

# Sterilizing Endodontic Files by four different sterilization methods to prevent cross-infection - An In-vitro Study

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## ABSTRACT

**Background:** Aim of the study was to compare 4 different methods of sterilizing endodontic files in dental practice.

**Materials & Methods:** The present study was performed on 100 K-files, 21 mm long and of size 25. Of these, 20 files were taken as control group, and the remaining 80 files were divided into 4 groups of 20 files each and they were tested for the efficacy of sterilization with different methods: Autoclave, glass bead, glutaraldehyde and CO<sub>2</sub> laser.

**Results:** The study showed that the files sterilized by autoclave and lasers were completely sterile. Those sterilized by glass bead were 90% sterile and those with glutaraldehyde were 80% sterile.

**Conclusion:** The study concluded that autoclave or laser could be used as a method of sterilization in clinical practice and in advanced clinics; laser can be used also as a chair side method of sterilization.

**Key Words:** Bacillus Stearothermophilus, endodontic files, endodontic instrument box, sterilization, Thioglycollate medium.

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## Introduction

Microorganisms induce a variety of infectious diseases in the human body. Infection control is a major topic of concern in medical and dental health care settings. Contamination directly or indirectly leads to transmission of infectious agents.<sup>1</sup> The prevention of cross-contamination of infectious diseases among dental staff and patients is a major problem which dental practitioners face. Sterilization prevents the

spread of infectious diseases. In dentistry, it primarily relates to processing reusable instruments to prevent cross-infection.<sup>2</sup> In endodontic, various instruments like files, reamers, gates glidden drill and peeso reamers are used for cleaning and shaping the root canal and to eliminate the bacterial population in pulp canal space. There are various methods to sterilize these instruments, such as dry heat sterilizer, autoclave, ethylene oxide gas, glass-bead sterilizer or hot-salt sterilizer, etc.<sup>3</sup> There are very few studies

which compare these methods of sterilizing of endodontic files. Keeping these points into consideration the present study was under taken to compare 4 different methods of sterilizing endodontic files used in dental practice.

### Aims and Objectives

The purpose of this study was to compare 4 methods of sterilizing endodontic files in dental practice and recommend the effective method from among these.

The aim of this study was to investigate the efficacy of 4 accepted methods of sterilizing endodontic instruments:

- (1) Autoclaving
- (2) Carbon dioxide laser sterilization
- (3) Chemical sterilization (with glutaraldehyde)
- (4) Glass-bead sterilization

### Materials and Methods

The present study was carried out on 100 K-files, 21 mm long, size 25. 20 K-files were taken as control group, and the remaining 80 files were divided into 4 groups of 20 files each in four different modes of sterilization: Autoclave, glass bead, glutaraldehyde and CO<sub>2</sub> laser.

In the present study all the 100 files were pre-sterilized in an endodontic instrument box by autoclaving for 30 minutes at 121°C at a pressure of 15 pounds, for standardization to eliminate any bias. Later the test files were divided into 5 groups of 20 files each and labeled as A (autoclave), B (glass bead), G (glutaraldehyde), L (CO<sub>2</sub> laser) and C (control) and were numbered 1 to 20.

The spore suspension was prepared by immersing the commercially available bacillus stearothermophilus strips into thioglycollate medium broth and incubated at 55°C for 48 hours. Growth that occurred in the test tube was confirmed by doing Gram's stain that showed the presence of gram-positive bacillus stearothermophilus.

All the pre-sterilized files were contaminated with bacillus stearothermophilus in a sterile Petri dish for 5 minutes. After 5 minutes of immersion, the files were transferred to another sterile Petri dish under vacuum

hood safety with the help of a sterile tweezer, following which the files were dried in an incubator for 10 minutes at 37°C and stored in an endodontic instrument box till they were sterilized by different methods.

### Method of sterilization

The 20 contaminated files in **Group A** were placed in an endodontic instrument box and subjected to autoclave at 121°C for 15 minutes at a pressure of 15 pounds. The 20 contaminated files in **Group B** were taken in 4 batches of 5 files each and wiped for 10 seconds with a gauze soaked with surgical spirit and placed in the periphery of the glass-bead sterilizer and sterilized for 45 seconds at 240°C. Similarly the 20 contaminated files in **Group G** were placed in a sterile plastic container containing 2.4% glutaraldehyde solution and were left in it for 12 hours for cold (chemical sterilization). The 20 contaminated files in **Group L** were irradiated for 3 seconds per surface at 10 watts using CO<sub>2</sub> laser system. The laser beam was moved along the length of the instrument during the 3-second period. A sterile tweezer was used to hold the handle of the file and change the surface for exposure. After completion of sterilization, the shaft of the instrument was removed from the handle by means of a sterile autoclaved wire cutter and each file was placed in separate tubes containing thioglycollate medium with the help of a sterile tweezer to check for any microbial growth and the efficacy of sterilization method. The 20 contaminated files in **Group C** (control group) were put in separate tubes containing thioglycollate medium by the method described above without doing any sterilization.

The test tubes containing files were labeled with the date and were kept for incubation at 55°C for 72 hours. After 72 hours the test tubes were removed from the incubator and each test tube was checked for any turbidity in the test tube. Presence of turbidity in a test tube indicated the presence of bacillus stearothermophilus and that the particular file was not sterilized completely. The test tubes were then further kept for incubation at 55°C for 21 days and again checked for microbial growth. The test tubes with turbidity were checked and confirmed for the presence of microorganisms by viewing under light microscope

after doing Gram's stain and culture test. The specificity of bacillus stearothermophilus stains was confirmed with the help of sugar test.

## Results

The study showed that the endodontic files sterilized by autoclaving in an instrument box at 121°C for 15 minutes at a pressure of 15 pounds (Group A) showed total sterility [Figure 1].

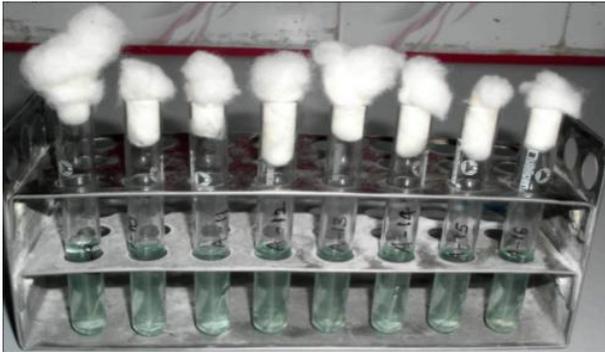


Figure 1: Test tubes after autoclave sterilization



Figure 3: Test tubes after glutaraldehyde sterilization

The files subjected to sterilization by glass-bead sterilizer after wiping for 10 seconds with a gauze soaked with surgical spirit and sterilized for 45 seconds at 240°C (Group B) showed presence of turbidity in 2 test tubes. Incomplete sterilization to the range of 10% was observed when the files were sterilized in glass-bead sterilizer [Figure 2].

The endodontic files sterilized by immersing in glutaraldehyde for 24 hours (Group G) showed sterilization up to only 80%. This method showed contamination of 4 files after incubation [Figure 3].

The files on sterilization by CO<sub>2</sub> laser for 3 seconds per surface at 10 watts (Group L) showed 100% sterility. There was total sterility seen by this method of

sterilization [Figure 4].

Statistical analysis of the 4 different sterilization methods showed a statistically significant difference between groups with regard to their efficacies in sterilization ( $P < 0.05$ ) [Table 1]. It was observed that auto-clave and laser sterilization showed 100% sterilization as compared to chemical and glass bead sterilization.

Comparison of the sterilized groups with the control



Figure 2: Test tubes after glass-bead sterilization

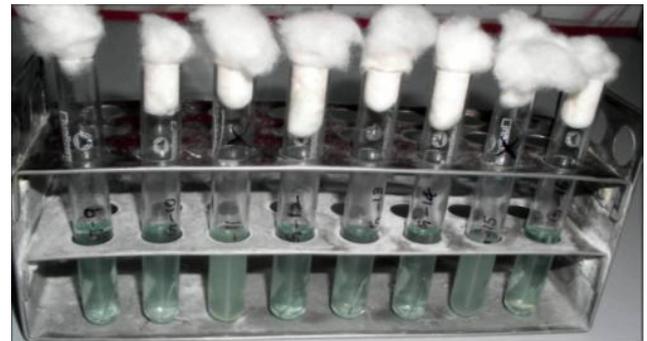
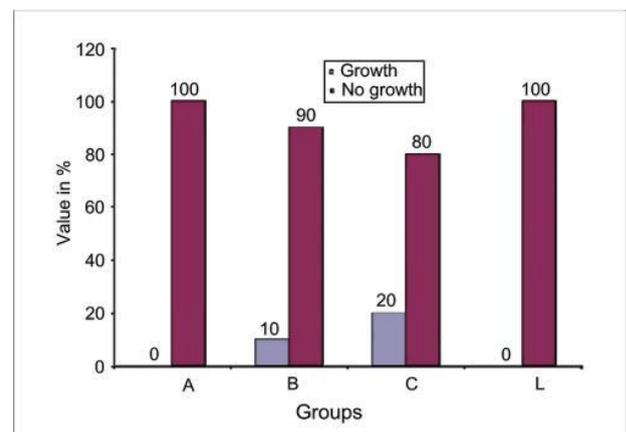


Figure 4: Test tubes after laser sterilization

group with regard to their efficacies in sterilization showed that the difference was statistically significant [Graph 1].



Graph 1: Graph of comparison of groups

Group	Growth		No Growth		P value
	Number	Percentage	Number	Percentage	
A	-	-	20	100	0.048
B	2	10	18	90	
G	4	20	16	80	
L	-	-	20	100	
C	20	100	-	-	

**Group A:** The 20 contaminated files were placed in an endodontic instrument box and subjected to autoclave at 121°C for 15 minutes at a pressure of 15 pounds.

**Group B:** The 20 contaminated files in were taken in 4 batches of 5 files each and wiped for 10 seconds with a gauze soaked with surgical spirit and placed in the periphery of the glass-bead sterilizer and sterilized for 45 98 seconds at 240°C.

**Group G:** The 20 contaminated files in were placed in a sterile plastic container containing 2.4% glutaraldehyde solution and were left in it for 12 hours for cold (chemical sterilization).

**Group L:** The 20 contaminated files in were irradiated for 3 seconds per surface at 10 watts using CO<sub>2</sub> laser system.

**Group C:** The 20 contaminated files were taken as control group.

## Discussion

Sterilization of instruments is done by three major methods: Steam under pressure (autoclave), dry heat and chemiclave. Lasers have also started being used as a method for sterilization. The bacillus stearothermophilus contaminated endodontic k-files in this study are heat-resistant bacteria spores. Various modes have been adopted for sterilizing endodontic instruments but out of them Steam autoclaving and glass-bead sterilizers are commonly recommended. Boyd<sup>4</sup> stated that moist heat generally kills microorganisms by coagulation of proteins. However, coagulation occurs only when overkill conditions are attained. Fewer changes are observed as changes in nucleic acids, inactivation of enzymes, and alteration of cytoplasmic membrane which probably kills the microorganisms before the coagulation occurs. In the present study it was observed that complete sterilization was possible by autoclaving the instruments in an endodontic box the results of this study are in agreement with the results of Rajkumar et al,<sup>2</sup> Hurtt et al,<sup>5</sup> and Velez et al.<sup>6</sup> Damaging alterations of proteins by dry heat are the result of oxidation, desiccation and changes in osmotic pressure owing to

evaporation of moisture. Dry heat is slow process which needs higher temperature as compared to that used for moist heat method. Glass-bead sterilizer showed sterilization up to only 90% and that total sterility was not found even after sterilizing for 45 seconds at 240°C. These results are not in agreement with the results found by Rajkumar et al,<sup>2</sup> but the results are in agreements with the study done by Hurtt et al.<sup>5</sup>

The present study showed that there was 80% sterilization by immersing the files in glutaraldehyde solution for 12 hours, the present results are similar to the study done by Hurtt et al.<sup>5</sup>

In the present study CO<sub>2</sub> laser latest technique was used as one of the mode of sterilization as it is commonly used nowadays in the dental office. CO<sub>2</sub> Laser used for sterilization showed complete sterilization of the endodontic instrument similar findings were also presented by Nammour et al.<sup>7</sup> Powell et al.<sup>8</sup> on the basis of a study stated that all 3 lasers (argon, CO<sub>2</sub> and NdYAG) were capable of sterilizing endodontic instruments, but the efficiency of argon laser was better even at lower energy level. The result of the present study is similar to that of the study done by Hooks et al.<sup>9</sup> and Adrian and Gross.<sup>10</sup>

### Conclusion

The present study it was observed that autoclaving and exposing to laser give complete sterilization, where as Glass-bead sterilizer can be used as an alternative when these two methods are not available, though autoclave is an effective method for sterilizing endodontic files, the time taken by it to sterilize is more.

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