Differential Antimicrobial Efficacy of Multipurpose Solutions against *Acanthamoeba* Trophozoites

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**SIGNIFICANCE:** This investigation examines the effectiveness of several common contact lens solutions in the disinfection of *Acanthamoeba*, which causes a serious eye infection most often resulting from dysfunctional or improper use of contact lens products.

**PURPOSE:** *Acanthamoeba* keratitis is an eye infection caused by a free-living amoeba, which can lead to extensive corneal damage and frequently blindness. *Acanthamoeba* keratitis is linked with contact lens use combined with noncompliance with contact lens care cleaning regimens. The patient's choice and use of multipurpose solutions (MPSs) continue to be a risk factor for *Acanthamoeba* keratitis. Thus, it is critical that the *Acanthamoeba* disinfection efficacy of the popular MPSs be determined. Here we compare the efficacy of seven major MPSs on the global market.

**METHODS:** Using standard methods of *Acanthamoeba* disinfection and quantification, *Acanthamoeba* ATCC 30461, 30868, 50370, and 50676 trophozoites were inoculated into each MPS and held for the manufacturer’s recommended disinfection time. *Acanthamoeba* recovery plates were incubated for 14 days, after which positive wells were identified and cell concentrations determined using the 50% endpoint method.

**RESULTS:** Members of the OPTI-FREE products (Express, Replenish, and Puremoist [Alcon, Fort Worth, TX]) demonstrated significantly higher percentages of antimicrobial activity compared with the renu Advanced Formula (Bausch + Lomb, Rochester, NY), Biotrue (Bausch + Lomb), Acuvue RevitaLens (Johnson & Johnson, Santa Ana, CA), and Lite products (Cooper Vision, Scottsville, NY) for four of the trophozoite strains tested.

**CONCLUSIONS:** Many of the popular MPS biocides maintain little or no antimicrobial activity against *Acanthamoeba* trophozoites, and the number of biocides in an MPS does not necessarily indicate its antimicrobial activity.
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METHODS

As previously described,14,15 axenic culture media (containing 20 g biosate peptone, 5 g glucose, 0.3 K2HPO4, 10 μg vitamin B12, and 15 mg L-methionine per liter of distilled deionized water) was used to produce homogenous populations of Acanthamoeba trophozoites. Axenic culture media was adjusted to a pH of 6.6 to 6.95 with 1 M of NaOH and autoclaved at 121°C for 20 minutes before storing at room temperature for use within 2 months. One-fifth Ringer's solution was used to harvest organisms and for seeding trophozoites into 96-well plates.

Antimicrobial efficacy of contact lens disinfecting solutions against Acanthamoeba trophozoites was conducted as previously published,14 per a modified version of ISO standard 14729. Acanthamoeba strains were obtained from ATCC (American Type Culture Collection, Manassas, VA): ATCC 30461 (Acanthamoeba polyphaga, group T4, isolated from human corneal scrapings; Houston, TX, 1973), ATCC 30868 (Acanthamoeba castellani, group T4, isolated from human cornea; Cambridge, England, 1974), ATCC 50370 (A. castellani, group T4, isolated from human eye infection; New York, NY, 1978), and ATCC 50676 (Acanthamoeba mauritaniensis, group T4, isolated from human eye infection; Namibia or South Africa, 1990). These strains belong to the T4 group, which is the most commonly associated genotype with Acanthamoeba keratitis.16 Acanthamoeba trophozoites were subcultured in axenic media with the final 24 hours of growth in fresh media to promote uniform Acanthamoeba trophozoite proliferation, before testing. After scale-up, cells were collected and centrifuged at 500g for 5 minutes at room temperature, and washed three times with Ringer's solution. Pellets were then resuspended in Ringer's solution, and the count seeding density was confirmed via hemocytometer. Trophozoites were inoculated into each multipurpose solution for a final cell density between 2 × 10^5 and 5 × 10^5 cells per well. Each multipurpose solution was held at room temperature for the manufacturer's soaking time (Table 1). At disinfection time, 1 mL of multipurpose solution was placed in a 12-well plate containing 2 mL of nonnutrient agar with 100 μL of Escherichia coli (10^5 colony-forming unit/mL; ATCC 8739). Plates were incubated for 14 days at 28°C ± 2°C. After incubation, positive wells were identified and surviving cells quantified using the 50% end point following the Reed and Muench computation.18 This computation calculates the concentration of a test substance or culture that produces an effect of interest in half of the test units. Antimicrobial efficacy was determined by calculating the log and percent reduction of the cell densities recovered from the multipurpose solution at disinfection time, compared with the inoculum control of the respective strain. Each Acanthamoeba strain was tested in one to two independent trials, in triplicate, on different days, and the results were averaged. All multipurpose solutions were tested simultaneously using the same inoculum stock as a direct comparison. To understand the differences between groups, log reduction quantifications were analyzed via the Student two-tailed t test and are represented as mean ± standard error.

To obtain images of the Acanthamoeba following disinfection efficacy of each solution, propidium iodide staining (as propidium iodide only binds to exposed cellular DNA of amoeba with damaged cell walls; Invitrogen, Carlsbad, CA) was used with three of the Acanthamoeba strains: ATCC 30461, ATCC 50370, and ATCC 50676.15 Propidium iodide staining was performed on separate cultures from those used for quantification via the 50% endpoint method. Briefly, Acanthamoeba were seeded into a black clear-bottom 96-well plate at a density of 1 × 10^4 cells per well. Cells were allowed to adhere for 2 hours. Media was removed, and multipurpose solutions were added to appropriate wells (0.2 mL/well) in 15 replicates to confirm that representative images were indicative of quantitative log calculations. Blanks and untreated Acanthamoeba wells were included as controls. Following the multipurpose solution manufacturer's listed disinfection time, multipurpose solutions were removed, and 0.2 mL of 2 μg/mL propidium iodide diluted in ¼ Ringer's solution was added to each well of the 15 replicates. This was followed by confocal imaging (Nikon Ti Eclipse Microscope; Nikon, Minato City, Tokyo, Japan) at ×20 magnification using the Nikon NIS-Elements platform.

RESULTS

To determine the Acanthamoeba disinfection efficacy of each multipurpose solution, trophozoites were tested in each multipurpose

| Contact lens care product | Manufacturer | Biocides | Disinfection time (h) |
|---------------------------|--------------|----------|-----------------------|
| OPTI-FREE Puremoist       | Alcon, Fort Worth, TX | Polyquaternium-1 (0.001%), myristamidopropyl dimethylamine (0.0006%) | 6 |
| OPTI-FREE Express         | Alcon, Fort Worth, TX | Polyquaternium-1 (0.001%), myristamidopropyl dimethylamine (0.0005%) | 6 |
| OPTI-FREE Replenish       | Alcon, Fort Worth, TX | Polyquaternium-1 (0.001%), myristamidopropyl dimethylamine (0.0005%) | 6 |
| Acuvue RevitaLens         | Johnson & Johnson, New Brunswick, NJ | Polyquaternium-1 (0.0003%), alexidine dihydrochloride (0.00016%) | 6 |
| renu Advanced Formula     | Bausch + Lomb, Rochester, NY | Polyquaternium (0.00015%), alexidine dihydrochloride (0.0002%), polyaminopropyl biguanide (0.00005%) | 4 |
| Biotru                    | Bausch + Lomb, Rochester, NY | Polyaminopropyl biguanide (0.00013%), polyquaternium (0.0001%) | 4 |
| Lite                      | CooperVision, Lake Forest, CA | Polyhexanide (0.0001%) | 6 |
FIGURE 1. OPTI-FREE products (Express, Replenish, and Puremoist Multi-Purpose Disinfecting Solutions) maintain a significantly higher percentage of *Acanthamoeba* antimicrobial efficacy vs. other global market products. Quantifications are represented as mean ± standard error percent reduction vs. inoculum controls. All seven products were tested against ATCC 30461 (A), ATCC 30868 (B), ATCC 50370 (C), and ATCC 50676 (D). n = 3 to 6 per group. *aP* < .05 vs. renu Advanced Formula, *bP* < .005 vs. renu Advanced Formula, *cP* < .005 vs. Biotrue, *dP* < .005 vs. Acuvue RevitaLens, and *eP* < .005 vs. Lite.
solution according to the manufacturer's stated disinfection time at room temperature (Fig. 1, Table 2). The disinfection efficacy of each multipurpose solution was determined by calculating the percent reduction compared with the inoculum control. Inoculum controls for each strain and replicate ranged between 2 × 10^8 and 3.8 × 10^9 log cells/mL. Each multipurpose solution was tested against ATCC 30461 (Fig. 1A), ATCC 30868 (Fig. 1B), ATCC 50370 (Fig. 1C), and ATCC 50676 (Fig. 1D). In all four strains, OPTI-FREE Express Multi-Purpose Disinfecting Solution and OPTI-FREE Puremoist Multi-Purpose Disinfecting Solution (Alcon, Fort Worth, TX) maintained significantly higher antimicrobial activity than the renu Advanced Formula (P < .05; Bausch + Lomb, Rochester, NY), Biotrue (P < .05; Bausch + Lomb), and Lite products (P < .05; Cooper Vision, Scottsville, NY). In the ATCC 30461 strain, OPTI-FREE Express Multi-Purpose Disinfecting Solution and OPTI-FREE Puremoist Multi-Purpose Disinfecting Solution maintained a significantly higher antimicrobial activity than Acuvue RevitaLens (P < .001; Johnson & Johnson, Santa Ana, CA). This was also true for strain ATCC 50370 (P < .001) and in strain ATCC 30868 (P ≤ .001). Similarly, for the ATCC 30461 strain, OPTI-FREE Replenish Multi-Purpose Disinfecting Solution demonstrated significantly greater antimicrobial activity versus Biotrue (P = .003); for the ATCC 30868 strain versus renu Advanced Formula (P = .04) and Lite (P = .003); for the ATCC 50370 strain versus renu Advanced Formula (P = .005), Biotrue (P < .001), Acuvue RevitaLens (P < .001), and Lite (P < .001); and for the ATCC 50676 strain versus Biotrue (P = .004) and Lite (P = .001).

For three of these strains, representative images of the antimicrobial efficacy were obtained using a novel rapid method, which was recently outlined. Propidium iodide, which stains red any cellular DNA components that are exposed because of cell death and loss of cell membrane integrity, or spilled because of cell lysis, was used to examine Acanthamoeba cultures immediately after completion of the multipurpose solution exposure time as a separate qualitative examination from the log reduction quantitative experiments. These representative examinations were performed for all seven multipurpose solutions with the ATCC 30461 strain (Fig. 2), the ATCC 50370 strain (Fig. 3), and the ATCC 50676 strain (Fig. 4). The control samples (after 6 hours of incubation) demonstrated minimal red staining. However, natural cell death is evident even in the control samples, indicating a base level of expected mortality without intervention. Like the quantifications noted in Fig. 1A, the Lite multipurpose solution demonstrated a strong degree of cell death within the ATCC 30461 strain (Fig. 2), but not in the ATCC 50370 or 50676 strains (Figs. 3, 4). Acuvue RevitaLens and renu Advanced Formula seemed to cause the highest amount of cell death within the ATCC 50676 strain (Fig. 4), but not the other two strains. Biotrue seemed to induce a minimal amount of cell death in all three of the tested strains compared with the other products. Among all three strains, the three OPTI-FREE products demonstrated a high amount of red staining, indicating high amounts of cell death. Overall, these visual observations were in line with the 50% endpoint quantifications found in Fig. 1.

**DISCUSSION**

Acanthamoeba keratitis is a dangerous ocular infection largely because of the difficulties in diagnosing, treating, and managing the disease. Critically, it can lead to severe corneal damage and, in many cases, permanent blindness without corneal transplant. Although it is a fortunately rare affliction, misuse of contact lens care products or ineffective contact lens care products have been directly linked to Acanthamoeba keratitis infections and outbreaks. Thus, it is integral to public health that information regarding the efficacy of common contact lens multipurpose solutions be both robust and available. The goal of this study was therefore to use widely reported methods of Acanthamoeba testing and quantification resulting from multiple replicates, using both standard and clinical strains of Acanthamoeba and a wide range of multipurpose solutions on the global market. Notably, this study focuses on the

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**FIGURE 1.** OPTI-FREE products (Express, Replenish, and Puremoist Multi-Purpose Disinfecting Solutions) maintain a significantly higher percentage of Acanthamoeba antimicrobial efficacy vs. other global market products. Quantifications are represented as mean ± standard error percent reduction vs. inoculum controls. All seven products were tested against ATCC 30461 (A), ATCC 30868 (B), ATCC 50370 (C), and ATCC 50676 (D). n = 3 to 6 per group. aP < .05 vs. renu Advanced Formula, bP < .005 vs. renu Advanced Formula, cP < .005 vs. Biotrue, dP < .005 vs. Acuvue RevitaLens, and eP < .005 vs. Lite.
trophozoite form of Acanthamoeba, as the biocides in non-hydrogen peroxide–based systems have been shown to be ineffective against the Acanthamoeba cyst.28

The field of Acanthamoeba keratitis research and contact lens disinfection agents sometimes contains contentious or differing results, often attributable to varying methods of investigation and quantification. Fortunately, the methods used in this investigation demonstrated similar results to what has been reported for other global products.29

Furthermore, despite a field of varied reports, some consistencies in the use of certain biocides against Acanthamoeba persist. For

### TABLE 2. CIs for multipurpose solution disinfection efficacy comparisons with \( P < .05 \), as related to Figs. 1 to 4

| Contact lens care product | Compared with | Strain tested  | 95% CI     |
|---------------------------|---------------|----------------|------------|
| OPTI-FREE Express MPS     | renu Advanced Formula | ATCC 30461 | −3.127 to −1.674 |
| OPTI-FREE Express MPS     | Biotrue | ATCC 30461 | −4.244 to −2.983 |
| OPTI-FREE Express MPS     | Acuvue RevitaLens | ATCC 30461 | −3.681 to −2.394 |
| OPTI-FREE Express MPS     | Lite | ATCC 30461 | −3.325 to −2.018 |
| OPTI-FREE Replenish MPS   | Biotrue | ATCC 30461 | −1.885 to −0.5141 |
| OPTI-FREE Puremoist MPS   | renu Advanced Formula | ATCC 30461 | −3.102 to −1.701 |
| OPTI-FREE Puremoist MPS   | Biotrue | ATCC 30461 | −4.215 to −3.014 |
| OPTI-FREE Puremoist MPS   | Acuvue RevitaLens | ATCC 30461 | −3.653 to −2.425 |
| OPTI-FREE Puremoist MPS   | Lite | ATCC 30461 | −3.253 to −2.092 |
| OPTI-FREE Express MPS     | renu Advanced Formula | ATCC 30868 | −3.459 to −1.079 |
| OPTI-FREE Express MPS     | Biotrue | ATCC 30868 | −4.129 to −2.045 |
| OPTI-FREE Express MPS     | Acuvue RevitaLens | ATCC 30868 | −4.129 to −2.045 |
| OPTI-FREE Express MPS     | Lite | ATCC 30868 | −4.938 to −2.917 |
| OPTI-FREE Replenish MPS   | renu Advanced Formula | ATCC 30868 | 0.067 to 1.535 |
| OPTI-FREE Replenish MPS   | Lite | ATCC 30868 | −1.235 to −0.479 |
| OPTI-FREE Puremoist MPS   | renu Advanced Formula | ATCC 30868 | −3.385 to −2.091 |
| OPTI-FREE Puremoist MPS   | Biotrue | ATCC 30868 | −3.852 to −3.260 |
| OPTI-FREE Puremoist MPS   | Acuvue RevitaLens | ATCC 30868 | −3.852 to −3.260 |
| OPTI-FREE Puremoist MPS   | Lite | ATCC 30868 | −4.457 to −4.246 |
| OPTI-FREE Express MPS     | renu Advanced Formula | ATCC 50370 | −1.789 to −0.560 |
| OPTI-FREE Express MPS     | Biotrue | ATCC 50370 | −2.113 to −1.237 |
| OPTI-FREE Express MPS     | Acuvue RevitaLens | ATCC 50370 | −1.841 to −0.349 |
| OPTI-FREE Express MPS     | Lite | ATCC 50370 | −2.117 to −1.780 |
| OPTI-FREE Replenish MPS   | renu Advanced Formula | ATCC 50370 | −1.598 to −0.373 |
| OPTI-FREE Replenish MPS   | Biotrue | ATCC 50370 | −1.920 to −1.055 |
| OPTI-FREE Replenish MPS   | Acuvue RevitaLens | ATCC 50370 | −1.648 to −0.753 |
| OPTI-FREE Replenish MPS   | Lite | ATCC 50370 | −1.958 to −1.623 |
| OPTI-FREE Puremoist MPS   | renu Advanced Formula | ATCC 50370 | −2.076 to −0.0660 |
| OPTI-FREE Puremoist MPS   | Biotrue | ATCC 50370 | −2.430 to −1.308 |
| OPTI-FREE Puremoist MPS   | Acuvue RevitaLens | ATCC 50370 | −2.155 to −1.008 |
| OPTI-FREE Puremoist MPS   | Lite | ATCC 50370 | −2.753 to −1.591 |
| OPTI-FREE Express MPS     | renu Advanced Formula | ATCC 50676 | −1.855 to −0.039 |
| OPTI-FREE Express MPS     | Biotrue | ATCC 50676 | −3.367 to −2.041 |
| OPTI-FREE Express MPS     | Lite | ATCC 50676 | −4.977 to −3.098 |
| OPTI-FREE Replenish MPS   | Biotrue | ATCC 50676 | −3.318 to −1.240 |
| OPTI-FREE Replenish MPS   | Lite | ATCC 50676 | −4.846 to −2.379 |
| OPTI-FREE Puremoist MPS   | renu Advanced Formula | ATCC 50676 | −1.913 to −0.575 |
| OPTI-FREE Puremoist MPS   | Biotrue | ATCC 50676 | −3.255 to −2.742 |
| OPTI-FREE Puremoist MPS   | Lite | ATCC 50676 | −5.045 to −3.618 |

CI = confidence interval; MPS = multipurpose solution.
instance, the OPTI-FREE products have been previously demonstrated as highly effective against *Acanthamoeba* trophozoites.\(^{12,30,31}\) OPTI-FREE products have also previously been found to produce little or no encystment,\(^{32,33}\) ensuring that the more vulnerable trophozoite form persists and is able to be acted upon by multipurpose solutions. Finally, the biocides used in the OPTI-FREE products, namely, polyquaternium-1 and myristamidopropyl dimethylamine, have been shown to have the greatest efficacy against *Acanthamoeba* (and other ophthalmological pathogens) as compared with other biocides.\(^{34-36}\)

The results of the current investigation indicate similar results: products containing polyquaternium-1 and myristamidopropyl dimethylamine demonstrated significantly greater *Acanthamoeba* trophozoite disinfection efficacy than other biocides or combinations of biocides. According to the results demonstrated here, less effective biocides include polyaminopropyl biguanide or alexidine dihydrochloride combined with polyquaternium or polyquaternium-1. In addition, although having a greater number of biocides does not necessarily infer greater antimicrobial efficacy, as in the case of renu

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**FIGURE 2.** Representative images of ATCC 30461 *Acanthamoeba* cell death after exposure to multipurpose solutions. Propidium iodide staining results in red stain indicating cell death or lysis, whereas gray color indicates living cells. Scale bar, 100 μm; ×20 magnification.

**FIGURE 3.** Representative images of ATCC 50370 *Acanthamoeba* cell death after exposure to multipurpose solutions. Propidium iodide staining results in red stain indicating cell death or lysis, whereas gray color indicates living cells. Scale bar, 100 μm; ×20 magnification.
Advanced Formula, the higher concentration of polyquaternium-1 in the OPTI-FREE products does seem to impart a higher percentage of disinfection. Indeed, products containing 0.001% polyquaternium versus those containing less (0.0003% in Acuvue RevitaLens, 0.00015% in Biotrue) maintained significantly greater antimicrobial activity. In addition, the OPTI-FREE products are the only products examined that contain myristamidopropyl dimethylamine. These data imply that either polyquaternium acts on *Acanthamoeba* in a dose-dependent manner, whereas other biocides are ineffective against this particular pathogen, or myristamidopropyl dimethylamine is a critical component to *Acanthamoeba* antimicrobial activity, or both.

The visual examination of the antimicrobial activity of these multipurpose solutions demonstrated similar results to the 50% endpoint quantifications performed here. Propidium iodide staining has been previously established as an efficient method for identifying dead or lysed amoeboid cells. Visually, it is easy to assess that there is very little cell death in control samples (although a minor amount of cell death persists because of natural cell cycles) based on the very small amount of red staining. Furthermore, the visual observations gathered from these representative stains are in agreement with published data regarding the morphological changes of *Acanthamoeba* after exposure to biocides, such as a rounding of the cell wall, retraction of pseudopodia, and encystment. The renu Advanced Formula, Biotrue, Acuvue RevitaLens, and Lite products (which contain a maximum of three parts per million of polyquaternium and/or any other biocide) overall demonstrated a low to moderate amount of propidium iodide staining between the three *Acanthamoeba* strains examined. In general, the antimicrobial quantifications aligned with the visual representations of *Acanthamoeba* cell death. Notably, within each of these multipurpose solutions, it was clear in both the 50% endpoint quantifications and the visual staining that the antimicrobial activity of each multipurpose solution can be altered by which *Acanthamoeba* strain is tested, as the results were not universal between strains. However, even within the differences noticed between strains, the OPTI-FREE products, which contain 10 parts per million of polyquaternium-1, consistently demonstrated both quantifiably high levels of antimicrobial activity and visually the greatest amount of propidium iodide-based cell death.

In conclusion, the difference and concentration of biocides between multipurpose solutions are critically important in the endeavor to determine the contact lens care product antimicrobial activity. After the examination of multiple laboratory and clinical isolates of *Acanthamoeba*, and seven of the most widely used multipurpose solutions on the global market, this investigation demonstrated that the biocides used within the OPTI-FREE products are the most efficacious regarding *Acanthamoeba* trophozoite disinfection.
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