# One-Visit Versus Two-Visit Root Canal Treatment: Effectiveness in the Removal of Endotoxins and Cultivable Bacteria

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## Abstract

**Introduction:** This clinical study was conducted to compare the effectiveness of 1-visit versus 2-visit root canal treatment in removing endotoxins and cultivable bacteria from primarily infected root canals. **Methods:** Forty-eight primarily infected root canals were selected and randomly divided into 4 groups: G1, 1% NaOCl; G2, 2% chlorhexidine (CHX) gel; G3, 1% NaOCl + $Ca(OH)_2$; and G4, 2% CHX gel + $Ca(OH)_2$ (all, n = 12). G1 and G2 involved 1-visit treatment, whereas G3 and G4 involved 2-visit treatment with the placement of $Ca(OH)_2$ medication for 14 days. Samples were collected before and after root canal procedures. A chromogenic *LAL* assay test was used to quantify endotoxins. Culture techniques were used to determine bacterial counts. **Results:** Endotoxins and cultivable bacteria were detected in 100% of the initial samples. All treatment protocols were effective in reducing bacterial load from infected root canals: G1 (1% NaOCl, 99.97%), G2 (2% CHX gel, 99.75%), G3 (1% NaOCl + $Ca(OH)_2$, 99.90%), and G4 (2% CHX gel + $Ca(OH)_2$, 96.81%), respectively ($P < .05$). No differences were found in bacterial load reduction when comparing 1-visit and 2-visit treatment groups, irrespective of the irrigant tested ($P > .05$). Higher median percentage values of endotoxin reduction were achieved in the 2-visit treatment groups (G3, 98.01% and G4, 96.81%) compared with 1-visit treatment groups (G1, 86.33% and G2, 84.77%) (all $P < .05$). **Conclusions:** Both 1-visit and 2-visit root canal treatment protocols were effective in reducing bacteria and endotoxins, but they were not able to eliminate them in all root canals analyzed. Furthermore, 2-visit root canal treatment protocols were more effective in reducing endotoxins than 1-visit root canal treatment protocols. *(J Endod 2013;39:959–964)*

## Key Words

Endotoxin, LAL, limulus amebocyte lysate assay, root canal

One-visit and 2-visit root canal treatments have gained attention during recent decades under different aspects, including healing rates, postobturation pain, bacterial disinfection, as well as patient preferences (1–4).

It has long been known that the infectious disease involved in apical periodontitis is the result of the interplay between number of bacterial cells, microbial virulence, and host defense (5). Therefore, ideal antimicrobial treatment protocol for teeth with apical periodontitis should be able to eliminate bacteria (3, 6, 7) as well as microbial virulence factors (7, 8), which might contribute to the perpetuation of periapical inflammation process.

Lipopolysaccharide (LPS), generally referred to as endotoxin, major constituent of the outer cell wall of gram-negative bacteria, is one of the most important virulent factors participating in the development and maintenance of apical periodontitis (9, 10). Clinical investigations have revealed the presence of endotoxin in 100% of the root canal samples in primary (7, 8, 11–14) and secondary (14, 15) infectious diseases showing apical periodontitis, with high levels related to the development of clinical symptoms and larger area of bone destruction (7, 11–13).

Because of the high toxicity of endotoxin *in vivo* (10, 16) and *in vitro* (17), even at very low concentrations, its removal/neutralization during endodontic treatment seems to be important for the healing process of periapical tissues of infected root canals (8, 11).

The root canal disinfection can be attained by treatment involving chemomechanical preparation, immediately followed by obturation (1-visit treatment) or supplemented by a previous interappointment intracanal medication (2-visit treatment) (3, 6).

Previous studies evaluating the efficacy of chemomechanical procedures in reducing/eliminating endotoxins from infected root canals by using sodium hypochlorite (NaOCl) (7, 8, 11) as well as chlorhexidine gel 2% (CHX) (11, 18) indicated that the mechanical debridement along with irrigation is able to reduce endotoxin contents ≈50% (by using conventional instrumentation) (7, 11, 18) and ≈98% by using rotary nickel-titanium files (8). However, endotoxins were still detected in 100% of the root canal samples of infected teeth after instrumentation (8, 11, 18).

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Calcium hydroxide [$Ca(OH)_2$], the most commonly used intracanal medication during 2-visit treatment, has been proven to be effective against endotoxins as demonstrated by *in vitro* studies (19–21). However, in clinical practice, controversy exists on whether $Ca(OH)_2$ medication can improve the removal or elimination of endotoxins from infected root canals (18, 22).

There is currently no clinical study comparing the effectiveness of 1-visit versus 2-visit root canal treatment protocol regarding the removal of endotoxins from primarily infected root canals with apical periodontitis.

Therefore, this clinical study was conducted to compare the effectiveness of 1-visit versus 2-visit root canal treatment in removing endotoxins and cultivable bacteria from primarily infected root canals.

## Materials and Methods

### Patient Selection

Forty-eight patients attending the São José dos Campos Dental School (UNESP), São José dos Campos (SP), Brazil for primary endodontic treatment were included in the present study. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the last 3 months or who had any general disease were excluded. The Human Research Ethics Committee of the São José dos Campos Dental School (UNESP) approved the protocol describing the sample collection for this investigation, and all volunteer patients signed an informed consent form.

All the selected teeth were single-rooted with primary endodontic infection, showing the presence of 1 root canal and absence of periodontal pockets deeper than 4 mm. None of the patients reported spontaneous pain. Teeth that could not be isolated with rubber dam were excluded. The following clinical/radiographic features were found in root canals with primary endodontic infections investigated: pain on palpation, 2 of 40, tenderness to percussion, 7 of 40, and size of radiolucent area >3 mm, 29 of 40.

### Sampling Procedures

Files, instruments, and all materials used in this study were treated with $Co^{60}$ gamma radiation (20 kGy for 6 hours) for sterilization and elimination of preexisting endotoxins (EMBRARAD; Empresa Brasileira de Radiação, Cotia, SP, Brazil). The method used for disinfection of the operative field has been previously described (8, 10, 16). Briefly, the teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% $H_2O_2$ (volume/volume for 30 seconds), followed by 2.5% NaOCl for the same period of time and then inactivated with 5% sodium thiosulfate. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it on blood agar plates, which were then incubated both aerobically and anaerobically.

A 2-stage access cavity preparation was made without the use of water spray but under manual irrigation with sterile/apyrogenic saline solution and by using sterile/apyrogenic high-speed diamond bur. The first stage was performed to promote a major removal of contaminants, including carious lesion and restoration. In the second stage before entering the pulp chamber, the access cavity was disinfected according to the protocol described above. Sterility of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically. A first endotoxin sampling was taken by introducing sterile/apyrogenic paper points (size #15; Dentsply-Maillefer, Balaigues, Switzerland) into the full length of the canal, which was determined radiographically, and retained in position during 60 seconds for sampling. Immediately afterward, the sample was placed in a pyrogen-free glass and immediately suspended in 1 mL limulus amebocyte lysate (LAL) water according to the endotoxin dosage by using the kinetic chromogenic LAL (Lonza, Walkersville, MD) assay. This sampling procedure was repeated with 3 paper points that were pooled in a sterile tube containing 1 mL VMGA III transport medium (23) for microbial cultivation.

After accessing the pulp chamber and subsequent first endotoxin sampling, teeth were randomly divided into 4 groups: G1, 1% NaOCl (n = 12); G2, 2% CHX gel (n = 12); G3, 1% NaOCl + calcium hydroxide [$Ca(OH)_2$] (n = 12) medication; and G4, 2% CHX gel + [$Ca(OH)_2$] medication (n = 12), with the first 2 groups involving 1-visit treatment and the latter 2 involving 2-visit treatment.

Next, the cervical and middle thirds of the root canals were prepared with the crown-down technique by using Endo-Eze files (Ultradent Products, South Jordan, UT) according to the manufacturer's instructions. The Endo-Eze files were adapted to the Endo-Eze contra-angle handpiece (Kavo do Brasil, Ltda, Saguaçú Joinville, SC, Brazil). K-files, size #15 or #20 (Dentsply-Maillefer), were always used between each instrumentation. The lumen of the canal was identified by using a K-file size #10 (Dentsply-Maillefer). Next, cervical interferences were eliminated with the 13/.60 instrument of the Endo-Eze system according to the same principles of the crown-down pressureless technique. Instrumentation was continued by using oscillating 13/.45 file, K-file (#15 or 20), oscillating 13/.35 file, K-file (#15 or 20), and oscillating 10/.25 file until reaching a depth 3 mm shorter than the full length of the root canal, as calculated from preoperative radiographs. During preparation of the cervical and middle thirds, the root canal was filled with selected auxiliary chemical substance, followed by irrigation and aspiration of 5 mL sterile apyrogenic saline after use of the oscillating instrument. This procedure was repeated at each file change. The apical preparation was performed by using 4 manual K-files (Dentsply-Maillefer), which ended in #35 to #45 size.

In the 1-visit 1% NaOCl group (G1), the use of each instrument was followed by irrigation with 5 mL 1% NaOCl solution by means of a syringe (27-gauge needle). Before the second sampling after instrumentation, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during 1-minute period, which was then removed with 5 mL sterile/apyrogenic water.

In the 1-visit 2% CHX gel group (G2), root canals were irrigated with a syringe (27-gauge needle) containing 1 mL of the substance before each instrument and immediately rinsed with 4 mL saline solution. Before the second sampling after instrumentation, 2% CHX activity was inactivated with 5 mL solution containing 5% Tween 80 and 0.07% (w/v) lecithin during 1-minute period, which was then removed with 5 mL sterile/apyrogenic water.

In the 2-visit 1% NaOCl group (G3) and 2-visit 2% CHX gel group (G4), root canal irrigation was performed as described earlier, and subsequently the canals were dried by using sterile/apyrogenic paper points and filled with freshly prepared paste of $Ca(OH)_2$ in propylene glycol for a period of 14 days. The paste was inserted into the canals with the aid of a lentulo spiral. Care was taken to properly fill the root canal with the calcium hydroxide paste without any radiographically visible air bubbles. The paste was condensed at the canal orifice level with the aid of a sterile cotton pellet. Next, the access cavities were properly closed with ionomer cement.

After 14 days with intracanal medication, the samples of G2 and G4 had their surgical field isolated and disinfected, including removal of the provisional restoration. Next, the root canals were irrigated with 10 mL saline solution, and calcium hydroxide antimicrobial activity was neutralized with 0.5% citric acid. Afterward, another collection of bacterial material and endotoxins was performed.

At the end of instrumentation, root canals in all 4 groups were flooded with 17% EDTA during 3-minute period. EDTA was activated