Sterilization monitoring by biological indicators and conventional swab test of different sterilization processes used in orthodontics: A comparative study

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Abstract

Introduction: The need of effective sterilization method and their monitoring is necessary. Biological indicators are specific microorganisms with high resistance toward particular sterilization methods. Their processes include steam autoclave, dry heat sterilizer, ethylene oxide sterilizer. This article has considered various methods to monitor the effectiveness of different sterilization methods used in orthodontics.

Materials and Methods: The parameters for comparison were the control and experimental instruments utilized in orthodontic treatment. The efficacy of sterilization was evaluated by comparison of bacterial growth obtained in monitoring by biological indicators and swab test method. Results: No spore growth was found when sterilization process was evaluated by biological indicators in comparison to swab test where spore growth was present. Instruments dipped in Bioclenz-G solution for 10 min showed spore growth, but no spore growth was seen in 10 h cycle. Discussion: The result of the study verifies the established effectiveness of biological indicators over conventional swab test method in monitoring various sterilization processes used in orthodontics. Bioclenz-G solution can be used as an effective cold sterilization method for sterilization.

Conclusion: For evaluating the effectiveness of sterilization, biological indicators preclude the drawbacks of incomplete verification of destruction of all vegetation and inordinate delay in procurement of results as is the case with chemical indicators and lab culture, respectively.

Key words: Biologic indicators, infection control, orthodontic instrument sterilization, swab test

INTRODUCTION

With the increased usage of implants as anchorage and growing popularity of surgically assisted orthodontics, the necessity for sterilization has increased manifold. Effective infection control is an essential requirement to prevent cross contamination at all levels in an orthodontic setup.¹ The most commonly used infection control methods are disinfection and sterilization. Disinfection reduces microbial contamination but is less lethal to pathogenic organisms as compared to sterilization. Disinfection does not remove all vegetative spores. Sterilization, however, removes all forms of microorganisms including viruses, bacteria, fungi, and spores.

The efficacy of a sterilization procedure may be monitored by various methods such as the use of chemical indicators, study of lab cultures, and biological indicators. Chemical indicators only ensure that instruments have been exposed to sterilization cycle by change in their color, but it cannot be verified that complete sterilization has been achieved, and all vegetative spores have been destroyed.

In conventional microbiological culture, the effectiveness of sterilization process is studied by spore growth, which can be seen by naked eye.²⁻³ This procedure requires experience and skill to determine spore growth; even airborne contamination can effect the result of culture method. Besides, this method is time-consuming. A minimum of 48–72 h are required for the spores to grow on any culture medium. Biological indicators provide the only real method of verifying the effectiveness of sterilization procedures.¹,⁴⁻⁵ Currently, they are only being...
used by pharmaceutical companies and some corporate hospitals. These Indicators consist of ampoules or strips enclosed in glassine envelopes that contain a known quantity of Bacillus stearothermophilus and/or Bacillus subtilis spores.[6] The indicators for monitoring steam autoclave or chemical vapor sterilization contain spores of B. stearothermophilus (Geobacillus stearothermophilus). Whereas those for monitoring dry heat or ethylene oxide sterilization contain spores of B. subtilis (Bacillus atrophaeus) [Figures 1 and 2].

### Aims
The aim of this study was to emphasize the role of bacterial spores in monitoring the effectiveness of various sterilization methods used in orthodontics.

### Objective
- Compare the efficiency of various sterilization procedures using conventional swab test and biological indicators
- Determine the efficiency of cold sterilization using Bioclenz-G (2% Glutaraldehyde) solution.

### Materials and Methods
The sample used in this study consisted of hinged and nonhinged orthodontic instruments.

After ultrasonic cleaning, the contaminated instruments were washed with distilled water so as to remove all the impurities present in the cleaning solution. The samples were then divided into two loads medium and heavy, this formed the experimental group. The medium load consisted of 15 instruments. The heavy load consisted of 30 instruments.

The instruments were then air-dried and divided into two groups for each load. One set was not passed through sterilization process and was directly sent to microbiology lab for culture. This set comprised of the control group. The other set of instruments were passed through different sterilization cycles (along with their respective biological indicators) which comprised the experimental group, like steam autoclave (121°C at 15 psi pressure for 18 min), hot air oven (171°C for 1 h), ethylene oxide (55°C for 8 h) sterilizer.

After the completion of sterilization cycles, the biological indicator was removed from the sterilizer.

The biological indicator vial was crushed so as to mix the culture medium and spore strip present inside the vial, and was incubated at 56° C. for B. stearothermophilus (steam autoclave), and 37° C for B. subtilis (dry heat oven, ethylene oxide), to determine the efficacy of each sterilizer.

Conventional swab test of the sterilized instruments was done so as to check the spore growth for sterilization efficacy.

Bioclenz-G solution was used for cold sterilization. Instruments were dipped for 10 min and 10 h and efficiency of sterilization was checked and compared using conventional swab test.

### Results
The baseline parameters for comparative results of all experimental and control group tested by conventional swab test method and biological indicator method reveal that by conventional swab test method in steam sterilization, no spore growth was found in medium load in all the 15 groups tested in experimental group. In heavy load, one group out of 15 groups showed spore growth, which indicates improper sterilization of that group of instruments. In dry heat sterilization out of 15 groups medium showed spore growth. Similarly one group out of 15 in heavy load showed spore growth, thus indicating in both cases lack of proper sterilization [Figure 3].

In ethylene oxide sterilization, no spore growth was found in each of the 15 groups tested for medium and heavy load in the experimental group, which confirms proper sterilization of all the groups of instruments. No spore growth was found in any of the biological indicators processed through all the three sterilization cycles in medium load and heavy load in experimental group [Table 1]. This indicates proper sterilization of all the hinged and nonhinged instruments.
Instruments dipped in Bioclenz-G solution for cold sterilization showed spore growth in 10 min cycle but showed no spore growth in 10 h cycle [Table 2 and Figure 4].

No statistics has been done in this study as it was a random trial study. Comparison was been done for evaluation of the effectiveness of different sterilization methods.

**DISCUSSION**

A variety of sterilization procedures are utilized in orthodontics for infection control. The type of sterilization can depend upon a variety of factors including critical, noncritical instruments, type of instruments that is, hinged, nonhinged, etc., The current study evaluated the following sterilization processes–steam autoclave, hot air oven, ethylene oxide sterilizer, and Bioclenz-G solution.

This study also considered the use of biological indicators and swab test method for evaluating the various processes of sterilization and their monitoring efficiency.

The result of this study verified the established effectiveness of biological indicators over swab test method for monitoring sterilization. Bioclenz-G can be used as a cold sterilization method if instruments are dipped for 10 h duration. The result of this study adds to the scientific knowledge of sterilization which can contribute to a safer and improved orthodontic health care benefits.

Palenik et al.,[7] 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 other hand, monitoring of spore growth by 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.,[8] discussed in their study that proper sterilization should be taken for culturing the instruments. Airborne contamination must be eliminated for proper results. They

![Figure 3: Growth found in three experimental groups](image)

![Figure 4: After incubation of agar medium Bioclenz-G solution](image)

| 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          |
Khattri, et al.: Sterilization monitoring of different sterilization processes used in orthodontics

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

| Monitoring method Time duration | Conventional laboratory method | Number of samples (n) |
|---------------------------------|---------------------------------|-----------------------|
| 10 min                          | Spore present                  | 15                    |
|                                 | Spore absent                    | 0                     |
| 10 h                            |                                 | 15                    |

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 al.,[9] performed a study to determine the ability of forced-air, Dry heat sterilizer to kill the spore 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,[6] Glutaraldehyde solution used at 2% concentration with a contact time of 10 h 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 h of duration.

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.

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 naked eye.[6] But it does not ensure eradication of all microorganisms not visible to the naked eye. This is confirmed by 100% spore growth of instruments of the control group.[10]

Steam autoclave can be used as the best and quickest method for sterilization of orthodontic instruments if proper measures are taken to prevent corrosion.

The limitation of this study was 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, their effectiveness in control of orthodontic sterilization. A multidisciplinary study including orthodontist, microbiologist, and pathologist can provide further insight into the use of biological indicators.

Conclusion

This article has considered the issue of effectiveness of biological indicators for different sterilization procedures used in orthodontics. This comparative study affirms the process superiority of biological indicators over conventional monitoring by swab test.

A thorough understanding of scientific principles of various sterilization processes and monitoring methods is a prerequisite for the establishment of validated sterilization in orthodontics.

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