# An *In Vitro* Comparison of New Irrigation and Agitation Techniques to Ultrasonic Agitation in Removing Bacteria From a Simulated Root Canal

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## Abstract

**Introduction:** This *in vitro* study compared 3 agitation and 2 irrigation devices to ultrasonic agitation at mechanically removing bacteria from a plastic simulated canal, instrumented to 35/.06. **Methods:** The plastic blocks were divided into seven groups. The control (C) group with brain-heart infusion (BHI) broth (sterile) received only needle irrigation. The remaining groups were incubated with BHI inoculated with *Enterococcus faecalis*. Irrigation and agitation techniques were ultrasonic, needle irrigation, EndoVac irrigation (Smart Endodontics; Discus Dental, Culver City, CA), EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK), F-File (Plastic Endo, Lincolnshire, IL), and sonic. Sterile water was the irrigant in all treatments. Remaining bacteria were stained with 0.1% crystal violet. The crystal violet was extracted using a detergent and measured spectrophotometrically. **Results:** The results of this study show that ultrasonic agitation was not significantly different ($p > 0.05$, Tukey test) from the control. There was no significant difference ($p > 0.05$, Tukey test) between the ultrasonic agitation and the use of EndoActivator, F-File, and sonic agitation. Ultrasonic agitation was significantly more effective at removing bacteria than needle irrigation and EndoVac irrigation ($p < 0.05$, Tukey test). **Conclusion** In a plastic simulated canal, ultrasonic agitation was significantly more effective than needle irrigation and EndoVac irrigation at removing intracanal bacteria. Ultrasonic, EndoActivator, F-File, and sonic agitation are similar in their ability to remove bacteria in a plastic simulated canal. *(J Endod 2009;35:1040–1043)*



## Key Words

Agitation technique, root canal irrigants


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An objective of conventional endodontic treatment is to remove pulp tissue and minimize the amount of pathologic debris in the root canal system (1). Mechanical instrumentation of the root canal without irrigants or dressings has been shown to minimally reduce the concentration of microorganisms; however, no instrument file system alone has been shown to eliminate bacterial contamination from the root canal (2, 3). To date, NaOCl remains the most effective endodontic irrigant because of its ability to dissolve tissue, its broad antimicrobial spectrum, and high efficacy against obligate and anaerobic facultative microorganisms (4). Mechanical instrumentation combined with antimicrobial irrigation further reduced the number of microorganisms by 100 to 1,000 times (5). However, several studies have shown that traditional mechanical preparations in conjunction with needle irrigation with different concentrations of NaOCl still do not predictably render a root canal free of bacteria (5–9).

This bacterial persistence within the canals of endodontically treated teeth as contained within the residual canal debris or attached to the canal wall has been shown to play a major role in continuing or creating apical periodontitis (10–14). Because apical periodontitis may be caused by colonization of the root canal system by microorganisms, removal of the critical mass of microbes is essential to endodontic success (15). Bacteria can attach to the root canal walls and organize into biofilms, thus resisting treatment (14, 16). The slow metabolic rate of microorganisms in biofilms as well as the extracellular matrix of the biofilm works to impede the effectiveness of many antimicrobials (17, 18). Despite the high success rates of endodontic therapy, it appears that current irrigant and file techniques alone do not achieve complete removal of microorganisms from within the root canal system. Therefore, reducing the bacterial community to a level below that required to induce or sustain diseases has become the accepted goal (14).

Ultrasonic and sonic agitation was introduced as a means to increase the effectiveness of chemomechanical preparation in hopes to more effectively clean the canal system and disrupt the bacterial communities. Carver et al (19) found that although ultrasonic agitation did not completely remove all bacteria, after hand and rotary cleaning and shaping, it did significantly reduce bacterial counts compared with hand and rotary instrumentation alone. There have been conflicting results for the increased efficacy and advantages of sonic or ultrasonic instrumentation as the primary technique to remove the smear layer and canal debris. Many of these studies incorporated 1- to 3-minute agitation times. However, Sabins et al (20) found that passive ultrasonic agitation produced significantly cleaner canals than did the passive use of sonic agitation during 30- and 60-second cleaning periods. Recently, several agitation devices and an irrigation technique have been introduced as proposed armamentarium to effectively clean the root canal system and replace ultrasonic agitation.

The purpose of this *in vitro* study is to determine whether needle irrigation, EndoVac irrigation, EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK), F-File (Plastic Endo, Lincolnshire, IL), and sonic agitation are as effective as ultrasonic agitation at mechanically removing bacteria in a simulated 30° canal.

## Materials and Methods

The first of three separate trials consisted of 42 plastic resin blocks (Sybron Endo, Glendora, CA), with a 30° simulated root canal (Fig. 1). They were prepared in a

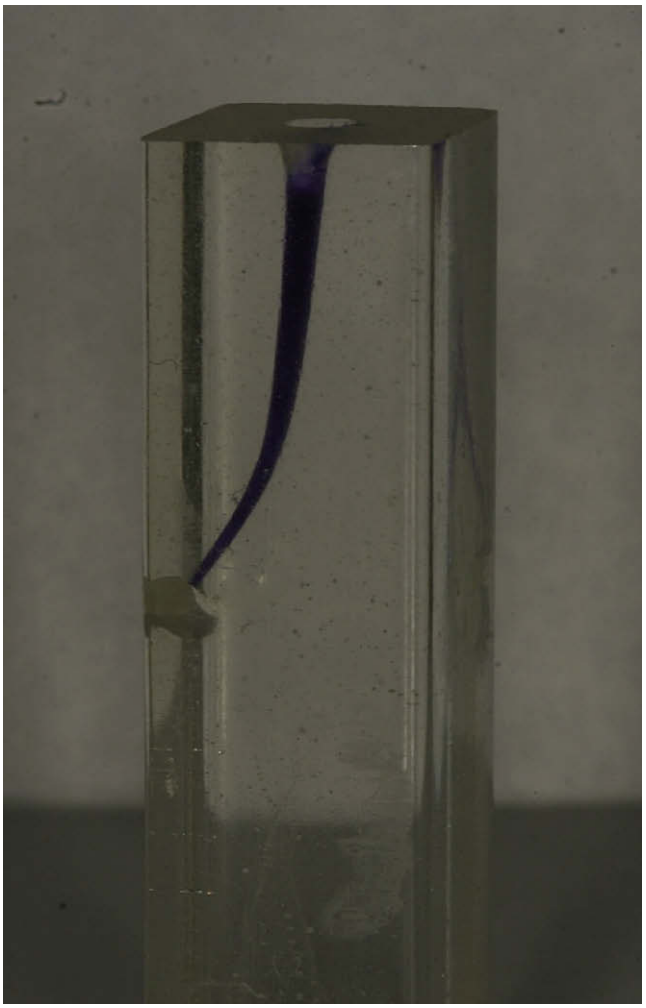

**Figure 1.** Plastic block with crystal violet.

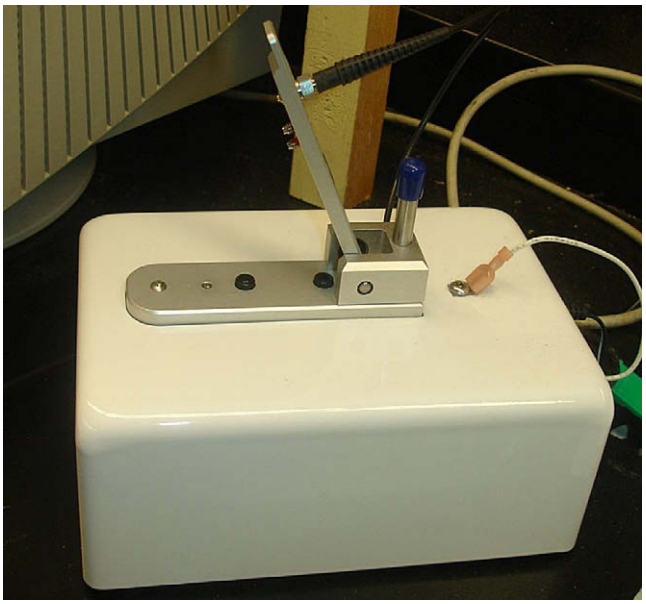

**Figure 2.** NanoDrop ND-1000 spectrophotometer.

crown-down technique. The coronal and middle thirds of the simulated canals were prepared with Race (Brasseler USA, Savannah, GA) 40/.10 and 35/.08 nickel-titanium rotary endodontic files. The apical third was prepared with a crown-down technique to a size 35/.06 using Brasseler Endo Sequence (Brasseler USA, Savannah, GA) rotary endodontic files (9, 21, 22). All canals were irrigated with sterile water using a monoject syringe with a 28-gauge needle (Max-i-Probe; Dentsply MPL, Elgin, IL). The irrigation needle was placed as far apically as possible without binding in the canal to simulate clinical conditions. The canals were then dried with intracanal suction and sterile paper points (Kerr USA, Romulus, MI). All prepared resin practice blocks were autoclaved with the Statim 2000S (SciCan, Toronto, Ontario) to ensure sterility.

The control of 6 blocks was filled with sterile brain-heart infusion (BHI) broth (Becton Dickinson and Co, Sparks, MD) 1 mm short of the coronal orifice. The remaining 36 blocks were filled 1 mm short of the coronal orifice with BHI broth inoculated with *Enterococcus faecalis* (American Type Culture Collection 4082). All plastic blocks were covered with parafilm "M" (American Can Company Greenwich, CT) and incubated aerobically at 37°C for 7 days. After 7 days, the plastic blocks were removed, and the control group was kept separate and labeled as group C. The remaining 36 blocks were randomly assigned to groups with six blocks per group as follows: (1) control (C*)* ($n$ = 6): this group received only needle irrigation with 6 mL of sterile water using a 28-gauge needle (Max-i-Probe; Dentsply MPL, Elgin, IL), (2) US ($n$ = 6): MiniEndo II ultrasonic unit (Spartan EIE Inc, San Diego, CA) agitation for 30 seconds 2 mm short of apex in short 2- to 3-mm cyclic axial motion on low speed as directed by the manufacturer; (3) NI ($n$ = 6): this group received needle irrigation with 6 mL of sterile water using a 28-gauge needle (Max-i-Probe); (4) EV ($n$ = 6): EndoVac (Smart Endodontics; Discus Dental, Culver City, CA) irrigation system as directed by the manufacturer using only sterile water as the irrigant; (5) EA ($n$ = 6): EndoActivator (Dentsply Tulsa Dental Specialties, Tulsa, OK) agitation for 30 seconds 2 mm short of apex in cyclic axial motion with the 15/.02 tip on the highest speed as directed by the manufacturer, (6) F ($n$ = 6): F-File agitation for 30 seconds at length in a short circumferential 2- to 3-mm cyclic axial motion at 900 rpm as directed by the manufacturer, and (7) S ($n$ = 6): Micromega 1500 sonic handpiece (Medidenta Int Co, Woodside, NY) agitation for 30 seconds 2 mm short of apex in short 2- to 3-mm cyclic axial motion as directed by the manufacturer.

Groups US, EA, F, and S all received 3 mL of irrigation before agitation and then received a final flush with 3 mL of sterile water to closely resemble clinical procedures. All canals were dried after irrigation with intracanal suction and sterile paper points.

After treatment, all canals were then filled 1 mm short of the canal orifice with 0.1% crystal violet (w/v in distilled water) for 10 minutes to stain the remaining bacteria (23). The canals were then irrigated with 2 mL of sterile water to remove excess crystal violet. A detergent of 2% Na-deoxycholate (w/v in distilled water) was placed in each canal for 5 minutes to extract the remaining crystal violet from attached bacteria. The solution of Na-deoxycholate/crystal violet was removed from the canals and placed in a sterile 1.5-mL microcentrifuge tube (VWR International Inc, Batavia, IL). A 2-$\mu$L quantity of Na-deoxycholate/crystal violet solution was removed from the microcentrifuge tube and measured by using the NanoDrop *ND*-1000 (Fig. 2) Spectrophotometer (NanoDrop Technologies, LLC, Wilmington, DE) at an absorbance of 605 nm. A higher absorbance measurement indicates a greater amount of retained post instrumented bacteria (23).

As stated previously, this study was conducted three separate times to ensure reproducibility and statistical relevance. A total of 126 plastic