Original Article

# Evaluation of the efficacy of sonic activation in root canal cleaning with an endocator adenosine triphosphate tester: An *in vitro* study

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## Abstract

**Context:** Evaluating the efficacy of sonic activation in root canal cleaning using an Endocator adenosine triphosphate (ATP) tester.

**Aim:** The aim of this study was to assess the cleaning efficacy of needle irrigation and sonic activation by measuring ATP values and colony-forming units (CFUs) during root canal procedure.

**Settings and Design:** The design of the study is an *in vitro* study.

**Materials and Methods:** Eighty ($n$ = 80) extracted mandibular molars were selected, and distal root canals were enlarged to F2 with ProTaper Gold rotary files. Canals were rinsed with 2 mL of 5.25% sodium hypochlorite (NaOCl), 2 mL of 17% ethylenediaminetetraacetic acid, and 2 mL of distilled water. Samples are sterilized, *Enterococcus faecalis* is inoculated and incubated at 37°C for 4 weeks. Baseline ATP values and CFU are measured. Samples are divided into four groups of $n$ = 20 and are irrigated. Group 1:2 mL of distilled water for 30 s. Group 2: 2 mL of distilled water activated with SmartLite Pro EndoActivator (SLP-EA) for 30 s. Group 3: 2 mL of 1% NaOCl for 30 s. Group 4: 2 mL of 1% NaOCl activated with SLP-EA for 30 s. The irrigation procedure was repeated for nine cycles. ATP values and CFU are recorded after each cycle. Friedman, Kruskal–Wallis, Wilcoxon, and Tukey's *post hoc* tests are used for statistical evaluation.

**Results:** A significant decrease in ATP values and CFU from baseline to residual level is observed in all groups ($P \leq 0.05$). Microbial load reduction is significant in Group 4, followed by Groups 2 and 3.

**Conclusions:** 1% NaOCl + SLP-EA showed better efficacy by recording lower ATP values and CFU.

**Keywords:** Adenosine triphosphate values; colony-forming units; endocator; *Enterococcus faecalis*; smartLite Pro EndoActivator

## INTRODUCTION

The primary goal of endodontic therapy is to eradicate the microbes by disinfection of root canal systems using irrigation solutions and agitation devices.[1] Persistent secondary intraradicular infections may be dominated by bacteria such as *Enterococcus faecalis*, Streptococcus species, Pseudoramibacter alactolyticus, and Candida albicans (yeast) at a range of $10^3$–$10^7$.[2]

*E. faecalis* is a Gram positive, facultative anaerobe associated with primary, posttreatment apical periodontitis and retreatment conditions, can penetrate upto 1200 $\mu$m into dentinal tubules. It can be evasive to irrigants and

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medicaments, and can survive upto an alkaline pH of 11.5.[3-5]

Irrigation plays a major role in disinfecting complex root canal systems.[6] Sodium hypochlorite (NaOCl) is the irrigant of choice (0.5%–8.25%),[7-9] which has a unique capacity to dissolve necrotic remnants and biofilm components with exceptional antibacterial activity.[10,11] NaOCl acts on lipoteichoic acids and endotoxins[12] with its readily available chlorine in the form of hypochlorite ($OCl^-$) and hypochlorous acid (HOCl).[13,14]

Activation techniques improve the removal of the smear layer[15] and enhance irrigants into the intricacies of the root canal system.[16] Sonic activation in the apical region eliminates vapor lock enhance irrigant penetration to the working length of the tooth, according to Agarwal *et al*.[17] SmartLite Pro EndoActivator (SLP-EA) (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) is recently introduced with ergonomic, contra-angled, sonic motor, flexible polymer tips with paddle-like parallelogram-shaped cross-section, available in small (yellow 15/02, 22 mm length), medium (red 25/04, 22- and 28-mm lengths), and works in elliptical motion at 3,000 cycles per minute (CPM) and 18,000 CPM.[18]

Conventionally, endodontic microflora are evaluated by culture-based methods which have limitations such as less sensitivity, being laborious and complex. Molecular methods require laboratory setup, are expensive, have potential for false positive and false negative results.[19]

The adenosine triphosphate (ATP) bioluminescence method is used to assess microbes in the root canal,[20,21] oral plaque,[22] food samples,[23] and in water.[24] Endocator assay relies on the Photinus (firefly) luciferase system.[25] In the presence of ATP, luciferin is activated to form luciferyl-adenylate and pyrophosphate. Luciferyl-adenylate further reacts with oxygen to form oxyluciferin and $CO_2$. Oxyluciferin, in its excited state, returns to the ground state with the release of luminescent light (ranging from green to yellow), which is detected by the Endocator and recorded relative light unit indicating ATP content in the sample.[26,27]

The current *in vitro* study aims to assess the efficacy of SLP-EA in reducing intraradicular bacterial count by evaluating ATP values and colony-forming unit (CFU) of *E. faecalis* at different time intervals.

## MATERIALS AND METHODS

### Sample size determination

The sample size is determined using G*power v 3.1.9.4 software (Heinrich-Heine-Universitat Dusseldorf, Dusseldorf, Germany). A total sample size of eighty ($n = 80$) is obtained with an effect size of 0.40, power of the study 0.80, and type-I ($\alpha$) error 0.05.

### Selection and preparation of samples

The institutional ethical committee reviewed (Pr. 115/IEC/SIBAR/2022) and approved. One hundred twenty-six extracted mandibular molars are collected and stored in 0.1% thymol. Of these, eighty teeth ($n = 80$) with intact distal roots and crowns are examined and sectioned buccolingually at the furcation area with the diamond disc.

### Root canal preparation

Neo Spectra ST HV and flow composites (Dentsply, Konstanz, Germany) are used to restore missing wall and to seal root apices. #15 K-file is used to ascertain the patency and length of the distal canal. Canals are instrumented with 25 mm ProTaper Gold rotary files (Dentsply Maillefer, Ballaigues, Switzerland) from S1 to F2. Irrigation is performed with a 30-gauge (G) side-vented needle by delivering 2 mL of 5.25% NaOCl for 3 min, 2 mL of 17% EDTA for 1 min, and a final rinse with 2 mL of distilled water. Samples are autoclaved, and one sample from each pouch[5] is checked with an Endocator ATP tester for sterility.

### Contamination of root canals

*E. faecalis* ATCC-29212 (American Type Culture Collection, Manassas, VA, USA) is cultured for 24 h on blood agar medium (Hy-Glass and Chemicals, India) at 37°C under aerobic conditions and a suspension with an optical density of 0.5 McFarland ($1.5 \times 10^8$ CFU/mL) is prepared.[28] A sterile insulin syringe with a 29G needle is used to inoculate bacterial suspension in samples. Each sample is placed in a sterile, flat bottom 12-well, cell culture plate with brain heart infusion (BHI) (Hy Glass and Chemicals, India) for 4 weeks at 37°C. The BHI is replaced every 3rd day. After 4 weeks, samples are rinsed with 5 mL of distilled water. The samples are randomly assigned into four groups, with each group of $n = 20$, and each sample is labeled from 1 to 20.

### Measurement of baseline adenosine triphosphate and colony-forming unit

100 µL of solution is aspirated from each sample for ATP testing using an Endocator ATP tester for baseline ATP and for culturing in the laminar air-flow cabinet. Blood agar medium is used for *E. faecalis* culturing under aerobic environment at 37°C, and CFU/mL is calculated after 24 h for baseline CFU. Sample collection and evaluation are performed by an endodontist who is blinded to the irrigation protocols.

#### *Irrigation methods*

Irrigation is done with a 30G side-vented needle.

- Group 1: Positive Control-distilled water: Root canal is irrigated with 2 mL of distilled water for 30 s
- Group 2: Distilled water+SLP-EA: Root canal is irrigated

with 2 mL of distilled water for 30 s by activation with SLP-EA at high speed with a medium tip (Red 25/04.22 mm) placed 2 mm short of working length for 30 s

- Group 3: 1% NaOCl: Root canal is irrigated with 2 mL of 1% NaOCl for 30 s
- Group 4: 1% NaOCl + SLP-EA: Root canal is irrigated with 2 mL of 1% NaOCl for 30 s by activation with SLP-EA at high speed using a medium tip (Red 25/04, 22 mm) placed 2 mm short of working length for 30 s.

The final rinse is done with 3 mL of distilled water for 2 min in all samples. For every sample, the irrigation protocol is repeated nine times for a total of 4.5 min. ATP values and CFU are measured after each activation, as mentioned above. After the ninth cycle, residual ATP value and residual CFU are recorded after agitation of the sample using SLP-EA placed 2 mm short of the working length and activated for 60 s with distilled water.

### Statistical analysis

The data are analyzed with IBM SPSS (Statistical Package for the Social Science, IBM Corporation Ltd., Armonk, New York, USA) software for Window version 26.0. The data are subjected to both descriptive and inferential statistics. Descriptive analysis is performed to obtain the mean and standard deviation. Intragroup comparisons are done with the Friedman test, Wilcoxon Signed-rank test and intergroup comparisons with the Kruskal–Wallis test and Wilcoxon rank sum test. Multiple pairwise comparisons are made using Tukey's *post hoc* test. For all the tests, the confidence level was set at 95% and level of significance at $P \leq 0.05$. The correlation between ATP and CFU is evaluated with Pearson's correlation coefficient = 0.9135 ($P = 0.0000$) [Figure 1].

## RESULTS

In intergroup analysis, the difference among the groups in ATP values and CFU at baseline was significant ($P = 0.041$ and 0.009) [Tables 1 and 2]. After the ninth level and residual level of activation, the difference in ATP values and CFU among groups was significant ($P = 0.000$ and 0.000) [Figure 2]. Pairwise comparison of ATP values showed significant difference between Group 1 and Group 2 ($P = 0.047$); Group 1 and Group 3 ($P = 0.007$). At the ninth level of activation, a significant difference in ATP values is observed between all the groups except Group 2 and Group 3 ($P = 0.084$). At the residual level, there is a significant difference in ATP values between all four groups. Pairwise comparison of CFU showed significant difference between Group 1 and Group 2 ($P = 0.022$); Group 1 and Group 3 ($P = 0.003$); and Group 3 and Group 4 ($P = 0.018$). At the ninth level of activation, a significant difference in CFU is observed between all groups except Group 2 and Group 3 ($P = 0.069$). At the residual level, there is a significant difference in ATP and CFU between all four groups [Table 3]. A high correlation was reported between ATP values and CFU in all groups. PRILE flowchart representing the experimental protocol is shown in Figure 3.

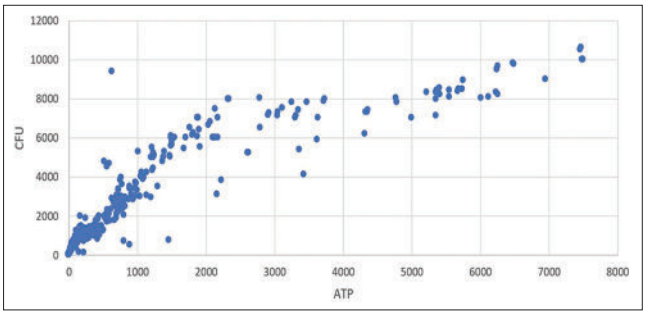


**Figure 1:** Correlation between adenosine triphosphate values and colony forming unit. Data from all groups and all time points. Pearson's Correlation Coefficient = 0.9135 ($P = 0.0000$). The correlation is high. However, looks like two parts

## DISCUSSION

Results from the study suggest that reduction in ATP levels and CFU was more effective with the agitation of 1% NaOCl and distilled water with SLP-EA than the irrigating effect of 1% NaOCl with significant difference. SLP-EA, with its unique paddle-shaped parallelogram cross-section, works in elliptical motion[18] with the formation of acoustic streaming and cavitations that might have enhanced the removal of inoculated bacteria from the root canal system. Similar results were reported with the activation of NaOCl with EndoActivator in the removal of microbial biofilm at a higher rate compared to needle irrigation.[29-32]

The time taken for 90% reduction of ATP mean value was 3.5 min from baseline in Group 1. In Groups 2 and 3, it is 1.5 min, and in Group 4, it is 1 min. Significance was observed between Groups 1 and 2, 1 and 3, 1 and 4, 2 and 4, and 3 and 4. However, significance was not observed between Groups 2 and 3. This finding is interesting and suggests that the efficacy of distilled water with SLP-EA is as effective as 1% NaOCl at 1.5 min interval in reducing the percentage of ATP values from baseline.

99.8%, 99.9%, and 100% reduction of ATP from baseline is observed in Group 4 at 2, 2.5, and 4.5 min, respectively, and is significant with other groups. This observation reflects that, even when the bacterial CFU are highest in the root canal, they can be eliminated within 4.5 min with the help of 1% NaOCl with SLP-EA. This observation indicates that sonic activation is needed for the highest percentage or complete eradication of bacteria from a root canal in less time.

**Table 1: Percentage change in adenosine triphosphate values from baseline**

| Group | Statistics | Timepoint (0=baseline) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 |
| Group 1 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −87.23 | −93.78 | −99.83 | −98.53 | −98.83 | −99.34 | −99.96 | −99.05 | −99.26 |
| | Median | −62.42 | −79.94 | −84.99 | −89.59 | −90.14 | −94.87 | −97.09 | −96.63 | −97.80 |
| | Maximum | −8.27 | −26.38 | −28.59 | −33.82 | −39.20 | −42.17 | −44.24 | −62.09 | −63.29 |
| | Mean | −56.45 | −72.34 | −75.17 | −84.02 | −83.04 | −86.35 | −91.12 | −92.48 | −92.66 |
| | Standard | 22.16 | 21.41 | 21.75 | 18.63 | 20.93 | 18.91 | 16.28 | 10.83 | 11.16 |
| | *P*[a] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 2 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −98.97 | −99.84 | −99.21 | −99.86 | −99.95 | −99.95 | −99.98 | −100.00 | −100.00 |
| | Median | −65.34 | −90.20 | −95.68 | −98.51 | −99.39 | −98.93 | −99.33 | −99.53 | −99.93 |
| | Maximum | 79.71 | 18.28 | −53.88 | −93.06 | −89.06 | −86.21 | −94.58 | −95.93 | −97.46 |
| | Mean | −47.58 | −73.67 | −91.95 | −97.89 | −98.14 | −98.10 | −98.94 | −99.10 | −99.51 |
| | Standard | 52.60 | 34.91 | 10.18 | 2.04 | 3.01 | 3.03 | 1.48 | 1.20 | 0.77 |
| | *P*[b] | 0.0026 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 3 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −93.32 | −98.67 | −99.62 | −99.80 | −99.83 | −99.92 | −99.93 | −99.97 | −99.97 |
| | Median | −42.66 | −90.21 | −94.36 | −97.84 | −98.60 | −99.28 | −99.43 | −99.68 | −99.60 |
| | Maximum | 17.85 | −54.56 | −83.77 | −89.81 | −94.00 | −92.86 | −95.50 | −97.76 | −98.50 |
| | Mean | −46.02 | −82.80 | −93.78 | −97.15 | −98.26 | −98.70 | −98.95 | −99.38 | −99.45 |
| | Standard | 31.96 | 16.94 | 5.35 | 2.82 | 1.62 | 1.97 | 1.27 | 0.64 | 0.49 |
| | *P*[c] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 4 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −99.49 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 |
| | Median | −88.20 | −99.41 | −99.93 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 |
| | Maximum | −52.55 | −84.87 | −97.63 | −99.27 | −99.34 | −99.87 | −99.95 | −99.93 | −100.00 |
| | Mean | −82.49 | −98.30 | −99.58 | −99.89 | −99.96 | −99.98 | −100.00 | −99.99 | −100.00 |
| | Standard | 15.81 | 3.47 | 0.71 | 0.21 | 0.15 | 0.04 | 0.01 | 0.02 | 0.00 |
| | *P*[d] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *P*[e] | 1=2=3=4 | 0.0002 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *P*[f] | 1=2 | 0.6443 | 0.1065 | 0.0023 | 0.0000 | 0.0000 | 0.0000 | 0.0001 | 0.0000 | 0.0000 |
| | 1=3 | 0.3652 | 0.0344 | 0.0013 | 0.0000 | 0.0000 | 0.0000 | 0.0005 | 0.0000 | 0.0000 |
| | 1=4 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | 2=3 | 0.2505 | 0.8989 | 0.7329 | 0.4248 | 0.1666 | 0.2851 | 0.8774 | 0.7720 | 0.1703 |
| | 2=4 | 0.0055 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | 3=4 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

[a,b,c,d]Wilcoxon signed rank test. two-sided, [e]Kruskal–Wallis. two-sided, [f]Wilcoxon rank sum test. two-sided

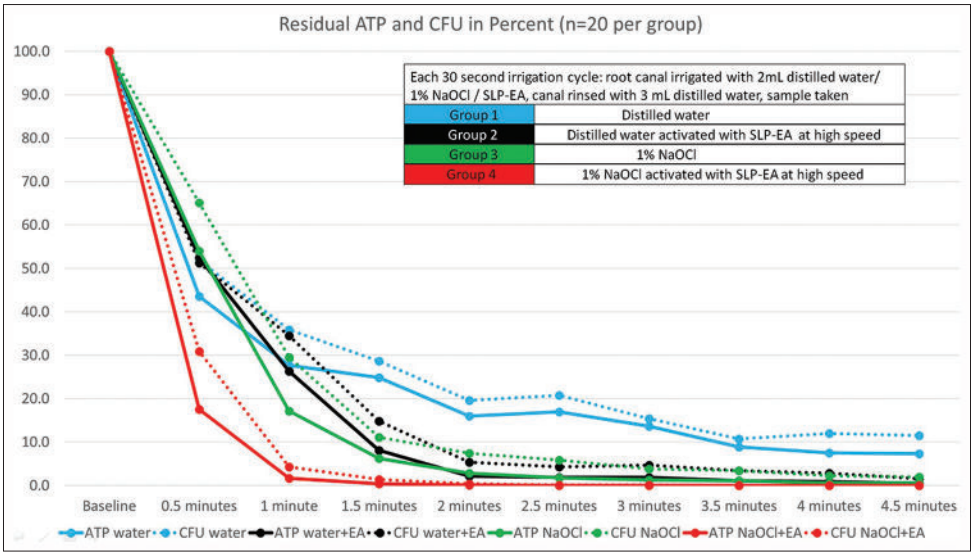


**Figure 2:** Residual adenosine triphosphate values and colony forming unit in percentage

In Groups 2 and 3, 99.8% and 99.9% reduction in ATP/CFU values from baseline is observed at 2 and 3 min, respectively; whereas a study conducted by Frough-Reyhani *et al*.[33] reported a reduction of 85.73% with a contact time of 10 min of NaOCl. However, 100% absolute change from baseline is observed only in Group 2 at 4 min and 4.5 min

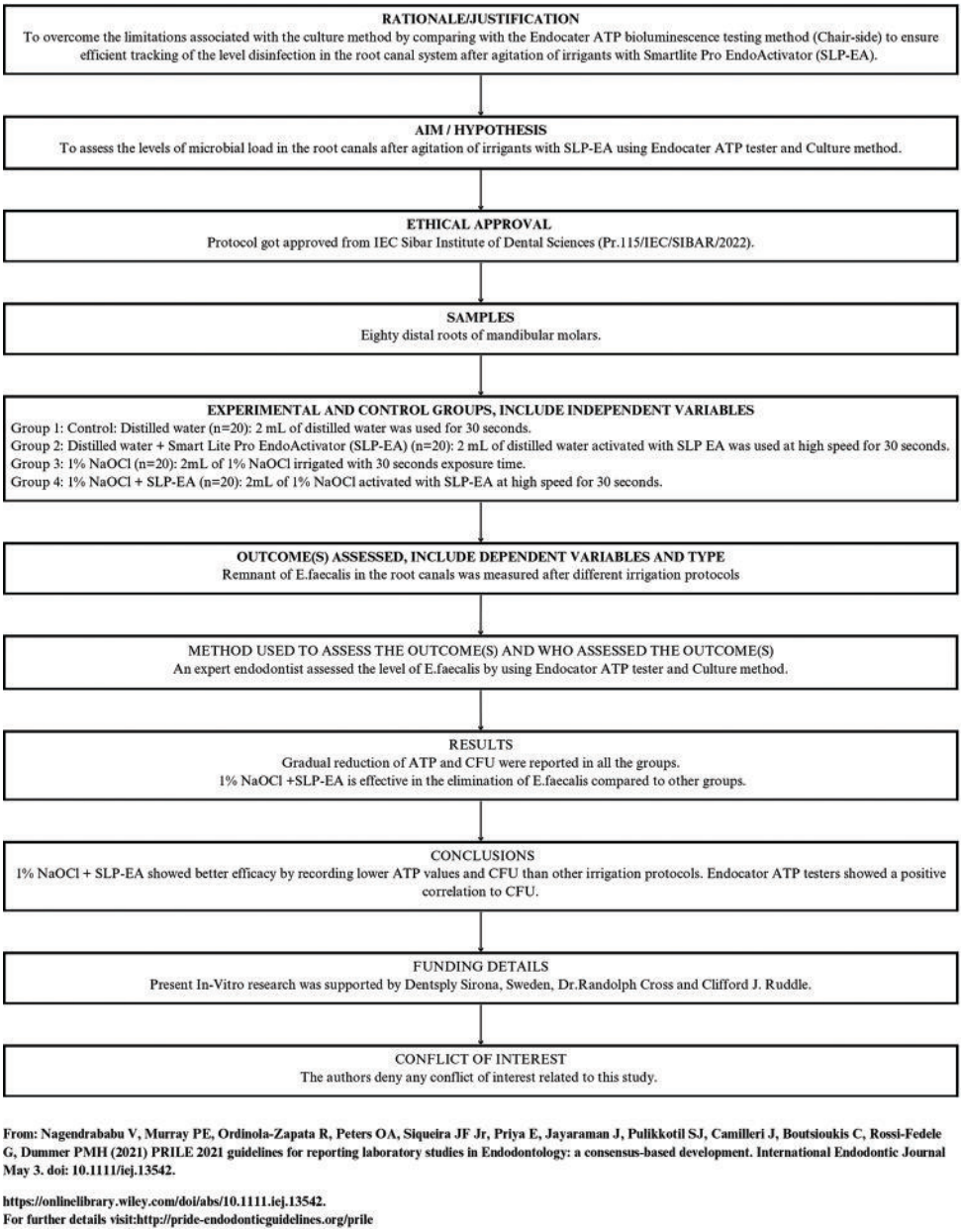


**Figure 3:** PRILE 2021 flow chart

but not in Group 3 without significance. This observation suggests that sonic activation of distilled water is as effective as 1% NaOCl and reiterates the importance of activation of irrigating solution during endodontic treatment.

Regarding 99.8%, 99.9%, and 100% reduction of minimum ATP values recorded at baseline in four groups, different findings were observed. A 100% change of ATP values was observed after two cycles of irrigation with 1% NaOCl and SLP-EA, and this finding was consistent up to the ninth cycle without relapse or increase of ATP/CFU values. This observation suggests that, in samples with low ATP/CFU baseline values, removal of bacteria from the root canal can be easy and at the earliest.

Reduction in CFU is highly significant between Groups 3 and 4 with complete elimination of CFU in Group 4 after the ninth cycle due to the effect of SLP-EA and indicates the importance of activation of the irrigating solution by mechanical means and eliminating CFU to the maximum percentage within 2.5–4.5 min; whereas Retamozo *et al*. stated that, for complete eradication of *E. faecalis,* 40 min is required with 5.25% NaOCl as irrigant.[34]

A high correlation is noted between ATP and CFU in all groups. ATP is reported to be present in bacteria that are in a viable but nonculturable state (VBNC).[35,36] Endocator testers are sensitive to identify the ATP from the bacteria

**Table 2: Percent change in colony forming units from baseline**

| Group | Statistics | Timepoint (0=baseline) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 |
| Group 1 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −82.05 | −87.92 | −98.62 | −94.25 | −95.44 | −98.38 | −99.88 | −96.63 | −97.56 |
| | Median | −45.95 | −66.11 | −74.45 | −84.19 | −85.01 | −88.26 | −92.90 | −92.12 | −93.27 |
| | Maximum | −7.50 | −34.09 | −31.24 | −42.46 | −48.86 | −52.27 | −56.82 | −60.67 | −55.20 |
| | Mean | −48.13 | −64.17 | −71.37 | −80.37 | −79.28 | −84.57 | −89.32 | −88.01 | −88.46 |
| | Standard | 20.84 | 15.96 | 18.48 | 14.13 | 14.58 | 11.88 | 11.05 | 10.23 | 12.26 |
| | *P*[a] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 2 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −94.54 | −99.33 | −97.50 | −98.95 | −99.60 | −99.71 | −99.85 | −100.00 | −100.00 |
| | Median | −53.49 | −85.30 | −89.16 | −95.60 | −96.76 | −96.49 | −97.87 | −98.08 | −99.64 |
| | Maximum | 7.89 | 51.89 | −60.00 | −89.07 | −80.81 | −87.46 | −84.81 | −87.84 | −92.70 |
| | Mean | −48.77 | −65.52 | −85.15 | −94.59 | −95.74 | −95.34 | −96.63 | −97.15 | −98.48 |
| | Standard | 31.43 | 38.18 | 11.32 | 3.23 | 4.09 | 3.82 | 3.64 | 3.02 | 2.24 |
| | *P*[b] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 3 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −87.14 | −96.35 | −99.03 | −99.45 | −98.96 | −99.44 | −99.53 | −99.69 | −99.72 |
| | Median | −24.71 | −80.41 | −88.23 | −91.82 | −94.80 | −97.00 | −97.99 | −98.21 | −98.59 |
| | Maximum | 30.25 | −31.80 | −78.48 | −82.93 | −88.20 | −86.68 | −90.06 | −94.71 | −95.59 |
| | Mean | −34.89 | −70.45 | −88.90 | −92.55 | −94.19 | −96.22 | −96.58 | −97.77 | −98.05 |
| | Standard | 33.88 | 21.97 | 5.69 | 4.75 | 3.39 | 3.20 | 3.00 | 1.68 | 1.23 |
| | *P*[c] | 0.0006 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Group 4 | *n* | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| | Minimum | −97.93 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 |
| | Median | −78.39 | −97.86 | −99.77 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 | −100.00 |
| | Maximum | −14.23 | −80.54 | −91.38 | −98.10 | −98.19 | −99.22 | −99.65 | −99.50 | −100.00 |
| | Mean | −69.07 | −95.67 | −98.60 | −99.60 | −99.85 | −99.92 | −99.97 | −99.95 | −100.00 |
| | Standard | 27.36 | 6.17 | 2.51 | 0.63 | 0.42 | 0.21 | 0.10 | 0.14 | 0.00 |
| | *P*[d] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *P*[e] | 1=2=3=4 | 0.0055 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| *P*[f] | 1=2 | 0.8568 | 0.1632 | 0.0050 | 0.0000 | 0.0000 | 0.0000 | 0.0015 | 0.0000 | 0.0000 |
| | 1=3 | 0.0774 | 0.1988 | 0.0004 | 0.0000 | 0.0000 | 0.0000 | 0.0015 | 0.0000 | 0.0000 |
| | 1=4 | 0.0085 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | 2=3 | 0.2506 | 0.8778 | 0.6157 | 0.1803 | 0.0498 | 0.7838 | 0.5691 | 0.8462 | 0.0525 |
| | 2=4 | 0.0278 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| | 3=4 | 0.0016 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

[a,b,c,d]Wilcoxon signed rank test. two-sided, [e]Kruskal–Wallis. two-sided, [f]Wilcoxon rank sum test. two-sided

**Table 3: Pair-wise comparison of adenosine triphosphate values and colony forming units counts at baseline, ninth level of activation, at residual level among four groups using Tukey's *post hoc* test**

| Groups | ATP/CFU | Baseline (*P*) | Ninth level of activation (*P*) | Residual level (*P*) |
|---|---|---|---|---|
| Group 1–Group 4 | ATP | 0.301 | 0.000* | 0.000* |
| | CFU | 0.556 | 0.000* | 0.000* |
| Group 1–Group 2 | ATP | 0.047* | 0.000* | 0.000* |
| | CFU | 0.022* | 0.000* | 0.000* |
| Group 1–Group 3 | ATP | 0.007* | 0.030* | 0.057 |
| | CFU | 0.003* | 0.034* | 0.054 |
| Group 4–Group 2 | ATP | 0.343 | 0.002* | 0.004* |
| | CFU | 0.090 | 0.002* | 0.000* |
| Group 4–Group 3 | ATP | 0.096 | 0.000* | 0.000* |
| | CFU | 0.018* | 0.000* | 0.000* |
| Group 2–Group 3 | ATP | 0.475 | 0.084 | 0.050* |
| | CFU | 0.507 | 0.069 | 0.052 |

*$P \leq 0.05$: Statistically significant. ATP: Adenosine triphosphate, CFU: Colony forming units

in VBNC and provide information in 10–15 s with an edge over the culture method.[20] This suggests that Endocator ATP testers can be used as a chair-side test to assess the microbial load in the root canals at different stages of the endodontic treatment.

The time taken to form CFU in this study is 4 weeks. In clinical cases with prolonged endodontic infections, complex biofilms involving *E. faecalis* and other species exhibit higher pathogenicity, making them more challenging to remove compared to isolated monoclonal forms.[33] This study's limitation is culturing isolated *E. faecalis*. To better understand CFU elimination during endodontic treatment, ATP values should be recorded across clinical scenarios, aiming for a 99%–100% reduction from baseline to assess treatment efficacy.

Further studies are needed to evaluate the sensitivity and specificity of Endocator ATP assay with different irrigation protocols required to eradicate microbes using various agitation devices.

## CONCLUSIONS

1% NaOCl+SLP-EA showed better efficacy by recording lower ATP values and CFU. Sonic activation influences the reduction of bacterial load. Endocator ATP testers can be used as chair-side evidence to evaluate the status of microbial load during endodontic treatment.

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## Conflicts of interest

There are no conflicts of interest.

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