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ORIGINAL RESEARCH

Evaluation of Various Sterilization Processes of Orthodontic Instruments using Biological Indicators and Conventional Swab Test Method: A Comparative Study

[1]Rajeev Lall, [2]Anshu Sahu, [3]Ankita Jaiswal, [4]Sunder Kite, [5]AR Sowmya, [6]MC Sainath

ABSTRACT

Aim: This study aimed to compare the efficiency of various sterilization procedures using conventional spore monitoring method, i.e., by using swab test and biological indicators and to determine the efficiency of cold sterilization by using Bioclenz-G (2% glutaraldehyde) solution.

Materials and methods: Each group was divided into medium load (containing 15 sets of instruments) and heavy load (containing 30 sets of instruments). Each group was tested 15 times for medium and heavy loads. Two groups are swab tested control group and experimental group with three different methods of sterilization: hot air oven, cold sterilization, and ethylene dioxide sterilization.

Results: Spores were present in all the groups tested for 10 minutes cycle, in comparison with no spore growth in any of the groups tested for a 10-hour cycle.

Conclusion: All methods of sterilization showed complete sterilization of instruments when monitored with biological indicators. One group of heavy load in steam autoclave and one group each of medium load and heavy load in hot air oven sterilizer showed sterilization failure when monitored with the conventional swab test method.

[1,2]Department of Orthodontics and Dentofacial Orthopedics Hazaribag College of Dental Sciences & Hospital, Hazaribagh Jharkhand, India

[3]Nisha Dental Healthcare Centre, Allahabad, Uttar Pradesh, India

[4]Department of Public Health Dentistry, Madha Dental College & Hospital, Chennai, Tamil Nadu, India

[5]Department of Public Health Dentistry, KGF College of Dental Sciences & Hospital, Kolar, Karnataka, India

[6]Department of Orthodontics and Dentofacial Orthopedics Madha Dental College & Hospital, Chennai, Tamil Nadu, India

Corresponding Author: Rajeev Lall, Department of Orthodontics and Dentofacial Orthopeadics, Hazaribagh College of Dental Sciences & Hospital, Hazaribagh, Jharkhand, India, e-mail: rajeevlall@hotmail.com

Clinical significance: This study proves the efficacy and durability of various sterilization procedures.

Keywords: Hot air oven, Steam autoclave, Sterilization.

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INTRODUCTION

Awareness of efficient sterilization techniques occupies the centerstage in the prevention of the spread of infectious diseases. Many oral and systemic disease-causing organisms are easily transmitted from the oral cavity having long latent period of incubation.[1]

Orthodontists are at an ever greater risk to exposure of serious pathogens and must take adequate precautions to guard themselves against their transfer. The preferred method to sterilize orthodontic pliers has always been debatable, with the common methods being moist heat by autoclave and dry-heat with hot air oven.[2]

Most commonly used infection control methods are disinfection and sterilization. Disinfection reduces the microbial contamination but is generally less lethal to pathogenic organisms than sterilization and does not remove all the vegetative spores. Sterilization destroys all forms of microorganisms including viruses, bacteria, fungi, and spores.[3]

The question arises as regards how much effectively or efficiently the sterilization procedure can be monitored by using chemical indicators, lab culture method, or biological indicators. The most frequently used method for checking the effectiveness of sterilization is the chemical

indicators. They are available in the form of strips. Their drawback is that they only ensure that the instruments have been exposed to sterilization cycle; they do not verify that complete sterilization has occurred and all vegetation has been destroyed.

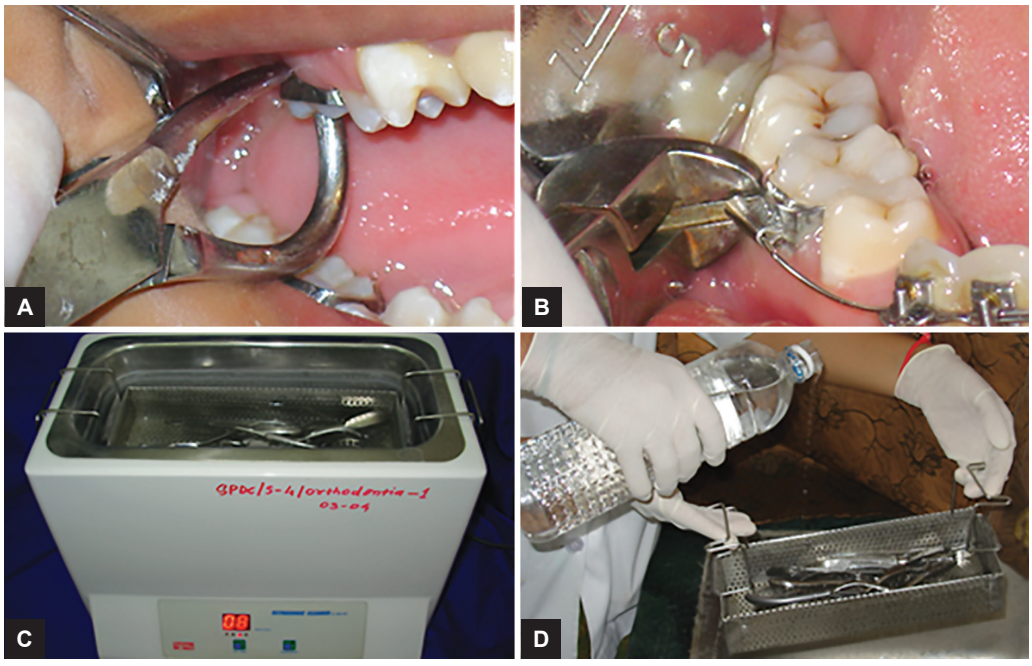
The conventional microbiological culture method can determine the effectiveness of sterilization process by spore growth which can be seen by the naked eye. The drawback of this procedure is that it requires lots of skill to determine the spore growth; even airborne contamination can affect the result of the culture method, and about 48 to 72 hours for spores to grow on the culture medium.[4-6] Biological indicators have been stated that they can provide a better method of verifying the effectiveness of sterilization procedures.

Biological indicators consist of ampules or strips enclosed in glassine envelope that contains a known quantity of *Bacillus stearothermophilus* and/or *Bacillus subtilis* spores.[7] Biological indicators for monitoring steam autoclave or chemical vapor sterilization contain spores of *B. stearothermophilus* (*Geobacillus stearothermophilus*). Biological indicators for monitoring dry heat or ethylene oxide sterilization contain spores of *B. subtilis* (*Bacillus atrophaeus*).

MATERIALS AND METHODS

One set of instruments was not passed through sterilization process and was directly sent to the microbiology lab for culture test which comprised the control group. The other set of instruments was passed through different sterilization cycles which comprised the experimental group. Each group was divided into medium load (containing 15 sets of instruments) and heavy load (containing 30 sets of instruments). Each group was tested 15 times each for medium and heavy loads (Fig. 1).

Figs 1A to F: Armamentarium



Figs 2A to D: Instrument contamination and precleaning

Control Group

The contaminated instruments were ultrasonically cleaned and air dried but was not processed through different sterilization procedures (Fig. 2).

Experimental Group

The contaminated instruments were ultrasonically cleaned and air dried and were processed through different sterilization procedures (Fig. 2).

The ampule was crushed and the crushed ampule was kept inside the incubator along with crushed control biological indicator at a temperature of 56°C for 24 hours in the steam autoclave sterilization method. In the steam autoclave swab procedure, after the sterilization cycle, the swab of the experimental group of instruments was taken along with the swab of the control group of instruments and was processed for lab culture. The sterilization cycle of ethylene oxide sterilizer was 8 hours at 55°C. The biological indicator was crushed along with the control biological indicator and was incubated for 24 hours at a temperature of 37°C.

In the ethylene oxide swab procedure, after the sterilization cycle, the swab of the experimental group of instruments was taken along with the swab of the control group of instruments and was processed for lab culture. In sterilization using the hot air oven, the sterilization cycle for the hot air oven was 171°C for 1 hour. The spore strips were incubated in soybean casein digestive culture medium at 37°C for 1 week.

In cold sterilization, no biological indicators were available, and no indicators were used. The swab of the experimental group and control group of instruments was taken for determining the spore growth.

After 24 hours of incubation, both the biological indicators (control and experimental groups) were removed from the incubator and were checked for change in the color of culture medium. If the culture medium changes color, it indicates the presence of spores or sterilization failure. If there is no change in color, it indicates no spore growth and sterilization was proper.

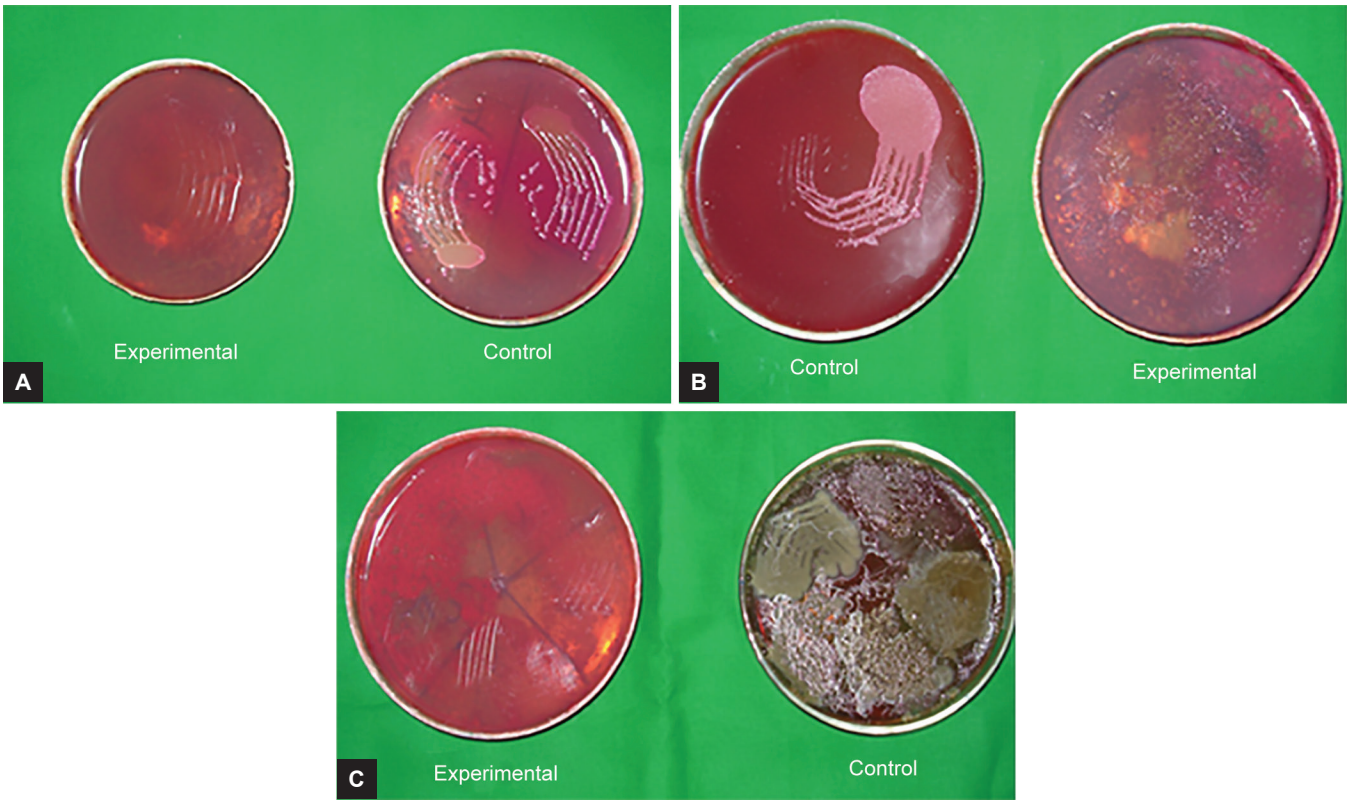
After 1 week of incubation of spore strip in the hot air oven, sterilizer change in turbidity of the culture medium was checked in both control and experimental groups. If the culture medium becomes turbid, it indicates sterilization failure. No change in turbidity indicates proper sterilization (Fig. 3).

After 48 hours of incubation of agar medium, the spore growth was determined. The spore growth can be seen with naked eyes (Fig. 4).

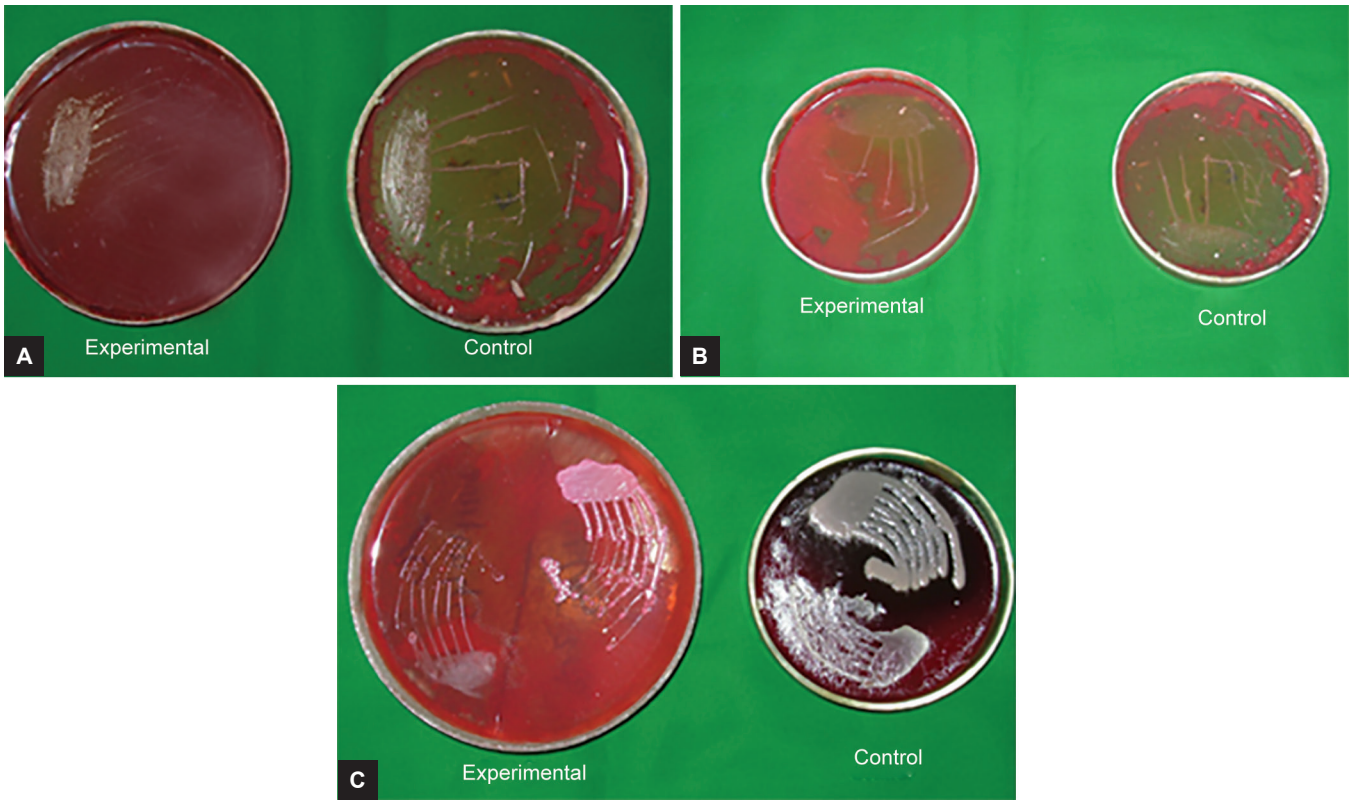
RESULTS

One out of 15 groups in steam autoclave showed the spore growth in a heavy load. In dry heat sterilization, one group both in medium load and in heavy load showed spore growth from all the fifteen groups (Table 1).

Instruments dipped in Bioclenz-G solution for 10 minutes of cycle showed spore growth. However, instruments dipped for 10 hours showed no spore growth (Table 2). The spore growth was seen in three of the groups tested by the conventional lab method, in comparison with no spore growth in groups tested by biological indicators in steam, dry heat, and ethylene oxide sterilization (Graph 1).



Figs 3A to C: After incubation of agar medium



Figs 4A to C: Growth found in three experimental groups

Spores were present in all the groups tested for 10 minutes cycle, in comparison with no spore growth in any of the groups tested for the 10-hour cycle (Graph 2).

DISCUSSION

One of the most important points to debate on as far as sterilization is concerned is the instrument damage caused in spite of proper sterilization protocol. The factors that

Table 1: Comparative evaluation of conventional laboratory method with biological indicators

Method of monitoring sterilization procedure		*Conventional laboratory method*		*Biological indicator method*		*Number of samples (n)*
	Load	*Spore present*	*Spore absent*	*Spore present*	*Spore absent*	
Steam autoclave	Medium load	0	15	0	15	15
	Heavy load	1	14	0	15	15
Dry heat oven	Medium load	1	14	0	15	15
	Heavy load	1	14	0	15	15
Ethylene oxide	Medium load	0	15	0	15	15
	Heavy load	0	15	0	15	15

Table 2: Evaluation of spore growth in cold sterilization by conventional laboratory method

Comparative evaluation of conventional laboratory method with biological indicators monitoring method time duration	*Conventional laboratory method*		*Number of samples (n)*
	Spore present	*Spore absent*	*15*
10 minutes	15	0	15
10 hours	0	15	15

influence instrument damage include the water quality, use of strong detergents, excessive heat exposure, and the presence of moisture after pre-sterilization cleaning, improper compositions, and concentrations of chemicals used and, last but not the least, the quality of pliers. It may be more appropriate to categorize the materials used in orthodontics under the following headings and discuss the practical guidelines for an effective process of sterilization.

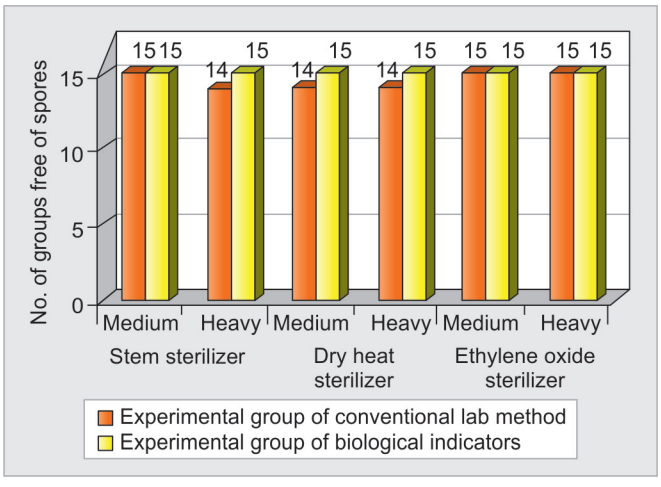
By ultrasonic cleaning of instruments, sterilization cannot be achieved. The debris, saliva, and blood may be cleaned off the instruments and are not visible to the naked eye.[7] The type of sterilization can depend upon a variety of factors including critical and noncritical instruments. The current study evaluated the following sterilization processes: hot air oven, cold sterilization, ethylene oxide sterilization, and also considered the use of biological indicators and the swab test method for evaluating the various processes of sterilization and their monitoring efficiency.

The results of this study verified the established effectiveness of biological indicators over the swab test method for monitoring sterilization. Bioclenz-G can be used as a cold sterilization method if instruments are dipped for 10 hours' duration.

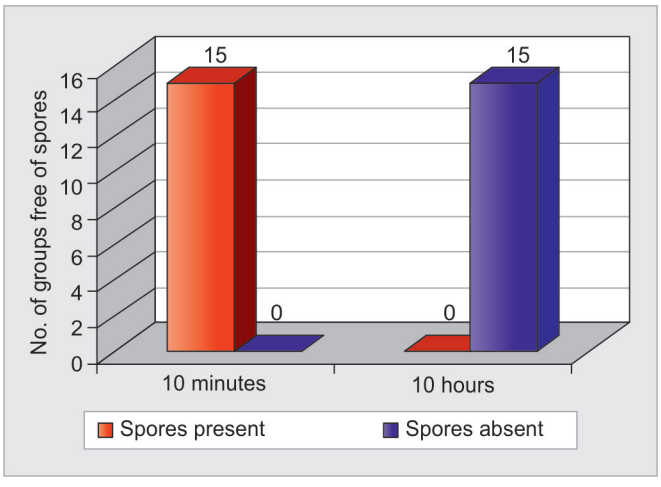
Palenik et al[8] discussed that the spores present in the biological indicators are highly resistant. If the spores are killed, it may be assumed that all the other microbes present on the dental instruments have also been killed. In the present study also, all the biological indicators processed through different sterilization techniques showed no spore growth which confirmed that all the instruments have been properly sterilized, and all the microbes have been killed.

On the contrary, the monitoring of spore growth by the conventional lab method revealed spore growth in three experimental groups, indicating sterilization failure. This could probably be due to airborne contamination or contamination of swab and culture while transferring.

Hohlt et al[9] discussed in their study that proper sterilization should be taken for culturing the instruments. Airborne contamination must be eliminated for proper



Graph 1: Comparison of conventional lab method with biological indicators



Graph 2: Number of groups free of spores by cold sterilization

results. They found that instruments and bands contaminated with blood and saliva showed no spore growth when the instruments were sterilized using steam autoclave, chemical vapor, and dry heat oven sterilizers. In their study, they used *B. stearothermophilus* and *B. subtilis* spores to monitor the sterilization cycle. In the present study also, all the spores used to determine the sterilization efficiency were killed, showing proper sterilization of instruments and the effectiveness of the sterilizers used.

Hohlt et al9 performed a study to determine the ability of forced air, dry heat sterilizer to kill the spores of *B. subtilis*. No sterilization failures were found. All the spores were killed. In our study, all the spores of *B. subtilis* and *B. stearothermophilus* were killed, indicating proper sterilization of contaminated instruments.

According to Miller and Sheldrake,[6] glutaraldehyde solution used at a 2% concentration with a contact time of 10 hours is also capable of killing bacterial spores and achieving sterilization. However, the microbial killing achieved using glutaraldehyde solution cannot be routinely verified using biological indicators as can be done with other methods of sterilization. In the present study, also all spores were killed when the instruments were dipped in the solution for 10 hours.

Biological indicators can be considered as the best method to check the sterilization efficiency as the spores present on them are highly resistant, and the inactivation of the spores determines the sterilization efficiency.[10-14]

A steam autoclave can be used as the best and quickest method for sterilization of orthodontic instruments if proper measures are taken to prevent corrosion.[15,16]

The limitation of this study is that biological indicators are not available for all sterilization procedures like cold sterilization. Further studies can be undertaken to evaluate and compare the various types of biological indicators and their effectiveness in the control of orthodontic sterilization. A multidisciplinary study including orthodontist, microbiologist, and pathologist can provide further insight into the use of biological indicators.

CONCLUSION

All methods of sterilization showed complete sterilization of instruments when monitored with biological indicators. One group of heavy load in the steam autoclave and one group each of medium load and heavy load in the hot air oven sterilizer showed sterilization failure when monitored with the conventional swab test method.

The efficiency of conventional swab test method in monitoring sterilization is questionable, as the results can vary due to airborne contamination and human error. The biological indicator is a more reliable and accurate method for monitoring sterilization. The American Dental Association recommends a weekly spore testing of dental office sterilizer to determine the sterilization efficiency.

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