

Comparative Evaluation of the Antibacterial Efficacy Of Metronidazole, Chlorhexidine and Normal Saline as Laser and Sonic Activated Intracanal Irrigants Against *Enterococcus faecalis* : An In vitro study

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Comparative Evaluation of the Antibacterial Efficacy Of Metronidazole, Chlorhexidine and Normal Saline as Laser and Sonic Activated Intracanal Irrigants Against Enterococcus faecalis : An In vitro study

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Abstract

Background: The persistence of *Enterococcus faecalis* is a significant issue in endodontic therapy, frequently leading to treatment failures. Its capacity to live under extreme conditions, penetrate dentinal tubules, and create resistant biofilms makes conventional antibacterial methods insufficient. Therefore, enhancing the efficacy of intracanal irrigants through advanced activation methods has become crucial for successful root canal disinfection. Despite the proven antimicrobial activity of metronidazole and chlorhexidine, the resilience of *Enterococcus faecalis* necessitates integrating activation techniques that improve irrigant penetration and disrupt biofilms. Laser and sonic activation methods show promise in enhancing the antibacterial performance of irrigants. However, limited studies exist comparing their effects on metronidazole, chlorhexidine, and saline. Hence, there is a need to evaluate the antibacterial efficacy of these irrigants when used in conjunction with laser and sonic activation techniques.

Objective: To compare the antibacterial efficacy of metronidazole, chlorhexidine, and normal saline as intracanal irrigants when activated by laser and sonic techniques against *Enterococcus faecalis* in extracted human mandibular premolars

Methods: Ninety freshly extracted single-rooted mandibular premolars will be obtained, decoronated, and biomechanically prepared using the ProTaper Universal rotary system up to F2. Canals will be inoculated with *Enterococcus faecalis* and incubated for 7 days to allow biofilm formation. The teeth will then be randomly divided into three main groups (metronidazole, chlorhexidine, and saline), each subdivided based on the activation method used (laser or sonic). Irrigation protocols will be standardized, and pre- and post-irrigation bacterial samples will be collected on paper points and cultured on Brain Heart Infusion (BHI) agar. Colony-forming units (CFUs) will be counted to evaluate antibacterial efficacy.

Results: Combining laser activation with metronidazole and chlorhexidine is anticipated to demonstrate superior antibacterial efficacy against *E. faecalis* compared to sonic activation and saline. The study is expected to reveal the most effective irrigant-activation combination for enhanced disinfection

Conclusions: This study aims to provide evidence-based insights into optimizing root canal disinfection protocols by evaluating and comparing the synergistic effects of antimicrobial agents and advanced activation techniques. The findings could contribute significantly to improving endodontic treatment outcomes and reducing the incidence of persistent infections caused by *E. faecalis*. Clinical Trial: This study does not require registration in the Clinical Trials Registry of India (CTRI) as it is a laboratory-based in vitro experimental study involving extracted human teeth, without any interventions on living human participants or clinical outcomes.

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Title: Comparative Evaluation of the Antibacterial Efficacy Of Metronidazole, Chlorhexidine and Normal Saline as Laser and Sonic Activated Intracanal Irrigants Against *Enterococcus faecalis*-An In vitro study

- **ABSTRACT**

Introduction:

Background :

The persistence of *Enterococcus faecalis* is a significant issue in endodontic therapy, frequently leading to treatment failures. Its capacity to live under extreme conditions, penetrate dentinal tubules, and create resistant biofilms makes conventional antibacterial methods insufficient. Therefore, enhancing the efficacy of intracanal irrigants through advanced activation methods has become crucial for successful root canal disinfection. Despite the proven antimicrobial activity of metronidazole and chlorhexidine, the resilience of *Enterococcus faecalis* necessitates integrating activation techniques that improve irrigant penetration and disrupt biofilms. Laser and sonic activation methods show promise in enhancing the antibacterial performance of irrigants. However, limited studies exist comparing their effects on metronidazole, chlorhexidine, and saline. Hence, there is a need to evaluate the antibacterial efficacy of these irrigants when used in conjunction with laser and sonic activation techniques.

Objective: To compare the antibacterial efficacy of metronidazole, chlorhexidine, and normal saline as intracanal irrigants when activated by laser and sonic techniques against *Enterococcus faecalis* in extracted human mandibular premolars.

Materials & Method: Ninety freshly extracted single-rooted mandibular premolars will be obtained, decoronated, and biomechanically prepared using the ProTaper Universal rotary system up to F2. Canals will be inoculated with *Enterococcus faecalis*

and incubated for 7 days to allow biofilm formation. The teeth will then be randomly divided into three main groups (metronidazole, chlorhexidine, and saline), each subdivided based on the activation method used (laser or sonic). Irrigation protocols will be standardized, and pre- and post-irrigation bacterial samples will be collected on paper points and cultured on Brain Heart Infusion (BHI) agar. Colony-forming units (CFUs) will be counted to evaluate antibacterial efficacy.

Expected Results:

Combining laser activation with metronidazole and chlorhexidine is anticipated to demonstrate superior antibacterial efficacy against *E. faecalis* compared to sonic activation and saline. The study is expected to reveal the most effective irrigant-activation combination for enhanced disinfection.

Conclusion: This study aims to provide evidence-based insights into optimizing root canal disinfection protocols by evaluating and comparing the synergistic effects of antimicrobial agents and advanced activation techniques. The findings could contribute significantly to improving endodontic treatment outcomes and reducing the incidence of persistent infections caused by *E. faecalis*.

CTRI Registration :

This study does not require registration in the Clinical Trials Registry of India (CTRI) as it is a laboratory-based in vitro experimental study involving extracted human teeth, without any interventions on living human participants or clinical outcomes.

Keywords: Enterococcus faecalis, Metronidazole, Chlorhexidine, Sonic Activation, Laser Activation, Root Canal Disinfection, Intracanal Irrigants, Endodontic Treatment



- **INTRODUCTION :**

BACKGROUND :

The combined action of intracanal drugs and chemomechanical preparation reduces or eliminates bacteria during root canal therapy.¹ The mechanical action of tools and the flow and backflow of irrigating solutions are used to remove bacteria and other irritants from the root canal's boundaries.² A common and successful endodontic treatment for pulpitis and apical periodontitis is conventional root canal therapy (RCT), which uses biomechanical equipment to remove affected tooth pulp and dentin tissue while using antibiotics and antibacterial materials to eradicate germs.³

Enterococcus faecalis (*E. faecalis*) has been detected in approximately 4% to 40% of primary endodontic infections, indicating its significant role in persistent and recurrent root canal infections, especially in cases of treatment-resistant apical periodontitis.^{4 5} Despite root canal treatment, up to 90% of teeth may retain residual infection. This bacterium's ability to invade dentinal tubules, endure nutrient-deprived environments, and resist conventional antimicrobial methods contributes to its persistence. Its biofilms are notably resilient to commonly used intracanal agents like sodium hypochlorite and calcium hydroxide.⁶

Many medications, including metronidazole (MTR) and chlorhexidine (CHX), have been used to treat oral diseases because they are excellent at inhibiting bacteria and biofilms. A broad-spectrum antibiotic that targets both anaerobic bacteria and protozoa, metronidazole is a nitroimidazole molecule. In addition to Gram-positive and Gram-negative bacilli, it exhibits strong antibacterial activity against anaerobic cocci. Both topical and systemic applications have been made in the treatment of periodontal disease. The anatomical complexity of the root canal system may, however, harbor germs and remaining pulp tissue following RCT, potentially leading to treatment failure.⁷

Chlorhexidine is a cationic biguanide with broad-spectrum coverage. It has activity against both Gram-positive and Gram-negative bacteria. At low concentrations with a range between 0.2%-0.06%, Chlorhexidine shows bacteriostatic properties. At high concentrations, more than 0.12%, it depicts bactericidal activity, antifungal properties, and some antiviral properties. Its effect is not hampered by biological fluids such as blood, and it works for up to 48 hours after application. It acts by penetrating the bacterial cell membrane, causing cytoplasmic leakage at lower concentrations and precipitating nucleic acid at higher concentrations .⁸

The distribution method in conjunction with the irrigant's characteristics affects the irrigation efficacy .⁹ Irrigant activation in endodontic procedures pertains to the utilization of various energy forms—mechanical, sonic, ultrasonic, or other physical modalities—to enhance the dynamic movement and distribution of irrigating solutions throughout the intricately structured root canal system. Evidence from systematic reviews and meta-analyses indicates that the use of activation, regardless of the specific device or mechanism employed, significantly improves the efficacy of debris and smear layer removal. Consequently, activation is recognized as an integral phase in the chemo-mechanical debridement process, contributing to the overall success of root canal disinfection .¹⁰ In contrast to ultrasonic irrigation, sonic irrigation uses a lower frequency (1–10 kHz). As a result, shear wall stresses and fluid velocity are reduced. Conversely, sonic activation produces noticeably more amplitude (horizontal tip displacement) .¹¹

In recent years, non-surgical root canal therapy has made greater use of laser technology. When used in conjunction with chemical solutions, its high power and distinct coherent rays are helpful during the root canal cleaning and disinfection phase. It has been demonstrated that a laser can effectively activate a root canal irrigant by causing mechanical disruptions .¹² The 940 nm diode laser is frequently utilized in

dentistry clinics due to its compact size, portability, affordability, and broad variety of oral medicine applications. Bacterial cell walls can be destroyed by the bactericidal photothermal disrupting impact of its radiation.¹³ Since water and hydroxyapatite are the primary components of dentin, the 940 nm wavelength has low absorption coefficients in water (about $\mu_a=0.04-0.05 \text{ cm}^{-1}$), which allows the laser beam to penetrate deeply into dentin (over 1000 μm) and kill distant bacteria.^{13 14}

According to the literature, *Enterococcus faecalis* during root canal therapy requires a multifaceted approach that includes effective irrigation techniques, appropriate antimicrobial agents like metronidazole and chlorhexidine, and advanced activation methods such as laser technology.

It has been observed in previous studies that the use of chlorhexidine and metronidazole as intracanal irrigants showed optimal results. However, studies with metronidazole as an intracanal irrigant with different activation methods need to be explored. So, there is a need for research that compares the antibacterial effectiveness of Metronidazole, Chlorhexidine, and normal saline as intracanal irrigants, which are activated by laser and sonic activation methods, against *Enterococcus faecalis*. This comparison will help determine the most effective activation technique for enhancing the antibacterial effectiveness of these irrigants, ultimately contributing to more reliable and durable endodontic treatments, improving treatment success rates, and reducing the incidence of persistent infections associated with this resilient bacterium.

- **RATIONALE:**

Enterococcus faecalis is a persistent and resistant microorganism commonly associated with root canal treatment failures. In the twenty-first century, with rising advancements in root canal treatment (RCT), the complete eradication of *E. faecalis* remains challenging due to its ability to survive under adverse conditions, with two main pillars

of its survival being penetrating dentinal tubules and forming biofilms that resist conventional antimicrobial strategies. Traditional irrigants such as metronidazole and chlorhexidine have demonstrated antibacterial effectiveness; however, their efficacy can be significantly enhanced by advanced activation methods. The combination of effective irrigants with activation techniques like laser and sonic methods may provide improved antibacterial outcomes. Hence, there is a need to evaluate and compare the antibacterial effectiveness of metronidazole, chlorhexidine, and saline when activated by laser and sonic methods to determine the most effective combination for eradicating *E. faecalis* and improving the success rates of endodontic treatments.

- **AIM & OBJECTIVES:**

- **AIM:** To compare the antibacterial efficacy of metronidazole, chlorhexidine, and saline as intracanal irrigants activated by laser and sonic techniques against *Enterococcus faecalis* in extracted mandibular human premolars

- **Objectives :**

- To evaluate the antibacterial efficacy of metronidazole as an intracanal irrigant by laser activation against *Enterococcus faecalis* in extracted mandibular human premolars
- To evaluate the antibacterial efficacy of metronidazole as an intracanal irrigant by sonic activation against *Enterococcus faecalis* in extracted mandibular human premolars.
- To evaluate the antibacterial efficacy of chlorhexidine as an intracanal irrigant by laser activation against *Enterococcus faecalis* in extracted mandibular human premolars.
- To evaluate the antibacterial efficacy of chlorhexidine as an intracanal irrigant by sonic activation against *Enterococcus faecalis* in extracted mandibular human premolars.
- To evaluate the antibacterial efficacy of normal saline as an intracanal irrigant by laser activation against *Enterococcus faecalis* in extracted mandibular human

premolars.

- To evaluate the antibacterial efficacy of normal saline as an intracanal irrigant by sonic activation against *Enterococcus faecalis* in extracted mandibular human premolars.

- **NULL HYPOTHESIS :**

There will be no significant effect of laser and sonic activation on the antibacterial efficacy of metronidazole, chlorhexidine, and saline as intracanal irrigants in eradicating *Enterococcus faecalis* in root canal treatments.

- **ALTERNATIVE HYPOTHESIS**

Laser and sonic activation will significantly enhance the antibacterial efficacy of metronidazole, chlorhexidine, and saline as intracanal irrigants in eradicating *Enterococcus faecalis* in root canal treatments.

- **MATERIAL & METHODOLOGY**

- **Materials:** The materials to be used in the current study are listed in Table 1

Table 1. Irrigants & Materials Used

- | |
|---|
| <ul style="list-style-type: none">• Microbiological sample preparation:<ol style="list-style-type: none">1) Strains of <i>E. faecalis</i>2) BHI Agar media |
|---|

3) Incubator
- Irrigants: 1) Chlorhexidine 2) Metronidazole 3) Normal Saline
- Activation devices: 1) Sonic activation device 2) Diode laser
- For Sample Preparation 1) Protaper Universal Treatment Rotary Files (for biomechanical preparation) 2) Paper points

- INCLUSION CRITERIA –**

Freshly extracted single-rooted permanent mandibular lower premolar teeth with closed apex, free from caries, fracture, and previous endodontic treatment.

- EXCLUSION CRITERIA –**

Teeth with visible cracks, abnormal morphology.

Teeth with developmental anomalies.

Teeth with external and internal resorption.

Teeth with multiple roots.

Teeth with calcified canals.

- SAMPLE SIZE FORMULA:**

$$n_1 = \{(\sigma_1^2 + \sigma_2^2 / \kappa) (Z_{1-\alpha/2} + Z_{1-\beta})^2\} \div \Delta^2$$

The notation for the formulae is:

- n_1 = sample size of Group 1

- n_2 = sample size of Group 2
- σ_1 = standard deviation of Group 1
- σ_2 = standard deviation of Group 2
- Δ = difference in group means
- κ = ratio = n_2/n_1
- $Z_{1-\alpha/2}$ = two-sided Z value (e.g., $Z = 1.96$ for 95% confidence interval)
- $Z_{1-\beta}$ = power

Mean CFU count in group II = 50.58

Mean CFU count in group III=27.58

σ_1 = SD of CFU count in group II=17.33

σ_2 = SD of CFU count in group II=2.81

For detecting mean difference of 23 i.e. $\Delta = 50.58 - 27.58 = 23$

$K = 1$

$$N = \frac{(17.33^2 + 2.81^2) (1.96 + 0.84)^2}{23^2}$$

=13.59 = 15 samples needed in each group.

Total sample size = 90

n = 15 samples are needed in each group

Reference: Jain S, Goel S, Nayar R, et al. Comparative evaluation of antibacterial efficacy of various root canal irrigants against *Enterococcus faecalis*: An in vitro study. J Conserv Dent. 2013;16(5):412-415. doi:10.4103/0972 0707.117504

Power of the Test: 80%

Level of significance: 5% (95% confidence interval)

• **SAMPLE SIZE:**

Distribution of sample size: 90

The specimens will be randomly divided into three groups and six subgroups (Table 2)

• **SAMPLE ALLOCATION:**

- **GROUPS:**

Table 2. Group Allocation Based on Irrigant & Activation Method

GROUPS	SUBGROUPS	SAMPLES
GROUP I Metronidazole as an irrigant	Sub-group IA: Laser Activation	n=15
	Sub-group IB: Sonic Activation	n=15
GROUP II Chlorhexidine as an irrigant	Sub-group IIA: Laser Activation	n=15
	Sub-group IIB: Sonic Activation	n=15
GROUP III Normal saline as an irrigant	Sub-group IIIA: Laser Activation	n=15
	Sub-group IIIB: Sonic Activation	n=15

- **STUDY DESIGN:** Comparative study design

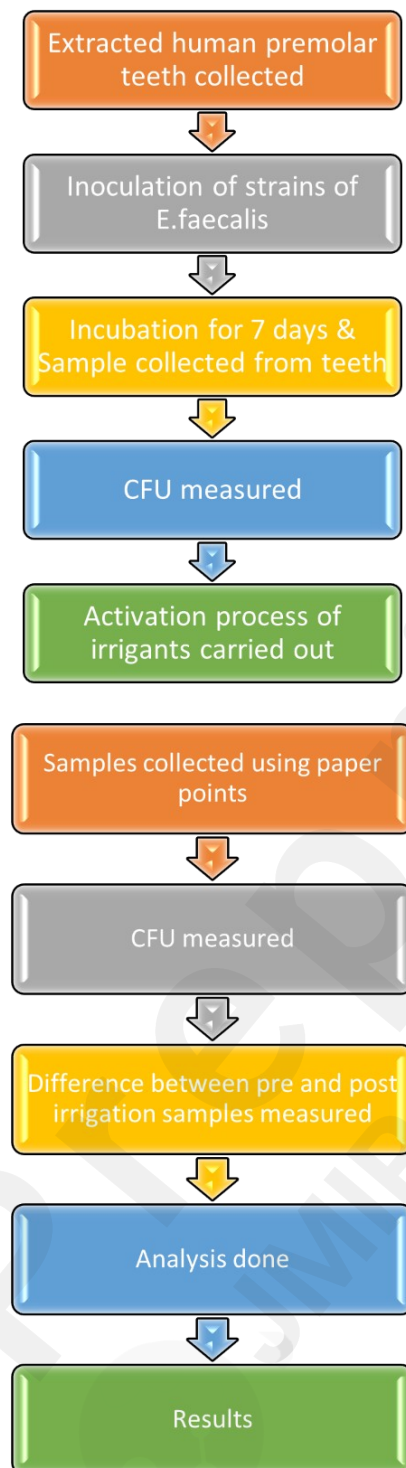
- **INTERVENTION:**

• This study aims to evaluate the antibacterial efficacy of three irrigant solutions—metronidazole, chlorhexidine, and normal saline—against *Enterococcus faecalis* using two activation methods (laser and sonic). The study will involve a total sample size of 90, divided into three main groups based on the intracanal irrigants used, and will further be divided into subgroups based on the activation protocol used. 90 extracted single-rooted premolar teeth are obtained from healthy patients with informed consent. The teeth will then be cleaned & sterilized, removing any residual tissue. Following the removal of any remaining tissue, the teeth will be washed and sterilized before being kept at room temperature in a 0.1% thymol solution until the experimental phase. To achieve a uniform root length of 13 mm, the crowns will thereafter be decoronated

using a diamond disc. To get the working length, one millimetre will be subtracted from the total root length.

One millimetre will be deducted from the total root length to establish the working length. Root canal shaping will be carried out up to the F2 instrument. *Enterococcus faecalis* will be cultured and inoculated into the prepared canals of all samples. The samples will be incubated for 7 days to allow biofilm formation. Pre-irrigation samples will be taken. The inoculated teeth will then be randomly assigned to their respective groups and subgroups. Irrigation will involve 3% sodium hypochlorite (Shivam, India), followed by 3 ml of 17% Ethylene diamine tetra acetic acid (EDTA) for one minute to remove the smear layer, and subsequent irrigation protocol of every group will then be followed by using the respective irrigants of metronidazole, chlorhexidine and saline accompanied by laser & sonic activation with the EndoActivator (Dentsply Maillefer, Switzerland). Post-irrigation samples will be collected with the help of paper points and deposited into BHI Agar media and incubated for 48 hours. The colonies will then be counted using the classic bacterial counting method to evaluate bacterial reduction. The data collected will then be subjected to statistical analysis. The procedure to be followed is depicted in the following flowchart (Figure 1)

Figure 1. Experimental protocol workflow (E.faecalis=*Enterococcus faecalis*, CFU=Colony forming units)



Statistical Analysis:

The dataset will undergo both summarization and advanced statistical interpretation. Analytical tools will include the Chi-square method, Student's t-tests (applicable for both related and independent samples), as well as one-way Analysis of Variance (ANOVA) followed by Tukey's post-analysis comparisons. All statistical procedures and visual data representations will be executed using Statistical Package for the Social Sciences (SPSS) (version 27.0) and GraphPad Prism (version 7.0). A probability value (p) below 0.05 will be treated as indicative of a statistically meaningful result. The statistical methods to be applied are illustrated in Figure 2 & Figure 3.

Figure 2. Statistical Test Flowchart

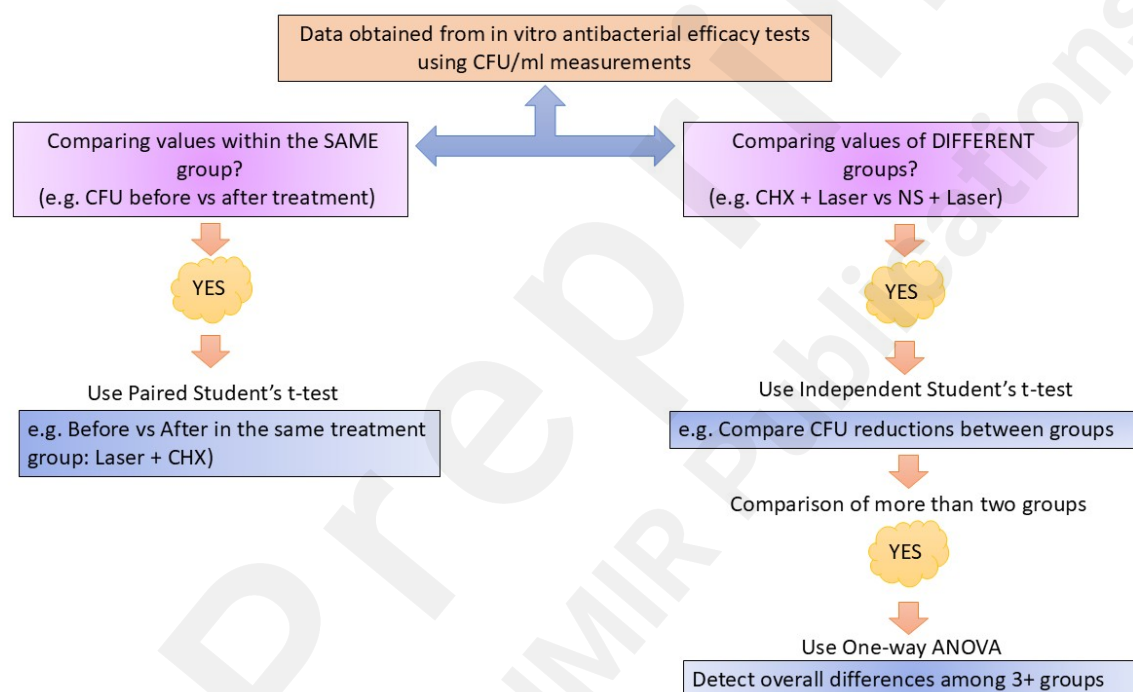
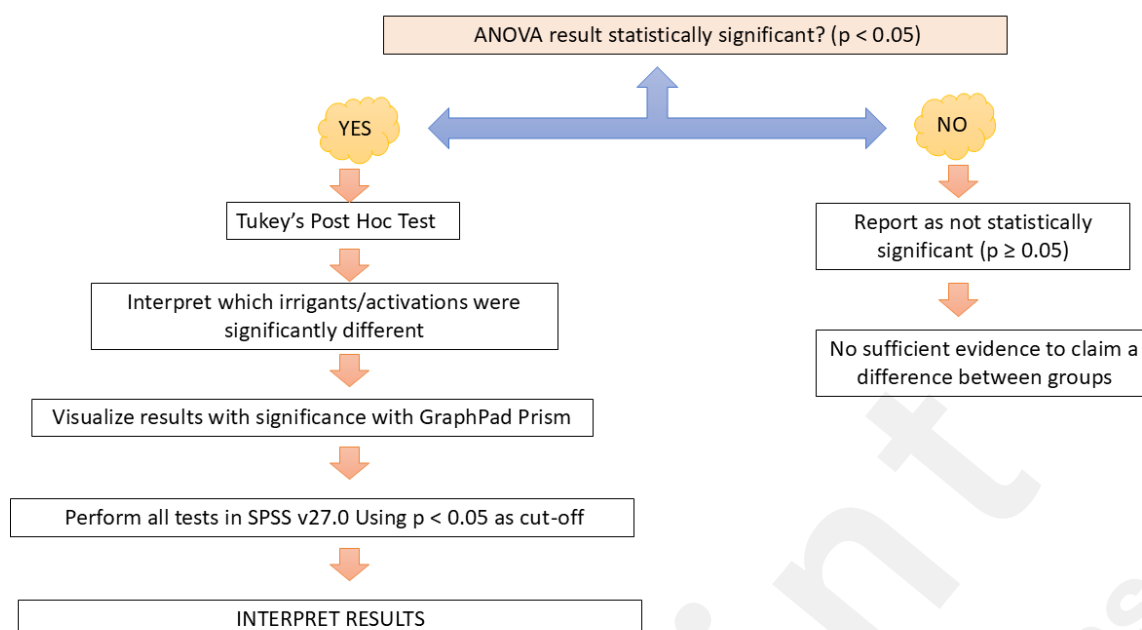


Figure 3. Post analysis steps



ETHICAL CONSIDERATIONS:

Ethical approval for the study was obtained from the Institutional Ethical Committee of Datta Meghe Institute of Higher Education & Research, Sawangi, Wardha (Approval number: DMIHER(DU)/IEC/2025/545 dated: 07/02/2025). Written informed consent will be obtained from patients prior to extraction regarding the use of extracted teeth for research purposes in this study. All extracted teeth will be de-identified before inclusion in the experimental protocol to maintain complete anonymity of the donors.

• OUTCOME MEASURE:

Primary outcome measure: Evaluation & comparison of reduction in colony forming unit count of *E. faecalis* following irrigation by metronidazole with sonic and laser activation techniques with that of chlorhexidine and normal saline.

Secondary outcome measure: To determine the most effective combination of irrigant and activation technique to target and eliminate *E. faecalis* residing in the root canal space effectively.

DISCUSSION :

Achieving successful root canal outcomes requires the meticulous elimination of all microorganisms harbored within the complicated architecture of the root canal system. Despite advancements in irrigation protocols and antimicrobial strategies, *Enterococcus faecalis* remains a major causative agent in treatment failures due to its exceptional resistance and adaptability.^{1 2} This facultative anaerobe is known for its ability to penetrate dentinal tubules, survive in nutrient-deprived environments, and resist common intracanal medicaments, including calcium hydroxide.³ These characteristics underscore the need for not only potent antibacterial agents but also effective activation techniques to enhance their intracanal performance.

In this context, metronidazole and chlorhexidine have emerged as promising irrigants. Metronidazole, a nitroimidazole derivative, is especially effective against anaerobic bacteria and has been utilized in both systemic and local applications in dental practice.⁴ Although its antibacterial spectrum includes *E. faecalis*, the clinical efficacy of metronidazole may be compromised when used without adjunct activation techniques due to its limited ability to penetrate biofilms.⁵ Chlorhexidine, on the other hand, is a broad-spectrum cationic biguanide that retains its activity in the presence of organic matter and has substantivity, remaining active for prolonged durations post-application.⁶ It has shown superior performance in reducing microbial load within the canal system, especially when used at concentrations above 0.12%.⁶

However, the delivery and penetration of these irrigants into canal irregularities are critically dependent on activation techniques. Sonic and laser activation have been shown to improve irrigant flow dynamics, resulting in deeper penetration and enhanced disinfection.^{7 8} Sonic activation systems such as the EndoActivator operate at lower frequencies (1–10 kHz), creating hydrodynamic agitation that improves smear layer and debris removal. Their greater amplitude allows more effective lateral displacement of irrigants within the root canal.⁹

Laser activation, particularly using diode lasers at 940 nm, represents a significant advancement in endodontic disinfection. The unique properties of laser energy—specifically its ability to induce photothermal and photoacoustic effects—result in deeper penetration into dentin and effective bacterial cell wall disruption.^{10 11} Unlike conventional irrigants whose efficacy is often limited to the main canal, laser activation facilitates disinfection in otherwise inaccessible microanatomical structures, including lateral canals and apical ramifications.¹² Furthermore, diode lasers are advantageous due to their compact size, cost-effectiveness, and compatibility with clinical settings.¹¹

The present investigation is designed to explore an under-researched area by directly assessing the antimicrobial performance of metronidazole, chlorhexidine, and saline when activated via laser and sonic methods. While previous studies have highlighted the individual efficacy of these irrigants and activation methods, few have explored their synergistic impact, particularly with metronidazole as the primary irrigant. Given that metronidazole's efficacy is enhanced in anaerobic conditions and that *E. faecalis* can adapt to both aerobic and anaerobic environments, activation becomes a pivotal factor in optimizing its bactericidal action.^{4 5}

Moreover, chlorhexidine's substantivity and ability to disrupt microbial membranes at higher concentrations may complement the mechanical benefits of sonic and laser activation, potentially yielding superior outcomes compared to passive irrigation techniques.^{6 8} In contrast, saline serves as a control, allowing for a clear demarcation of the role of activation in enhancing antibacterial performance.

Previous studies support the antibacterial potential of Metronidazole as an endodontic irrigant. Alaa A Amin et al. (2019) reported significant bacterial reduction with 0.5% Metronidazole and 2% Chlorhexidine in primary teeth, with saline showing no effect.¹⁵ Dubey et al. (2017) found Metronidazole effective against *B. fragilis* and *P. acnes*, while BioPure MTAD showed greater efficacy against *E. faecalis* and *C.*

albicans, highlighting Metronidazole's limited spectrum.¹⁶ Shweta et al. (2013) demonstrated enhanced antibacterial action of Metronidazole and Glutaraldehyde over saline, with ultrasonic activation outperforming hand irrigation.¹⁷ These findings justify evaluating activated Metronidazole and Chlorhexidine in the present study to enhance efficacy against *E. faecalis*.

Overall, the findings of this study are anticipated to contribute significantly to clinical endodontics by providing evidence for more effective disinfection protocols. If metronidazole, when activated, proves comparable or superior to chlorhexidine, it may represent a cost-effective and biocompatible alternative for routine endodontic practice. This integrated approach, combining pharmacological efficacy with physical activation, holds the promise of reducing persistent infections and improving long-term treatment outcomes.

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CONFLICTS OF INTEREST :

None declared.

ABBREVIATIONS:

E.faecalis: Enterococcus faecalis

BHI: Brain Heart Infusion

CFU: Colony-Forming Units

CHX: Chlorhexidine

EDTA: Ethylenediaminetetraacetic Acid

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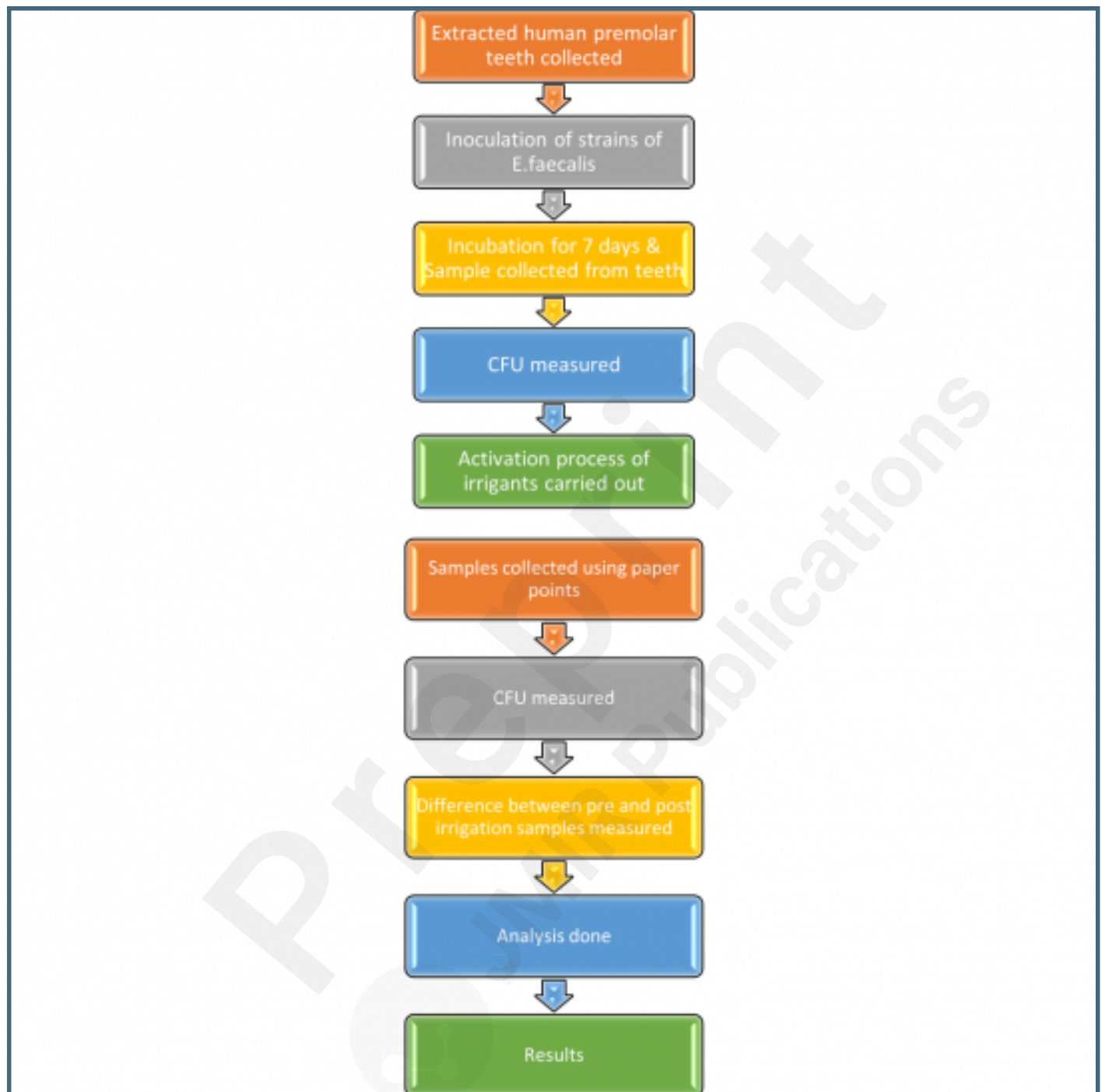
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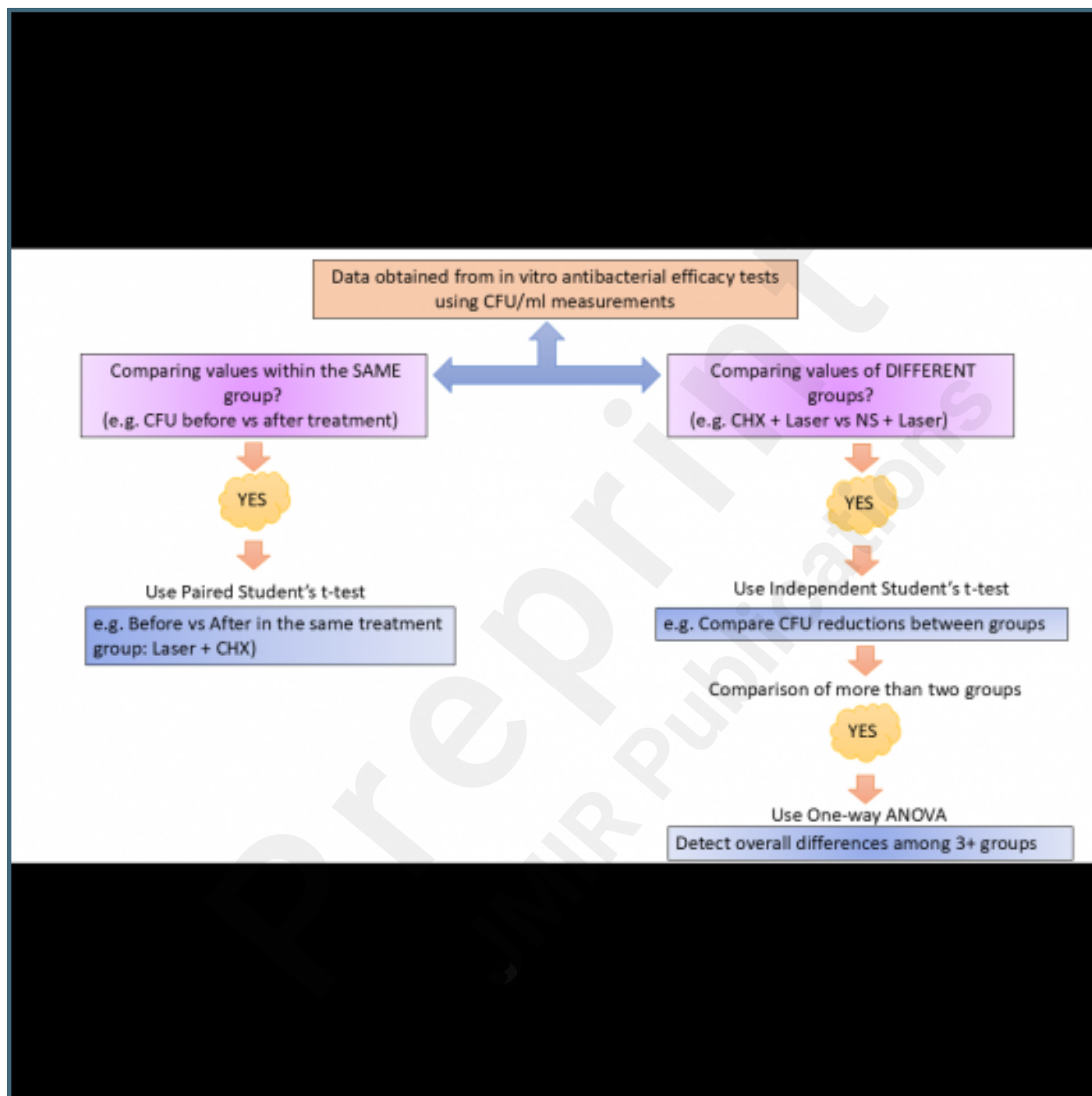
Supplementary Files

Figures

Experimental protocol workflow.



Statistical test flowchart.



Post analysis steps.

