



Short report

Ozonated water is inferior to propanol-based hand rubs for disinfecting hands

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SUMMARY

Ozone is a strong oxidizing biocide that has broad-spectrum antimicrobial properties. The aim of the study was to compare the efficacy of ozone to a propanol-based hand rub for hand disinfection. Twenty subjects were enrolled in an in-vivo cross-over trial (prEN 12791). Subjects treated their hands with the reference procedure (propan-1-ol 60%) or with ozone (4 ppm). Post-wash bacterial counts were determined from one hand (immediate effect), and from the other hand that had been gloved for 3 h (delayed effect). The investigation indicated that ozone is inferior to propan-1-ol 60% hand rub for hand asepsis.

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Introduction

Many agents available for hand asepsis are alcohol-based and are used with good results but may provoke skin reactions.¹ Alternatives to alcohol for hand asepsis that do not cause skin reactions but still provide effective hand asepsis would facilitate workplace compliance.

Ozone is a strong oxidizing biocide that has broad-spectrum antimicrobial properties.⁴ The ozone molecule acts as an oxidant via direct oxidative effects and through release of free

radicals when ozone decomposes. It interacts with bacterial cell walls, enzymes and nucleic material and destroys them.^{2–4} Ozone is used in various workplace settings such as in hospitals, dentistry and extensively in water and food treatment.^{5–7}

This study assessed ozonated water as a potential substitute for a propan-1-ol-based hand rub for hand asepsis, examining specifically the immediate reduction in bacterial loads on the fingertips. It was proposed that ozone was at least as effective in reducing the bacterial load on the fingertips as the alcohol-based rub, such that ozone attained an immediate post-wash bacterial load on the fingertips at least as low as that after application of the alcohol-based rub.

We hypothesized that application of ozonated water will result in a colony-forming unit (cfu) count on the fingertips that falls within a predetermined equivalence range for the

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immediate and delayed post-wash count after application of propan-1-ol 60% hand rub.

Methods

Products

The following products were used in this study:

- propan-1-ol (Chem Supply Pty Ltd, Gillman, South Australia), contains 99.5% propan-1-ol which was diluted to 60% propan-1-ol with sterile saline;
- Bigwell Infection Control: ozonated water generator (product no. BTG120235);
- Dominant Soft Hands Soap Batch 85488, containing glycerine and lanolin (Dominant Australia Pty Ltd, Brompton, South Australia);
- D/E neutralizing broth (product no. MIMOPM133 Thermo Fisher Scientific Australia Pty Ltd);
- tryptone soya agar (TSA) (product no. PP 2157 Thermo Fisher Scientific Australia Pty Ltd).

Test principle and prerequisites

The in-vivo bactericidal efficacy of ozonated water was assessed according to the European test method (prEN 12791) by using 20 veterinary student participants in a cross-over trial.⁸ Ethical consent was obtained from the University of Adelaide (H-2013-050). The subjects had not used any substances with antibacterial activity or antibacterial soap for one week prior to testing.

Wash phase

To eliminate transient bacteria and external particles, the subjects washed their hands with 10 mL non-medicated soap and water for 1 min using a standardized handwashing procedure.⁸ They then rinsed their hands with running tap water and dried them with non-sterile paper towels.

Determination of pre-wash cfu counts

The fingertips of the left and right hand, including the thumb, were rubbed for 1 min in two Petri dishes (9 cm diameter) containing 10 mL of tryptic soy broth (TSB). The Petri dishes were labelled indicating the left or right hand. From the sampling fluid for each hand, 1:10 and 1:100 dilutions were made, of which 0.1 mL aliquots of both solutions were spread over tryptic soy agar (TSA) dishes (two plates per hand per participant). Dishes were incubated at 36°C for 24 h and the cfu were counted at 24 h. Only plates with <300 cfu were counted.

Hand asepsis phase

Each subject was treated with a reference product (propan-1-ol 60%), or ozonated water (4 ppm). A period of at least one week elapsed between each product application to allow the reconstitution of normal skin flora. The ozonized water concentration in the handwash solution produced by the ozone generator was confirmed to be 4 ppm prior to and during testing. The water temperature was maintained at 18°C.

Propan-1-ol 60%-based hand rub was applied and reapplied in 3 mL portions to ensure that the hands were moist for a period of 3 min and hands were rubbed according to a standard handwashing technique.⁸ The hands were then allowed to air dry.

Ozonated water was allowed to run over the hands for 3 min while using a standard handwashing technique.⁸ The hands were then dried with sterile paper towels.

Determination of post-wash cfu counts

Immediately after disinfection, the subjects rubbed the fingertips of the left hand for 1 min into a Petri dish containing D/E neutralizing broth. This solution was used to calculate the immediate post-wash cfu count. The participants were gowned and the right hand was gloved for 3 h, using standard surgical gloving technique, during which the subjects attended university lectures. After removing the glove, sampling was done identically to that performed previously. This solution was used to calculate the delayed post-wash cfu count.

From each sample (immediate and delayed), 1 mL and 0.1 mL of undiluted aliquots and a 0.1 mL aliquot of a 1:10 dilution were seeded into separate TSA Petri dishes. Dishes were incubated at 36°C for 24 h and the cfu were counted 24 h after sampling.

To indicate the actual cfu count on the fingertips, the number of cfu/mL in the original sampling liquid was calculated by multiplying the cfu count for each dilution by its dilution factor. There were three plates (with the three dilution factors) per hand totalling six plates per student. For accuracy, only plates with <300 cfu were counted and included in calculations. All pre- and post-wash cfu counts were expressed as log₁₀ counts.

Statistical analysis

The log₁₀ cfu count was the response of interest and found to be normally distributed using the Shapiro–Wilk test with failure to reject the null hypothesis of normality. Thus, the log₁₀ cfu count was summarized as mean [95% confidence interval (CI)].

The pre-wash log₁₀ cfu count for each hand was first tested between products using an unpaired *t*-test (equal variances) against a two-sided hypothesis with *P* < 0.05 considered significant, in order to verify that the trials were not different in the initial bacterial load. Failure to find a difference allowed further comparison of absolute log₁₀ cfu counts between products.

The log₁₀ cfu count was compared from pre-wash to immediate post-wash for the right hand and pre-wash to delayed post-wash for the left hand, for each product, using a paired *t*-test. Comparisons between immediate post-wash and delayed post-wash for each product were made using an unpaired *t*-test. To adjust for multiple comparisons, a restricted *P* < 0.01 was considered significant.

Based on the hypothesis that ozone was at least as effective as the propan-1-ol 60% hand rub, demonstrated by attaining an immediate post-wash log₁₀ cfu count on the fingertips at least as low as that after alcohol-based asepsis, a non-inferiority analysis was performed.

For the immediate post-wash log₁₀ cfu count, an equivalence range was established based on the mean ±10% of the

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