Original Research
Microbiology

# Stability of antimicrobial activity of peracetic acid solutions used in the final disinfection process

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**Declaration of Interests:** The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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**DOI:** 10.1590/1807-3107BOR-2015.vol29.0038

Submitted: May 29, 2014
Accepted for publication: Nov 14, 2014
Last revision: Jan 15, 2015

**Abstract:** The instruments and materials used in health establishments are frequently exposed to microorganism contamination, and chemical products are used before sterilization to reduce occupational infection. We evaluated the antimicrobial effectiveness, physical stability, and corrosiveness of two commercial formulations of peracetic acid on experimentally contaminated specimens. Stainless steel specimens were contaminated with *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, blood, and saliva and then immersed in a ready peracetic acid solution: 2% Sekusept Aktiv (SA) or 0.25% Proxitane Alpha (PA), for different times. Then, washes of these instruments were plated in culture medium and colony-forming units counted. This procedure was repeated six times per day over 24 non-consecutive days. The corrosion capacity was assessed with the mass loss test, and the concentration of peracetic acid and pH of the solutions were measured with indicator tapes. Both SA and PA significantly eliminated microorganisms; however, the SA solution was stable for only 4 days, whereas PA remained stable throughout the experiment. The concentration of peracetic acid in the SA solutions decreased over time until the chemical was undetectable, although the pH remained at 5. The PA solution had a concentration of 500-400 mg/L and a pH of 2-3. Neither formulation induced corrosion and both reduced the number of microorganisms ($p$ = 0.0001). However, the differences observed in the performance of each product highlight the necessity of establishing a protocol for optimizing the use of each one.

**Keywords:** Peracetic Acid; Exposure to Biological Agents; Cross Infection; Disinfection; Corrosion.

## Introduction

The instruments and materials used in health establishments are frequently exposed to contamination with microorganisms. Sterilization of these instruments prevents cross-infection, and the appropriate procedure before sterilization can reduce soils and organic residues and, consequently, occupational infection risk.[1,2,3]

Final disinfection is performed by immersing contaminated medical and dental instruments containing organic residues, microorganisms,

and other contaminants in a disinfection solution, with the aim of eliminating or reducing the quantity of microorganisms before mechanical cleaning with soap and water.[3]

For many years, glutaraldehyde has been used for final disinfection. Although it is an effective disinfectant, aldehydes that remain on instruments after cleaning can promote tissue damage, especially to proteins involved in controlling cellular differentiation, thereby reducing the capacity for nucleic acid repair.[4,5] Thus, glutaraldehyde use has been limited in health services, and professionals have been searching for a product with the same effectiveness but lacking the toxicity.

Peracetic acid is considered a potent biocide, even at low concentrations (0.0001% to 0.2%). Peracetic acid has the advantages of remaining effective even in the presence of organic residues, and it decomposes into nontoxic and nonmutagenic substances (acetic acid and oxygen) and provides excellent disinfection in a short period.[6,7,8] Parameters for the correct use of peracetic acid in disinfection procedures should thus be defined. We therefore assessed the antimicrobial effectiveness, the physical stability, and corrosiveness of two different commercial formulations of peracetic acid in a simulated final disinfection process using stainless steel specimens.

## Methodology

This project was approved by the local Research Ethics Committee (CEP/UNITAU nº 038/11).

### Microorganism suspensions

*Staphylococcus aureus* (ATCC 25923) was plated in mannitol salt agar (Oxoid, Hampshire, UK) and *Escherichia coli* (ATCC 25922) in MacConkey agar (Oxoid) for 24 h at 37 °C. Growing colonies were transferred to sterilized saline solution (0.9% NaCl) until a suspension compatible with the 0.5 McFarland scale standard (approximately $1.5 \times 10^8$ cells/mL) was obtained. *Candida albicans* (ATCC 18804) was plated in Sabouraud Agar (Difco, Detroit, USA) for 24 h at 37 °C, and the suspension was adjusted to $10^6$ cells/mL after counting in a Newbauer chamber.

### Saliva and blood samples

On each day of the experiment, 5 mL saliva from the same person was collected in sterile disposable collectors (J Prolab, São José do Pinhais, Brazil). Also, blood samples were obtained from the same blood bag (Compoflex CPDA-1, Fresenius Kabi, Itapecerica da Serra, Brazil) collected from a blood donor by a local hospital. The blood bag would have been disposed of because of the presence of anti-erythrocyte antibodies. These saliva and blood samples were used to simulate the presence of organic materials that could interfere with the antimicrobial activity of the tested solutions.

### Experimental simulation of contamination of the specimens

Stainless steel specimens (5 cm long, 2 mm diameter) were scratched and the corners were rounded with a sander (DPU 10, Panambra Industrial e Técnica S/A, São Paulo, Brazil). In a 150-mL sterile beaker, 4 mL of each microbial suspension, 2 mL saliva, and 0.4 mL blood were mixed. Fourteen specimens that had been previously sterilized were immersed in this contaminant suspension for 30 min.

### Control specimens

After contamination, two specimens were transferred aseptically to tubes containing 8 mL sterile water with peracetic acid neutralizing solution (sodium thiosulfate, $Na_2S_2O_3$, VETEC, 2 g/L, Kunigk and Almeida[6]) and glass beads. The solution was mixed for 1 min using a Vortex (Vortex-Phoenix, Araraquara, Brazil). Next, 100 µL was plated in BHI medium (Difco) for total microorganism counts. The solution was also plated in mannitol salt Agar, MacConkey Agar, and Sabouraud Agar (Oxoid) with 0.1 mg/mL chloramphenicol (Quemicetina Succinato/Carlo Erba®, Milano, Italy) to confirm the amounts of *S. aureus*, *E. coli*, and *C. albicans*, respectively.

### Disinfection procedures

After contamination, the other 12 specimens were transferred aseptically to peracetic acid solutions: six were transferred to a ready commercial liquid solution of 0.25% peracetic acid (Proxitane Alpha® (PA) Thech Desinfecção Ltda., São Paulo,

Brazil; composition: peracetic acid 0.25%, hydrogen peroxide 5%, acetic acid 4%, and stabilizing vehicle q.s.p. 100%), and the other six were transferred to 100 mL of a 2% peracetic acid solution prepared with Sekusept Aktiv® powder (SA; Henkel-Ecolab, Dusseldorf, Germany; composition: > 30% oxygen-based bleaching agents, < 5% nonionic surfactants, 30%–50% sodium perborate monohydrate, 15%–20% citric acid, < 5% fatty alcohol ethoxylate, activator for sodium perborate (TAED), complexing agent, corrosion inhibitor, fragrances) and sterile water. The manufacturer's recommendations about the use-life of these products were not clear or specific. The solutions were stored in 250-mL closed plastic containers (Plasvale, São Paulo, Brazil).

Two specimens were immersed for 10 min (the minimum according to the manufacturer's instructions), two for 15 min, and two for 30 min. The specimens were then removed and processed in the same way as the control specimens. Following vortex agitation, 100 µL suspension was plated on BHI agar in duplicate. All plates were incubated for 48 h at 37 °C, and the colony-forming units (CFU) were then counted.

All procedures were repeated six times per day, four times per week, over 24 non-consecutive days (15 days of experiment) with the same disinfectant solutions.

### Physical analysis

The peracetic acid concentration and pH were measured with indicator tape (Merckoquant 100-500, Merck & Macherey-Nagel, Düren, Germany, respectively) at the beginning and end of each day's experiment. Storage, initial, and final room temperatures were determined each day of the experiment using an analog environmental thermometer scale with a range of -30 to +50 °C (Incoterm, Porto Alegre, Brazil).

### Corrosion analysis

The potential corrosiveness of peracetic acid solutions was verified using the mass loss test. Each specimen was weighed before and after peracetic acid exposure for 96 h at 60 °C in a closed receptacle to simulate a high stress environment.

### Statistical analysis

Room temperature variation was analyzed using analysis of variance followed by the Student's t test. The Mann-Whitney test was used to analyze the effects of peracetic acid by comparing the log CFU/mL of the control to the average log CFU/mL after exposure to the product for every day of the experiment and to the log CFU/mL, regardless of exposure time, between the different experimental days. The Kruskal-Wallis test was used to compare each exposure time between the different experimental days. A level of 5% was considered significant.

## Results

The total CFU/mL counts of the microorganisms from the control group and after exposure to peracetic acid solutions on each day are shown in Table 1. A significant reduction in microorganisms ($p = 0.0001$) was observed in both experimental groups compared with the control group up to day 11. After that, the SA solution was no longer tested because the solution no longer inhibited microorganism growth. The PA group retained significant microorganism-reducing potential until the last day of the experiment.

No significant change was observed in the inhibition of microorganism growth from day 1 to 4 with the SA solution. However, a significant decrease in inhibition occurred from day 4 to 8 ($p = 0.0164$) and from day 8 to 9 ($p = 0.0015$). No significant difference was observed in terms of time of exposure to SA for 10, 15, or 30 min ($p = 0.5008$; data not shown).

Although an increase in microbial growth was observed throughout the experiment with the PA solution, the rate of inhibition of microorganism growth was not significant ($p > 0.05$). Similar to SA, no significant difference was observed with PA in terms of time of exposure for 10, 15, or 30 min ($p = 0.9498$; data not shown).

Throughout the experimental period, the storage temperature of the peracetic acid, Sekusept Aktiv® or Proxitane Alpha®, ranged between 23 and 27 °C (25.7 ± 1.4 °C). The initial room temperature varied between 24 and 31 °C (27.1 ± 2.2 °C), and the final temperature was between 25 and 31 °C (27.7 ± 1.6 °C). The maximum temperature for each day ranged

**Table 1.** Counts of colony-forming units per milliliter of microorganisms obtained from the control group (without disinfectant), Sekusept Aktiv® peracetic acid (SA), and Proxitane Alfa® peracetic acid (PA) for each day of the experiment. Colony-forming units per milliliter are expressed as the logarithm.

| Day of the experiment | Control | SA | PA |
|---|---|---|---|
| 1 | 4.51 | 0 | 0 |
| 2 | 4.32 | 0.18 | 0 |
| 3 | 4.75 | 0.15 | 0 |
| 4 | 4.83 | 0.34 | 0.2 |
| 8 | 4.17 | 0.94 | 0.16 |
| 9 | 4.68 | 2.01 | 0.04 |
| 10 | 4.14 | 2.12 | 0 |
| 11 | 4.15 | 2.41 | 0.22 |
| 15 | 4.2 | - | 0.17 |
| 16 | 4.28 | - | 0.23 |
| 17 | 3.75 | - | 0.14 |
| 18 | 4.49 | - | 0.04 |
| 22 | 3.93 | - | 0.72 |
| 23 | 4.25 | - | 0.52 |
| 24 | 3.87 | - | 0.33 |
| Mean | 4.29 | 1.02 | 0.18 |
| Median | 4.25 | 0.64 | 0.16 |
| Standard deviation | 0.32 | 1.01 | 0.21 |

Mann-Whitney, $p = 0.0001$ (control vs. experimental groups)

between 26 and 33 °C (29.1 ± 2.1 °C). A significant variation in temperature ($p = 0.0230$) occurred during the trial period.

The concentration of peracetic acid in the SA solution ranged from 250 mg/L to undetectable during the experimental period. These values were 250 mg/L on day 1, 200 mg/L on day 2, 100 mg/L on day 3, between 100 and 0 on day 4, and undetectable at later times. The peracetic acid in the PA solution was 500 mg/L on day 1 and 400 mg/L from days 2 to 24. The SA solution had a pH of 5.0 throughout the experimental period, whereas PA had a pH of 2.0 on day 1 of the experiment, 2.5 from days 2 to 18, and 3.0 at later times.

Both solutions showed no corrosion capacity because the weight of the specimens was the same before and after peracetic acid exposure.

## Discussion

The effectiveness of low concentrations and short incubation times with peracetic acid in the process of sterilization and disinfection has been demonstrated.[9,10] However, little is known about the stability of the antimicrobial action of this product when in contact with instruments contaminated with microorganisms and organic material such as blood and saliva. These contaminated instruments must undergo a final disinfection prior to washing, which is known as "purging" in the hospital environment, to eliminate many or all pathogenic microorganisms.[11]

Sudhaus *et al*.[12] reported the influence of temperature on the microbicidal action of peracetic acid. The authors observed that higher temperatures result in lower antimicrobial action. The results of the present study showed that, during the experimental period of 24 days, although significant variations in room temperature occurred (23 to 33 °C, $p = 0.0230$), both peracetic acid solutions retained disinfectant potential for at least 4 days. This temperature variation occurred because of normal daily oscillations and because of the use of a Bunsen burner during the microbiological investigation procedures. We did not attempt to stabilize the temperature to more closely simulate the real situation.

While evaluating the shelf life of peracetic acid, Kunigk *et al*.[13] showed that, at 45 °C, the concentration of peracetic acid was reduced by half in 72 h, whereas at 25 °C the loss after 10 days was only 33%. In our present work, the commercial product SA showed a 20% loss in concentration after 24 h and 60% after 72 h, whereas the product PA lost 20% of the concentration only within the first 24 h, and no further decreases were observed. In particular, the concentration of PA indicated by the manufacturer was 2,500 mg/L, whereas the indicator tape used in this work detected no more than 500 mg/L. If the value of 2,500 mg/L is considered as the starting concentration, an 84% loss in concentration was detected on day 2 of the experiment, and the concentration then remained stable at 400 mg/L until the end of the experimental period. Peracetic acid indicator tape in the range 100 to 500 mg/L was chosen for this experiment, because it is often accurately used to verify minimum quantities of peracetic

acid, and this concentration range is considered sufficient for the disinfection process.[14]

According to Kitis[14] and Zhao *et al*.[15], a pH in the range of 5.5 to 10.2 results in spontaneous decomposition of peracetic acid into acetic acid and oxygen. This may have been an important factor in the difference in stability between the commercial products we evaluated, because although SA had a pH of 5.0, which is only 0.5 below the range of decomposition, this pH favors self-sustained decomposition. In contrast, PA had a pH between 2.0 and 3.0 throughout the experimental period, which is well below the peracetic acid decomposition range; this probably contributed to its greater stability.

Weak acids have potent antimicrobial activity because the non-dissociated forms pass freely through the cell membrane. When the cytoplasmic pH is higher than that of the growth medium, the weak acid dissociates, releasing a proton and acidifying the cytoplasm.[16] Thus, a low pH, which was lower in the PA solution than the SA solution, may also be responsible for microorganism elimination.

The results of this work demonstrated that the product SA maintained its disinfectant effectiveness until day 4 of use, whereas PA was effective until day 24 and perhaps later. Considering the microorganism growth on days 22 and 23, to guarantee a lower risk during the washing process of medical instruments, the results suggest that the PA solution can be re-used for a maximum of 18 non-consecutive days of use or 12 consecutive days for final disinfection.

Although the PA product (0.25%) had a lower concentration of peracetic acid, the better results observed in this study may be due to differences in the composition of the products, which may have interfered with the stability. The exposure times did not significantly impact microbial reduction, suggesting that peracetic acid solutions can be used for only 10 min, making this process faster and avoiding other problems with exposure for longer times. This shorter exposure time compared to what is necessary with glutaraldehyde (30 min) permits faster disinfection of biomedical instruments and materials.

Although peracetic acid has been thought to perhaps induce corrosion of biomedical instruments, our results confirmed the manufacturers' information that corrosion of stainless steel materials when using the specific conditions in this study does not occur. A temperature of 60 °C was used in the analysis because an increase in temperature reduces the resistance of these materials and increases the susceptibility to corrosion.[17] The high temperature may have contributed to the decomposition of peracetic acid, influencing the real corrosion capacity of the products. Thus, tests using lower temperatures should be performed to confirm these data.

Other types of stainless steel with different qualities are sometimes used, and these may be affected differently by the disinfection process. The stainless steel used in this study was an austenitic standard grade, AISI Type 304 L, which is considered to have high resistance to corrosion.

Healthcare professionals should be reminded that the final disinfection process (the process that aims to reduce the presence of microbes, thereby reducing the risk of contamination during instrument washing) differs from the process of disinfection, because the instruments are usually soiled (*i.e.*, blood, saliva, or fluids in addition to microorganisms are commonly present). An instrument must be free of organic material before an effective disinfection process can begin.

Analysis of the results of this work also demonstrated an important difference in the stability of the commercial products used in the final disinfection process, suggesting that further studies should be conducted with other peracetic acid products, both by the manufacturers and by independent researchers, to establish a reliable and secure time period for the re-use of each product.

## Conclusion

The peracetic acid products were stable and provided effective disinfection for at least 4 days with no corrosion capacity. However, the differences observed in the performance of each product highlight the necessity of establishing a protocol for the optimal use of each one.

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