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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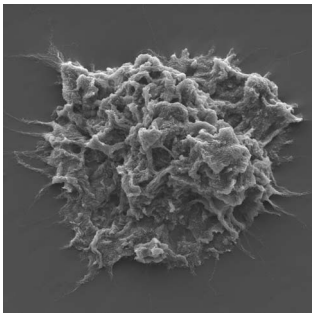

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Acanthamoeba is a pervasive microorganism. However, *Acanthamoeba* keratitis is a fortunately rare condition, as this infection can lead to blindness in as many as 41% of the afflicted patients.[1,2] Outbreaks of *Acanthamoeba* keratitis in the United States in 2007[3] and in the United Kingdom since 2010[4] have been directly linked to particular multipurpose solution contact lens care products.[3–6] These outbreaks resulted in the withdrawal of those products.[3–6] Although the current International Standard (ISO 14729)[7] does not presently recommend acanthamoebicidal testing, this standard is currently being updated by the American National Standards Institute and the International Standards Committee.[8]

The differences in the products' formulations and the biocides used in each product maintain the clear differentiation in the *Acanthamoeba* disinfection capabilities of each multipurpose solution. In general, *Acanthamoeba* is susceptible to some common biocides, which may have a variety of disinfection mechanisms. For instance, *Acanthamoeba* pseudopodia, nucleolar structure, mitochondria, and endoplasmic reticulum are susceptible to chlorhexidine gluconate.[9] Chlorhexidine diacetate has also been shown to produce shrinkage from the cyst wall, whereas polyhexamethylene biguanide induced both withdrawal of the cytoplasm from the cyst wall and swelling of cysts.[10] Finally, chlorine treatment has led to size reduction, permeabilization, and retraction of pseudopods.[11]

The biocides available for use within any multipurpose solution provide a unique challenge. Alongside the use of cleaning, wetting, and comfort agents, the biocides included in every multipurpose solution reach toward the goal of maintaining sufficient antimicrobial activity against potentially pathogenic microorganisms while promoting ocular health. Biocides effective against *Acanthamoeba* may not be suitable for patient safety as part of a multipurpose solution because of the potential for corneal damage. Furthermore, because *Acanthamoeba* is a unique pathogen in the field of potentially infectious ocular agents, biocides that may be effective against other common microorganisms and can be included in multipurpose solutions may have little to no effect against amoeba in either the trophozoite or cyst formation.[12,13] Therefore, the goal of this study was to determine the *Acanthamoeba* disinfection efficacy of seven of the most common multipurpose solutions on the global market.

METHODS

As previously described,[14,15] axenic culture media (containing 20 g biosate peptone, 5 g glucose, 0.3 KH_2PO_4, 10 µg vitamin B_{12}, 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-fourth 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 castellanii*, group T4, isolated from human cornea; Cambridge, England, 1974), ATCC 50370 (*A. castellanii*, 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 500*g* 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 9 mL of neutralizing broth (Ringer's solution with lecithin and polysorbate 80 to neutralize quaternaries, phenolics, hexachlorophene, and formalin)[17] and serially diluted in Ringer's solution. Each dilution was plated in quadruplicate on a 12-well plate containing 2 mL of nonnutrient agar with 100 µL of *Escherichia coli* (10^8 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

TABLE 1. Multipurpose solutions used and their manufacturers, biocides, and stated disinfection times

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
Biotrue	Bausch + Lomb, Rochester, NY	Polyaminopropyl biguanide (0.00013%), polyquaternium (0.0001%)	4
Lite	CooperVision, Lake Forest, CA	Polyhexanide (0.0001%)	6

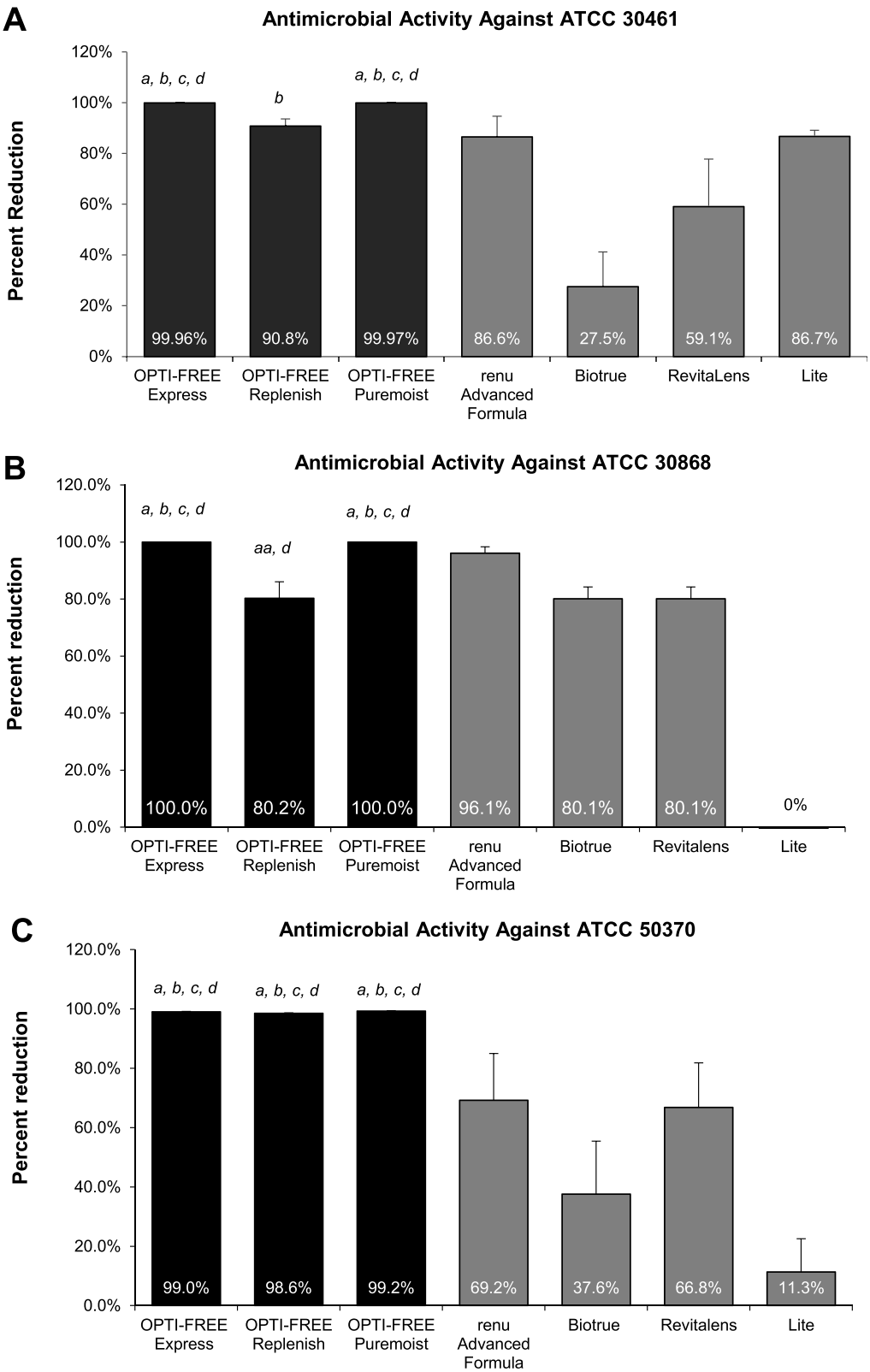
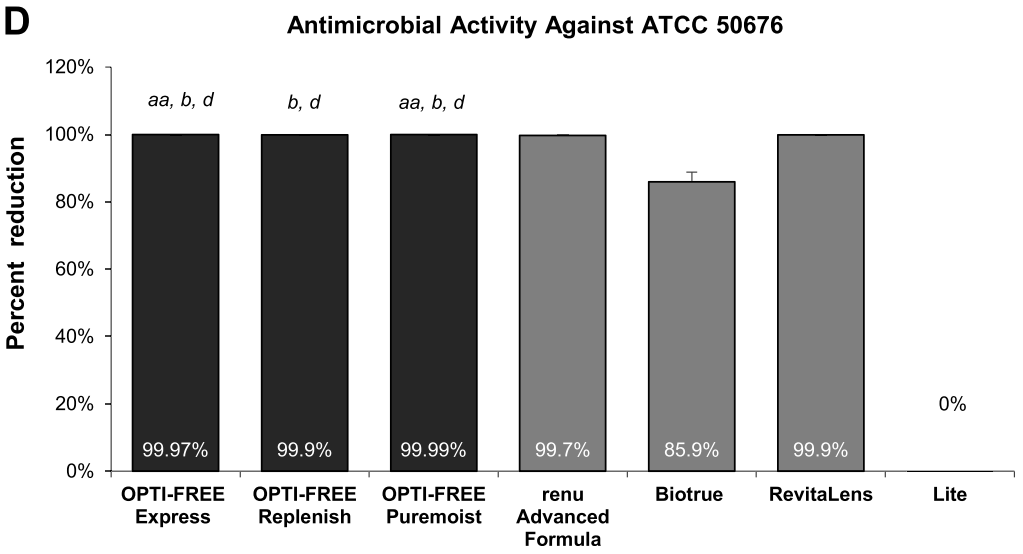


FIGURE 1. OPTI-FREE products (Express, Replenish, and Puremoist Multi-Purpose Disinfecting Solutions) maintain a significantly higher percentage of *Acanthamoeba* antimicrobial efficacy vs. other global market products. Quantifications are represented as mean ± standard error percent reduction vs. inoculum 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. $^{aa}P < .05$ vs. renu Advanced Formula, $^{a}P < .005$ vs. renu Advanced Formula, $^{b}P < .005$ vs. Biotrue, $^{c}P < .005$ vs. Acuvue RevitaLens, and $^{d}P < .005$ vs. Lite.



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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 2×10^5 and 3.8×10^5 log cells/mL. Each multipurpose solution was tested against ATCC 30461 (Fig. 1A), ATCC 30868 (Fig. 1B), ATCC 50370 (Fig. 1C), 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 ($P \leq .001$). 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.[15] 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.[1,2] 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.[3–6] 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[19–24] resulting from multiple replicates, using both standard and clinical strains of *Acanthamoeba,*[25–27] and a wide range of multipurpose solutions on the global market. Notably, this study focuses on the

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.

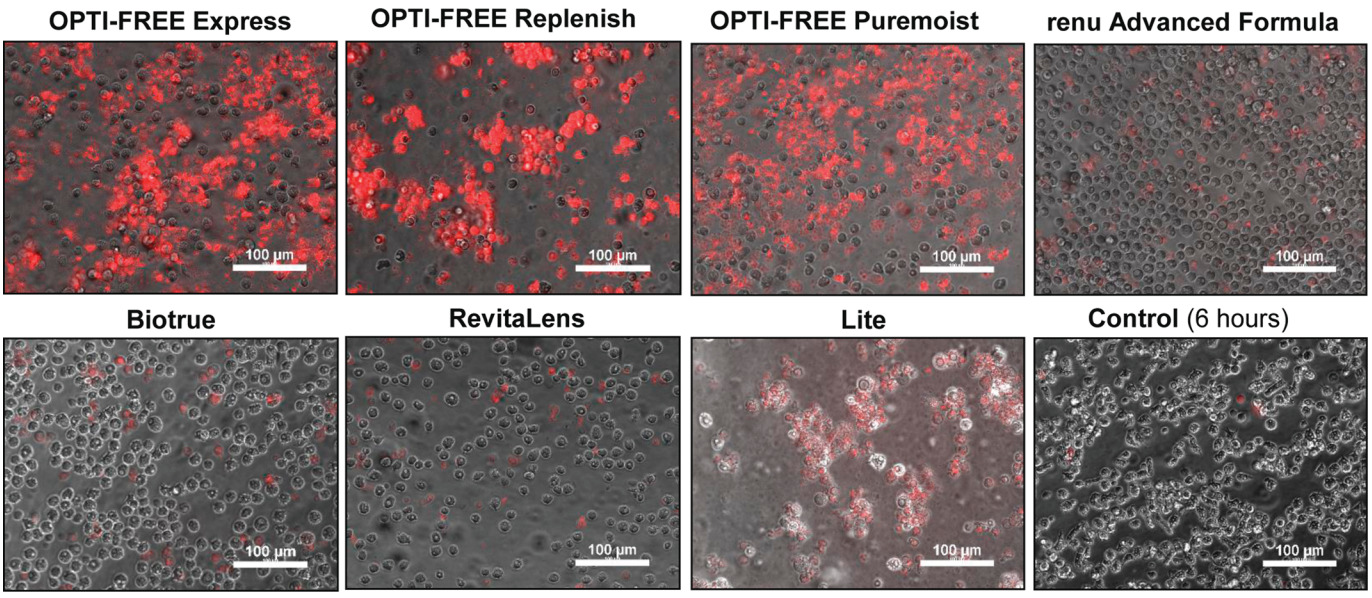

FIGURE 2. Representative images of ATCC 30461 *Acanthamoeba* cell death after exposure to multipurpose solutions. Propidium iodide staining results in red stain indicating cell death or lysis, whereas gray color indicates living cells. Scale bar, 100 µm; ×20 magnification.

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

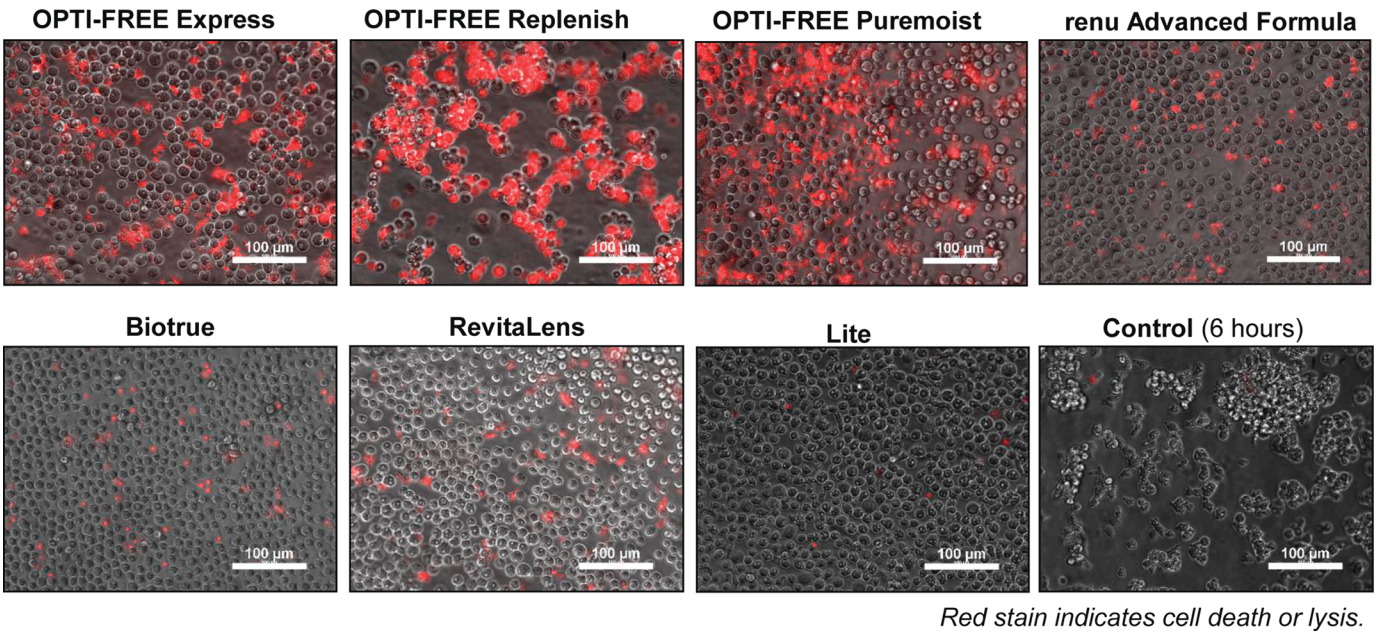

FIGURE 3. Representative images of ATCC 50370 *Acanthamoeba* cell death after exposure to multipurpose solutions. Propidium iodide staining results in red stain indicating cell death or lysis, whereas gray color indicates living cells. Scale bar, 100 µm; ×20 magnification.

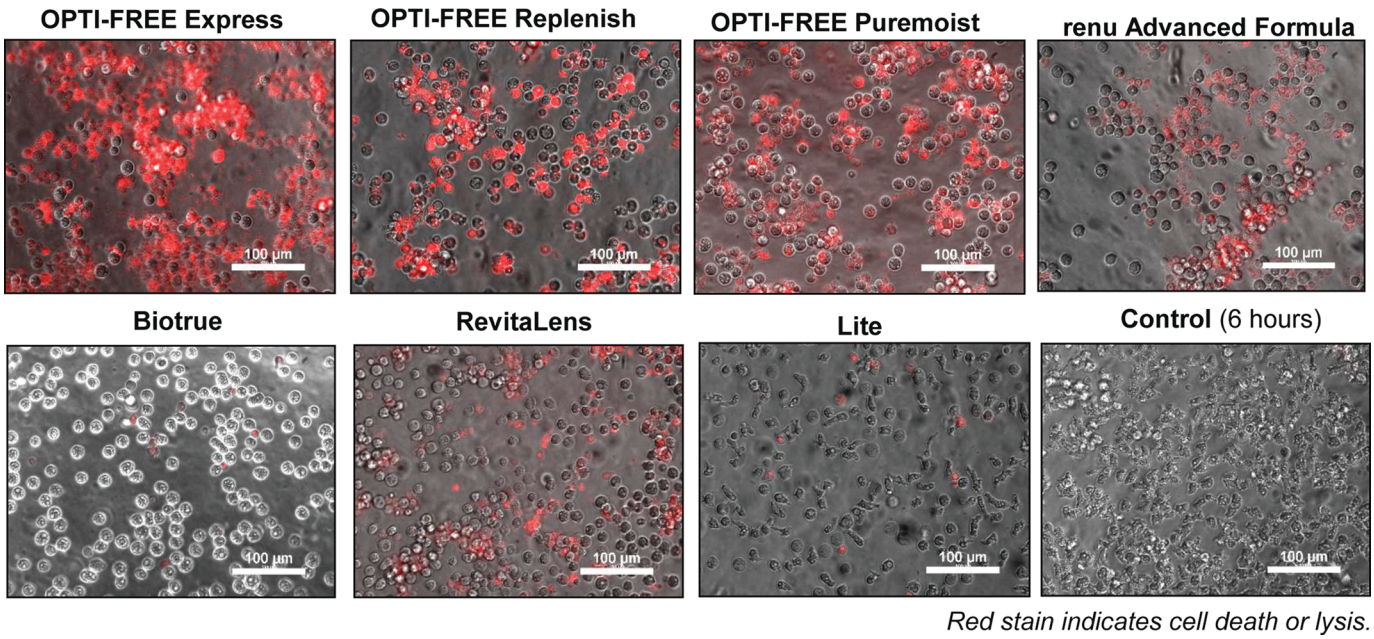

FIGURE 4. Representative images of ATCC 50676 *Acanthamoeba* cell death after exposure to multipurpose solutions. Propidium iodide staining results in red stain indicating cell death or lysis, whereas gray color indicates living cells. Scale bar, 100 µm; ×20 magnification.

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 renu Advanced Formula, 0.0001% 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.[15,37,38] 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.[11,39,40] 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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REFERENCES

1. Szentmary N, Daas L, Shi L, et al. *Acanthamoeba* Keratitis—Clinical Signs, Differential Diagnosis and Treatment. J Curr Ophthalmol 2019;31:16–23.

2. Scruggs BA, Quist TS, Salinas JL, et al. Notes from the Field: *Acanthamoeba* Keratitis Cases—Iowa, 2002–2017. MMWR Morb Mortal Wkly Rep 2019;68:448–9.

3. Verani JR, Lorick SA, Yoder JS, et al. National Outbreak of *Acanthamoeba* Keratitis Associated with Use of a Contact Lens Solution, United States. Emerg Infect Dis 2009;15:1236–42.

4. Carnt N, Hoffman JJ, Verma S, et al. *Acanthamoeba* Keratitis: Confirmation of the UK Outbreak and a Prospective Case-Control Study Identifying Contributing Risk Factors. Br J Ophthalmol 2018;102:1621–8.

5. Datta A, Willcox MD, Stapleton F. *In Vivo* Efficacy of Silver-impregnated Barrel Contact Lens Storage Cases. Cont Lens Anterior Eye 2021;44:101357.

6. Tu EY, Joslin CE. Recent Outbreaks of Atypical Contact Lens–related Keratitis: What Have We Learned? Am J Ophthalmol 2010;150:602–8.e2.

7. International Organization for Standardization (ISO). Ophthalmic Optics—Contact Lens Care Products—Microbiological Requirements and Test Methods for Products and Regimens for Hygienic Management of Contact Lenses; ISO 14729:2001/A1:2010. Geneva, Switzerland: ISO; 2010.

8. American National Standards Institute (ANSI). Minutes of the Parent Committee Meeting, February 27, 2018, Clearwater Beach, Florida; ASC Z80. Available at: https://www.thevisioncouncil.org/sites/default/files/ASCZ80_ParentCommitteeMinutes_February_27_2018_FINALMar19-2018.pdf. Accessed June 14, 2021.

9. Fatima H, Nakisah MA. Visualization on the Effect of Chlorhexidine Gluconate, a Biocide on *Acanthamoeba* sp. by Electron Microscopy. Malaysian J Microscopy 2013;9:154–9.

10. Khunkitti W, Hann AC, Lloyd D, et al. Biguanide-induced Changes in *Acanthamoeba castellanii*: An Electron Microscopic Study. J Appl Microbiol 1998;84:53–62.

11. Mogoa E, Bodet C, Morel F, et al. Cellular Response of the Amoeba *Acanthamoeba castellanii* to Chlorine, Chlorine Dioxide, and Monochloramine Treatments. Appl Environ Microbiol 2011;77:4974–80.

12. Borazjani RN, Kilvington S. Efficacy of Multipurpose Solutions against *Acanthamoeba* species. Cont Lens Anterior Eye 2005;28:169–75.

13. Shoff ME, Joslin CE, Tu EY, et al. Efficacy of Contact Lens Systems against Recent Clinical and Tap Water *Acanthamoeba* Isolates. Cornea 2008;27:713–9.

14. Crary MJ, Walters R, Shannon P, et al. Variables Affecting the Recovery of *Acanthamoeba* Trophozoites. Pathogens 2021;10:221.

15. Campolo A, Shannon P, Crary M. Evaluating Alternate Methods of Determining the Antimicrobial Efficacy of Contact Lens Care Products against *Acanthamoeba* Trophozoites. Pathogens 2021;10:126.

16. Arnalich-Montiel F, Lumbreras-Fernández B, Martín-Navarro CM, et al. Influence of Acanthamoeba Genotype on Clinical Course and Outcomes for Patients with *Acanthamoeba* Keratitis in Spain. J Clin Microbiol 2014;52:1213–6.

17. Healthcare Infection Control Practices Advisory Committee (HICPAC), Rutala WA, Weber DJ, Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. US Department of Health and Human Services, Centers of Disease Control and Prevention (CDC). Available at: https://www.in.gov/health/files/Tab_1_Resource_CD.pdf. Accessed June 14, 2021.

18. Reed LJ, Muench H. A Simple Method of Estimating Fifty per Cent Endpoints. Am J Epidemiol 1938;27:493–7.

19. Niszl IA, Markus MB. Anti-*Acanthamoeba* Activity of Contact Lens Solutions. Br J Ophthalmol 1998;82:1033–8.

20. Hughes R, Kilvington S. Comparison of Hydrogen Peroxide Contact Lens Disinfection Systems and Solutions against *Acanthamoeba polyphaga*. Antimicrob Agents Chemother 2001;45:2038–43.

21. Hiti K, Walochnik J, Haller-Schober EM, et al. Viability of *Acanthamoeba* After Exposure to a Multipurpose Disinfecting Contact Lens Solution and Two Hydrogen Peroxide Systems. Br J Ophthalmol 2002;86:144–6.

22. Kilvington S, Lam A. Development of Standardized Methods for Assessing Biocidal Efficacy of Contact Lens Care Solutions against *Acanthamoeba* Trophozoites and Cysts. Invest Ophthalmol Vis Sci 2013;54:4527–37.

23. Johnston SP, Sriram R, Qvarnstrom Y, et al. Resistance of *Acanthamoeba* Cysts to Disinfection in Multiple Contact Lens Solutions. J Clin Microbiol 2009;47:2040–5.

24. Kolar SS, Manarang JC, Burns AR, et al. Contact Lens Care Solution Killing Efficacy against *Acanthamoeba castellanii* by *in Vitro* Testing and Live-imaging. Cont Lens Anterior Eye 2015;38:442–50.

25. Thomson S, Rice CA, Zhang T, et al. Characterisation of Sterol Biosynthesis and Validation of 14α-Demethylase as a Drug Target in *Acanthamoeba*. Sci Rep 2017;7:8247.

26. Dobrowsky PH, Khan S, Khan W. Resistance of *Legionella* and *Acanthamoeba mauritaniensis* to Heat Treatment as Determined by Relative and Quantitative Polymerase Chain Reactions. Environ Res 2017;158:82–93.

27. Alves Dde S, Moraes AS, Alves LM, et al. Experimental Infection of T4 *Acanthamoeba* Genotype Determines the Pathogenic Potential. Parasitol Res 2016;115:3435–40.

28. Rayamajhee B, Willcox MD, Henriquez FL, et al. *Acanthamoeba* Keratitis: An Increasingly Common Infectious Disease of the Cornea. Lancet Microbe 2021;2:E345–6.

29. Borazjani RN, Kilvington S. Effect of a Multipurpose Contact Lens Solution on the Survival and Binding of *Acanthamoeba* Species on Contact Lenses Examined with a No-rub Regimen. Eye Contact Lens 2005;31:39–45.

30. Lakhundi S, Khan NA, Siddiqui R. Inefficacy of Marketed Contact Lens Disinfection Solutions against Keratitis-causing *Acanthamoeba castellanii* Belonging to the T4 Genotype. Exp Parasitol 2014;141:122–8.

31. Kal A, Toker MI, Kaya S. The Comparison of Antimicrobial Effectiveness of Contact Lens Solutions. Int Ophthalmol 2017;37:1103–14.

32. Lonnen J, Heaselgrave W, Nomachi M, et al. Disinfection Efficacy and Encystment Rate of Soft Contact Lens Multipurpose Solutions against *Acanthamoeba*. Eye Contact Lens 2010;36:26–32.

33. Padzik M, Chomicz L, Szaflik JP, et al. *In Vitro* Effects of Selected Contact Lens Care Solutions on *Acanthamoeba castellanii* Strains in Poland. Exp Parasitol 2014;145(Suppl):S98–101.

34. Codling CE, Maillard JY, Russell AD. Aspects of the Antimicrobial Mechanisms of Action of a Polyquaternium and an Amidoamine. J Antimicrob Chemother 2003;51:1153–8.

35. Moon EK, Lee S, Quan FS, et al. Effect of 2, 6-Dichlorobenzonitrile on Amoebicidal Activity of Multipurpose Contact Lens Disinfecting Solutions. Korean J Parasitol 2018;56:491–4.

36. Gabriel MM, McAnally C, Bartell J. Antimicrobial Efficacy of Multipurpose Disinfecting Solutions in the Presence of Contact Lenses and Lens Cases. Eye Contact Lens 2018;44:125–31.

37. Hillmann F, Novohradská S, Mattern DJ, et al. Virulence Determinants of the Human Pathogenic Fungus *Aspergillus fumigatus* Protect against Soil Amoeba Predation. Environ Microbiol 2015;17:2858–69.

38. Radosa S, Ferling I, Sprague JL, et al. The Different Morphologies of Yeast and Filamentous Fungi Trigger Distinct Killing and Feeding Mechanisms in a Fungivorous Amoeba. Environ Microbiol 2019;21:1809–20.

39. Khunkitti W, Lloyd D, Furr JR, et al. *Acanthamoeba castellanii*: Growth, Encystment, Excystment and Biocide Susceptibility. J Infect 1998;36:43–8.

40. Mogoa E, Bodet C, Legube B, et al. *Acanthamoeba castellanii*: Cellular Changes Induced by Chlorination. Exp Parasitol 2010;126:97–102.