

# Evaluation of glutaraldehyde as antimicrobial agent used in intraoral x-ray film holder bite block

*Avaliação do glutaraldeído como agente antimicrobiano usado em blocos de mordida de posicionadores para filmes radiográficos*

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## Abstract

The aim of the present study was to evaluate sterilization of intraoral X-ray film holder bite blocks by immersion in 2% glutaraldehyde solution for 10 hours. Seventeen intraoral X-ray film holder bite blocks were inoculated in Tryptic Soy Broth media containing one of the tested microorganisms (*S. aureus* and *B. subtilis*) and were incubated at 37 °C for 48 hours. One bite block was used as a positive control. The other blocks were immersed in 2% glutaraldehyde for 10 hours and one block was used as a negative control. Fifteen bite blocks were removed and five of them were washed with 70% alcohol, five blocks were washed with distilled water and the others were washed in running water. Bacterial growth was observed after incubation. No bacterial growth was found in any block, however, in the blocks that were washed with running water and distilled water, various colonies of fungi were observed, while no growth was found on the blocks which underwent a final wash with 70% alcohol. The 2% glutaraldehyde solution was effective for the sterilization of intraoral X-ray film holder bite blocks when they were immersed for 10 hours using 70% alcohol during the last wash.

Key words: Radiology. Microbiology. Dental infection control.

## Introduction

It has been reported that non-disposable instruments, including intraoral film holders, beam aligning devices, and panoramic bite-blocks should be sterilized with heat or gas<sup>1</sup>. Cold sterilization requires hours of immersion and it is considered unacceptable. The term "Cold Sterilization" is nominated "Immersion Sterilization". Immersion treatment requires items to be sterilized by completely immersing in the sterilizing solution. However, the American Dental Association (ADA)<sup>2</sup>, the Centers for Disease Control (CDC)<sup>3</sup>, Occupational Safety and Health Administrator (OSHA) and the Environmental Protection Agency (EPA) emphasize that chemical sterilizers should be only used when it is not possible to sterilize or dispose of items that become contaminated during treatment<sup>4,7</sup>.

It is important to recognize that the efficacy of both immersion and surface sterilizers is dependent on factors, including: concentration and type of microorganisms; chemical concentration; length of exposure time; and amount of bioburden<sup>5,7</sup>.

The Brazilian Health Department's<sup>8</sup> guideline named "General Orientation for Sterilization Centers", recommends the use of a 2% glutaraldehyde solution as a sterilizer between 8 and 10h. They also alert that the glutaraldehyde solution suffers alterations at temperatures over 25°C, being toxic and non-biodegradable. The 2% glutaraldehyde solution allows sterilization and high-level disinfection, according to the ANVISA<sup>9</sup>. This signifies that the products formulated with these substances should not

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present confirmable mutagenic, teratogenic or carcinogenic effects in mammals.

The aim of this study was to evaluate the sterilization method of intraoral X-ray film holder bite blocks by immersion in 2% glutaraldehyde solution for 10h for *Staphylococcus aureus* and *Bacillus subtilis* using different final washing methods and to confirm the shelf life of the glutaraldehyde solution preparation for the 1<sup>st</sup>, 14<sup>th</sup> and 28<sup>th</sup> day of use.

## Materials and method

For this study, seventeen intraoral x-ray film holder bite blocks were used (Hanshin Technical Laboratory, Japan). At the beginning, the bite blocks were disinfected with 2% glutaraldehyde solution by immersing for 10h. After this procedure, they were washed in 70% alcohol<sup>3,8,10</sup> and then they were dried in non-sterile paper towels and stored in a clean container.

Microorganisms used in this study were the American Type Culture Collection Strain (ATCC) of *Staphylococcus aureus* (25923) and *Bacillus subtilis* (6633). Approximately 1.0 mL of a standardized strain suspension (10<sup>6</sup> Colony Forming Unit – CFU) of *S. aureus* and *B. subtilis* was inoculated individually in sterile 500 mL of the Tryptic Soy Broth (TSB) and this suspension was incubated for 48h at 37 °C.

All seventeen bite blocks disinfected in 2% glutaraldehyde solution were placed in inoculated culture (TSB) containing one of the tested microorganisms for contamination. The bite blocks were incubated for 48h at 37 °C.

In order to confirm the contamination in this procedure, one bite block was used as positive control. Thereafter, sixteen blocks were removed from the culture (TSB) and, then, they were immersed in a solution of 2% glutaraldehyde for 10h in an opaque plastic container<sup>5,11,12</sup>. The container was plastic in

order to avoid corrosion and opaque to avoid alterations from any interaction with light.

One block was used as a negative control to confirm the efficacy of this procedure. After 10h, the bite blocks were removed from the solution and five of them were washed with running water, another five were washed with distilled water and the rest with 70% alcohol. Each block was placed individually in a sterile tube containing 4.5 mL of sterile saline solution and all the tubes were submitted to vibration in a shaker (Vortex, Marconi, Piracicaba, São Paulo, Brazil) for 1min to free the microorganisms from the block.

The saline solution (0.5 mL) containing *S. aureus* or *B. subtilis* was diluted in a decimal series 10<sup>-1</sup> to 10<sup>-4</sup>. In order to test the efficacy of the different final washes, aliquots of 25 µL of each dilution were transferred to plates of selective media (Acumedia): Manitol Salt Agar for *S. aureus* and Tryptic Soy Agar for *B. subtilis*. The plates were incubated at 37 °C for 48h. After incubation, a single microbiologist interpreted the cultures as positive or negative growths of the corresponding microorganism.

The experiment was divided into three stages to test its shelf life: the 1<sup>st</sup>, 14<sup>th</sup> and 28<sup>th</sup> day after the preparation of the 2% glutaraldehyde solution.

## Results

The macroscopic analyses of the agar plates in the positive control groups of *S. aureus* and *B. subtilis* demonstrated microbial growths of the respective microorganisms, while no evidence of bacterial growth was observed on the plates of the negative control groups of *S. aureus* and *B. subtilis* (Table 1).

Furthermore, no growth of the *S. aureus* or *B. subtilis* was observed on plates of their selective media following washing with running water, distilled water or 70% alcohol at all three stages of the experiment (Table 2).

Table 1 - Microbial growth of *S. aureus* and *B. subtilis* after 48 hours incubation at 37 °C using 2% glutaraldehyde solution

| Groups           | 1 <sup>st</sup> day          |                          | 14 <sup>th</sup> day         |                          | 28 <sup>th</sup> day         |                          |
|------------------|------------------------------|--------------------------|------------------------------|--------------------------|------------------------------|--------------------------|
|                  | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> |
| Positive control | +                            | +                        | +                            | +                        | +                            | +                        |
| Negative control | -                            | -                        | -                            | -                        | -                            | -                        |
| Current water    | -                            | -                        | -                            | -                        | -                            | -                        |
| Distilled water  | -                            | -                        | -                            | -                        | -                            | -                        |
| 70% Alcohol      | -                            | -                        | -                            | -                        | -                            | -                        |

(+) Growth of *Staphylococcus aureus* or *Bacillus subtilis*.

(-) No growth of *Staphylococcus aureus* or *Bacillus subtilis*.

Table 2 - Other microorganisms (fungi) growth after 48 hours incubation at 37 °C using 2% glutaraldehyde solution

| Washing         | 1 <sup>st</sup> day          |                          | 14 <sup>th</sup> day         |                          | 28 <sup>th</sup> day         |                          |
|-----------------|------------------------------|--------------------------|------------------------------|--------------------------|------------------------------|--------------------------|
|                 | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> | <i>Staphylococcus aureus</i> | <i>Bacillus subtilis</i> |
| Current water   | -                            | +                        | +                            | -                        | -                            | +                        |
| Distilled water | -                            | +                        | +                            | -                        | -                            | +                        |
| Alcohol 70%     | -                            | -                        | -                            | -                        | -                            | -                        |

(+) Growth of fungi.

(-) No growth of fungi.

Various colonies of fungi were observed on the plates from the bite blocks washed with running water or distilled water. The bite blocks washed with 70% alcohol did not present colonization by any other kind of microorganisms.

## Discussion

The aim of the infection control procedures is the prevention of disease transmission from patient to operator, operator to patient, and patient to the following patient. During the routine intraoral radiographic examination, oral microorganisms may be transferred from the patient's oral cavity to the radiographic equipment by the X-ray operator and may be transmitted to others patients<sup>13</sup>. Thus, the infection control protocol guidelines are used to minimize disease transmission<sup>1,4,5</sup>. In dentomaxillofacial Radiology, the chemicals used for immersion have been utilized for the disinfection of semicritical instruments and, according to the Brazilian Health Department<sup>8</sup>, the 2% glutaraldehyde solution is effective as a sterilizing solution when items are immersed for 10h. The results of this study indicated that the 2% glutaraldehyde solution effectively eliminates the microorganisms selected from intraoral X-ray film holder bite blocks, although no bacterial growths were observed in the tubes of the negative control group.

It should be pointed out that one manufacturer of the glutaraldehyde solution recommends final washing with sterile water or with physiological saline, but considering the lack of resources during the clinic routine, it was chosen to wash the bite blocks with 70% alcohol and, subsequently, they were dried with non-sterile paper towel to simulate clinic procedure.

No bacterial and fungi growth was observed on any plates when blocks were washed with 70% alcohol, suggesting that this protocol is efficient. The microorganism contamination observed in the groups that were washed with running water and with distilled water, probably resulted from water contamination, since there was no growth on the plates of the negative control group. This suggests that non-sterile paper towel did not influence the results, considering that fungi identification was not the aim of this study.

A study<sup>11</sup> has observed that the steam autoclave is considered the most efficient sterilization method for non-disposable intra-oral X-ray film holders, followed by immersion in 2% glutaraldehyde solution, which was the method used in this study. This author alerted that the solution has a limited shelf life and it represents a sterilizing solution only when fresh and at full strength<sup>11</sup>. Considering his report, this study tested the 2% glutaraldehyde solution on the 1<sup>st</sup>, 14<sup>th</sup> and 28<sup>th</sup> day following its dilution to confirm its strength during its 28 days shelf life.

When sterilization by heat is not possible due to the physical limitations of the device, the best alternative is sterilization by immersion in an EPA-registered and ADA-approved chemical sterilizer<sup>7,12</sup>.

However, non-disposable instruments, including intraoral X-ray film holders should be sterilized only by heat or gas, considering the sterilization by immersion unacceptable<sup>1</sup>. Furthermore, the American Dental Association<sup>9</sup> does not recommend this method for several reasons, due to the impossibility of biological management of the chemical solutions and the need to handle the sterilized instruments aseptically followed by washing with distilled water and drying with sterile towels. In contrast, other study indicated that the majority of Michigan dental surgeries used the 2% glutaraldehyde solution in order to prevent contamination of devices and patients during intraoral radiographic examination<sup>14</sup>.

Nevertheless, in Brazil, probably due to the high cost of intraoral X-ray film holders considered by some dentists, sterilization by immersion in 2% glutaraldehyde solution for 10h is still used. It should be taken into account that distilled water and sterile towels for drying are not available in the majority of dentomaxillofacial radiologic clinics.

A previous study also evaluated the efficacy of cross-infection control procedures in dental radiology during the bitewing examination. In this study, it was used phosphor plates and CCD based sensors system to show that the cross contamination represents a minor problem for both systems, when a standard asepsis procedure is followed<sup>15</sup>. This fact highlights the need for an infection control protocol application.

Another study using questionnaires compared asepsis procedures adopted by radiological department of different dental school in the U.S. and Canada. The author observed that the majority of the dental schools followed the guidelines for infection control established by the ADA<sup>2</sup> and "The Centers for Disease Control"<sup>3</sup> however, the investigator concluded that the application of these guidelines is difficult. Thus each department should develop its own protocol for cross infection control according to the risk of contamination<sup>16</sup>, being in agreement<sup>13</sup> with the protocol applied in our Radiological Clinic Department.

The contamination observed by other microorganisms, except for *B. subtilis* and *S. aureus* commented above in the discussion, was probably due to the unavailability of sterile water for use in practice. In order to avoid contamination by other microorganisms, the Radiological Clinic of our Dental School is using autoclaved film holder devices. Unfortunately the immersion sterilization in 2% glutaraldehyde solution is still used by the majority of Brazilian dentists.

## Conclusion

In conclusion, the sterilization of X-ray film holder bite blocks by immersion in 2% glutaraldehyde solution for 10h was efficient against *Bacillus subtilis* and *Staphylococcus aureus*, when blocks underwent a final wash with 70% alcohol and the shelf life of the glutaraldehyde solution did not present alteration.

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## Resumo

*O objetivo do presente estudo foi avaliar a esterilização de blocos de mordida de posicionadores para filmes radiográficos intrabucais, por imersão em solução de glutaraldeído a 2% por 10h. Dezesete blocos foram inoculados em meio Trytic Soy Broth contendo um dos microrganismos testados (S. aureus e B. subtilis), tendo sido incubados a 37 °C por 48h. Dentre os blocos, um deles foi usado como controle positivo; outro, como controle negativo, e os demais foram imersos em solução de glutaraldeído a 2% por 10h. Quinze blocos de mordida foram avaliados, sendo cinco lavados com álcool 70%, cinco com água destilada e cinco em água corrente. Nenhum crescimento bacteriano foi encontrado nos blocos, porém, naqueles que tiveram a lavagem final realizada em água corrente e água destilada, várias colônias de fungos foram observadas. Nenhum crescimento foi encontrado nos blocos que foram submetidos à lavagem final com álcool 70%. A solução de glutaraldeído a 2% foi eficiente para esterilização por imersão dos blocos de mordida de posicionadores radiográficos intrabucais quando ficaram imersos por 10h, usando-se álcool 70% na lavagem final.*

Palavras-chave: Radiologia. Microbiologia. Controle de infecções dentárias.

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