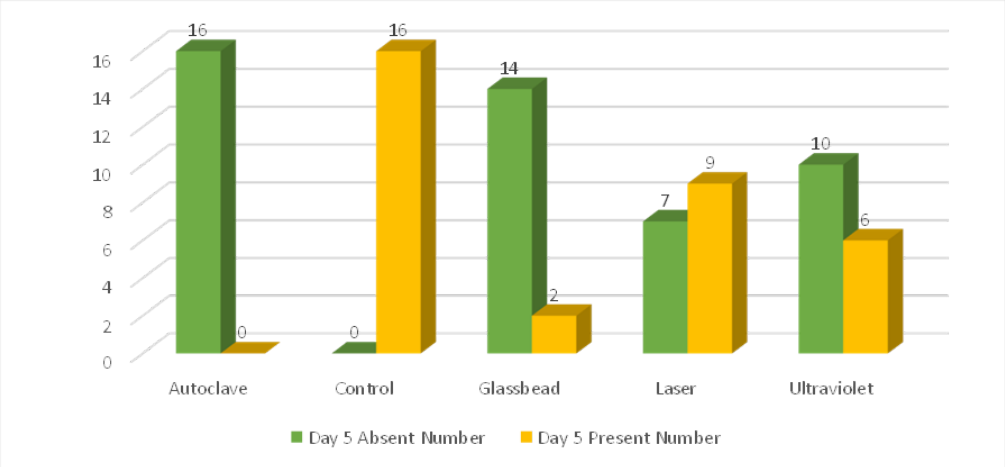


Table v: Comparison of turbidity with Autoclave at different time intervals

Days	Autoclave			
	Absent		Present	
	Number	N %	Number	%
Day 1	16	100.00%	0	0.00%
Day 2	16	100.00%	0	0.00%
Day 3	16	100.00%	0	0.00%
Day 5	16	100.00%	0	0.00%

Graph v: Comparison of turbidity with Autoclave at different time intervals

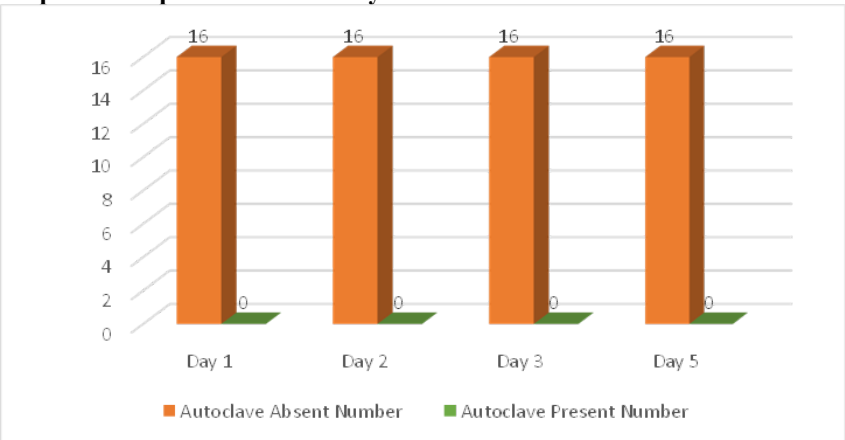


Table VI: Comparison of turbidity for Control group at different time intervals

Days	Autoclave			
	Absent		Present	
	Number	N %	Number	%
Day 1	0	0%	16	100%
Day 2	0	0%	16	100%
Day 3	0	0%	16	100%
Day 5	0	0%	16	100%

Graph VI: Comparison of turbidity for Control group at different time intervals

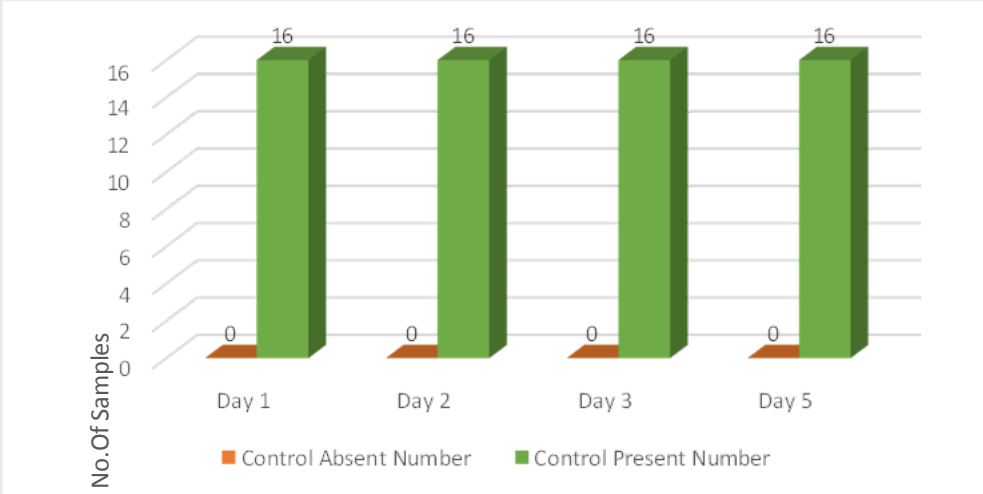


Table vii: Comparison of turbidity with Glass Bead at different time intervals

Days	Autoclave			
	Absent		Present	
	Number	N %	Number	%
Day 1	14	87.50%	2	12.50%
Day 2	14	87.50%	2	12.50%
Day 3	14	87.50%	2	12.50%
Day 5	14	87.50%	2	12.50%

Graph vii: Comparison of turbidity with Glass Bead at different time intervals

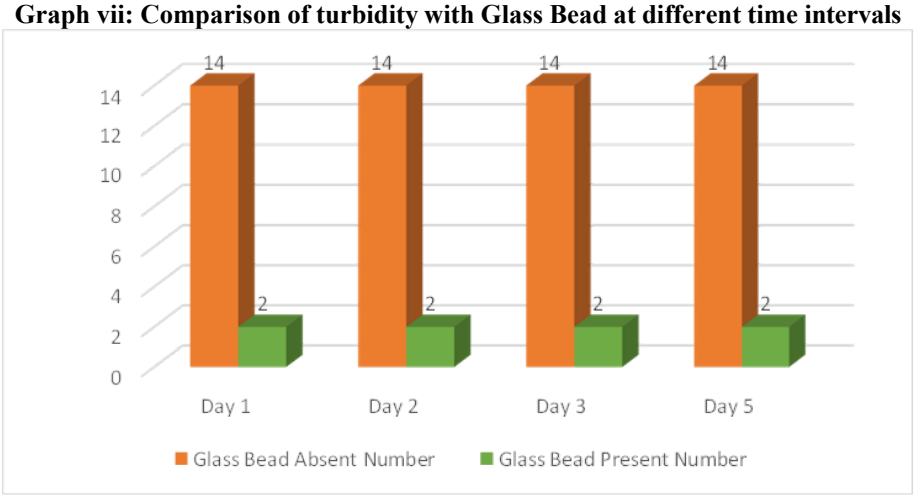


Table viii: Comparison of turbidity with Laser at different time intervals

Days	Autoclave			
	Absent		Present	
	Number	N %	Number	%
Day 1	10	62.50%	6	37.50%
Day 2	12	75.00%	4	25.00%
Day 3	7	43.80%	9	56.30%
Day 5	7	43.80%	9	56.30%

Graph viii: Comparison of turbidity with Laser at different time intervals

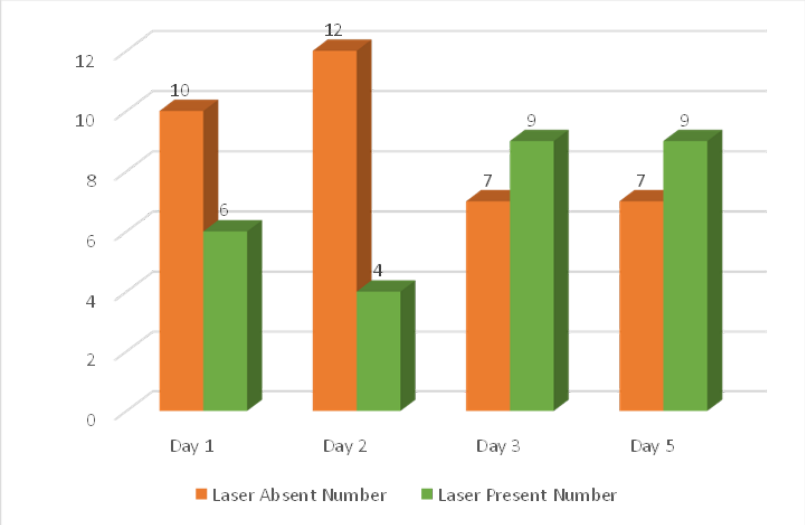
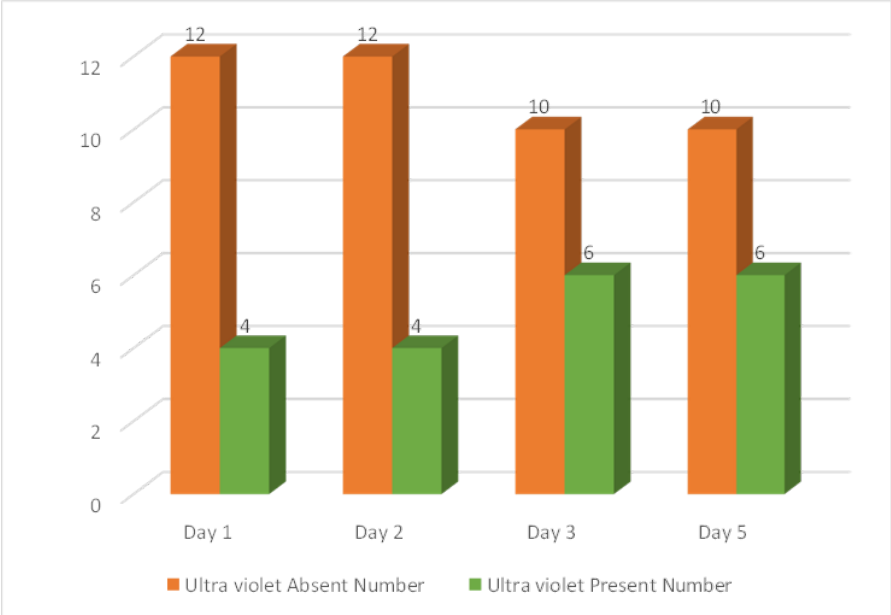


Table ix: Comparison of turbidity with Ultra Violet at different time intervals

Days	Autoclave			
	Absent		Present	
	Number	N %	Number	%
Day 1	12	75.00%	4	25.00%
Day 2	12	75.00%	4	25.00%
Day 3	10	62.50%	6	37.50%
ay 5	10	62.50%	6	37.50%

Graph ix: Comparison of turbidity with Ultra Violet at different time intervals



IV. Discussion

Dental caries begins as a small lesion but can progress to pulp involvement, necessitating endodontic treatment. Endodontics aims to preserve the tooth by eliminating infection and restoring function. *Streptococcus mutans* is a key pathogen because of its ability to adhere to dentin, form biofilms, and colonize root canals, making contamination of instruments a major concern. Hence, effective sterilization of endodontic files is critical for treatment success.

Sterilization aims to eliminate all microbial life, and methods must be chosen based on efficiency, safety, and practicality. Hedstrom files (H-files), with their spiral design and intricate surface, are difficult to sterilize completely. This study compared four methods— autoclave, glass bead, ultraviolet (UV), and diode

laser—using turbidity testing at multiple intervals to evaluate sterility.

Autoclave sterilization, regarded as the gold standard, uses steam under pressure (121°C, 15 psi) to denature proteins and disrupt microbial membranes. In this study, it achieved 100% sterility across all intervals. Similar findings were reported by Ameer et al9, Manhas et al10, and Chawla et al11, reaffirming that autoclaving provides complete microbial elimination. Its main drawback lies in being time-consuming and less feasible for chairside application, though its effectiveness remains unmatched.

Glass bead sterilization is a rapid chairside method using dry heat transfer from small heated beads. It achieved 87.5% sterility in this study, making it the second most effective method. Incomplete sterilization may result from inadequate insertion depth, bead size, or variable heat penetration. Previous research, such as Rani et al12, confirms its effectiveness for small hand instruments, though it is unsuitable for larger or heat- sensitive devices. Despite limitations, it remains a practical option when rapid turnaround is required.

UV sterilization, specifically UV-C radiation (254 nm), inactivates microorganisms by damaging DNA and preventing replication. In this study, it achieved 75% sterility with 5 minutes exposure, inferior to autoclave and glass bead methods. Studies by Enwemeka et al13 and other microbiological experiments show that longer exposure of 30–60 minutes achieves higher efficacy. Its inability to penetrate beyond surfaces reduces reliability for complex instruments such as H-files. While UV is useful for heat-sensitive instruments, its short exposure in this study was insufficient.

Diode laser sterilization showed the least efficacy, with only 43.7% sterility. Lasers act by photothermal and photochemical effects, producing localized heating and reactive oxygen species that damage microbial structures. However, the limited penetration depth and energy scattering reduce sterilizing power on intricate surfaces. Kumar et al14 and Gutknecht et al15 reported similar limitations, concluding that diode lasers are better suited as adjunct disinfectants rather than primary sterilization tools.

The findings of this study highlight the consistent superiority of autoclaving, which remains the most reliable method. Glass bead sterilization, though less effective, provides a practical chairside solution where rapid sterilization is needed. UV sterilization may be valuable for heat-sensitive instruments if longer exposure is permitted. Diode lasers, despite being portable and easy to operate, currently lack the efficacy to replace conventional techniques.

This aligns with existing literature supporting autoclave sterilization as the gold standard^1–3. However, given the limitations of conventional methods in chairside situations, alternative approaches such as glass bead and UV may serve as useful adjuncts. Future research should focus on optimizing chairside techniques, including newer laser technologies and modified UV systems, to achieve more complete microbial elimination without compromising efficiency.

In summary, this study reinforces that autoclave sterilization is the most effective method for endodontic instruments. Glass bead sterilization remains a valuable alternative for chairside use, UV requires longer exposure for reliable results, and diode lasers cannot yet substitute conventional sterilization. Clinicians should select sterilization methods based on clinical context, balancing efficacy with convenience and practicality.

V. Conclusion

Sterilization of dental instruments is essential to prevent cross-contamination and ensure successful endodontic treatment. Among the tested methods, autoclave remains the most reliable and is considered the gold standard due to its simplicity, efficiency, and wide applicability. Glass bead sterilization, though slightly less effective, offers a convenient and rapid chairside alternative. UV chambers and diode lasers showed some effectiveness but were less consistent, likely due to lack of standardized protocols. Continuous advancements in sterilization technologies highlight the importance of strict adherence to effective methods, enabling dental professionals to enhance safety, efficiency, and overall patient care.

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