

**Effectiveness of
Multipurpose Contact
Lens Solutions
Against *Acanthamoeba*
Trophozoites**

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Key Highlights

- *Acanthamoeba* keratitis (AK) can cause devastating damage to the eye and permanent vision loss
- Most cases of AK occur in contact lens wearers and exposure of lenses to water has been identified as a key risk factor
- OPTI-FREE® PureMoist® showed superior antimicrobial activity against two common strains of *Acanthamoeba* trophozoites (ATCC 30461 and ATCC 50370) compared to Biotrue, Acuvue RevitaLens and renu Advanced Formula based on traditional log reduction kill and an alternative method using Propidium Iodide staining to show cell death

Acanthamoeba

Acanthamoeba is a free-living amoeba (single-celled microorganism), that can be found in the environment such as in air, water and soil. It can cause rare, but severe infections of the eye, skin, and central nervous system, although most *Acanthamoeba* infections affect the cornea.^{1,2}

Acanthamoeba exist in two forms – trophozoite and cyst – and can change back and forth over time depending on the environmental conditions. Trophozoites are the active mobile form that have the potential to invade tissue and cause infection (Figure 1A). The cyst form is dormant, encased in a double layer cell wall and can withstand extreme environmental conditions (Figure 1B). While the cyst form itself does not pose an immediate threat, it can convert back to a trophozoite at any given time, potentially causing harm.²

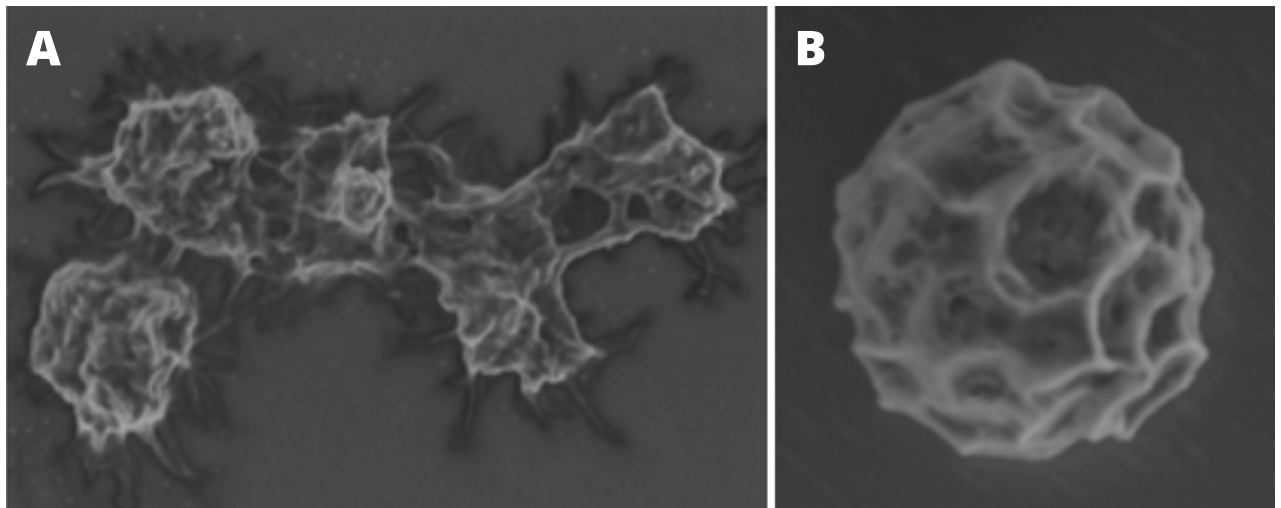


Figure 1 A & B. A) *Acanthamoeba* trophozoite showing extended pseudopodia (feet). B) *Acanthamoeba* cysts in its characteristic round/encased form. (images from Alcon Research, Ltd)

Acanthamoeba Keratitis

Acanthamoeba keratitis (AK) is one of the most severe types of corneal infections and can cause devastating damage to the eye and permanent vision loss, possibly requiring a corneal transplant in an attempt to restore vision.¹ *Acanthamoeba* release proteases that can quickly degrade corneal surface cells and penetrate deeply into corneal stromal tissue causing extreme inflammation making it hard to manage. Therefore, early diagnosis and early treatment are critical in order to minimize the severity of the infection and to provide the best possible outcome. However, even mild forms can take up to 6 months to resolve, while those that are more severe can take years.^{3,4}

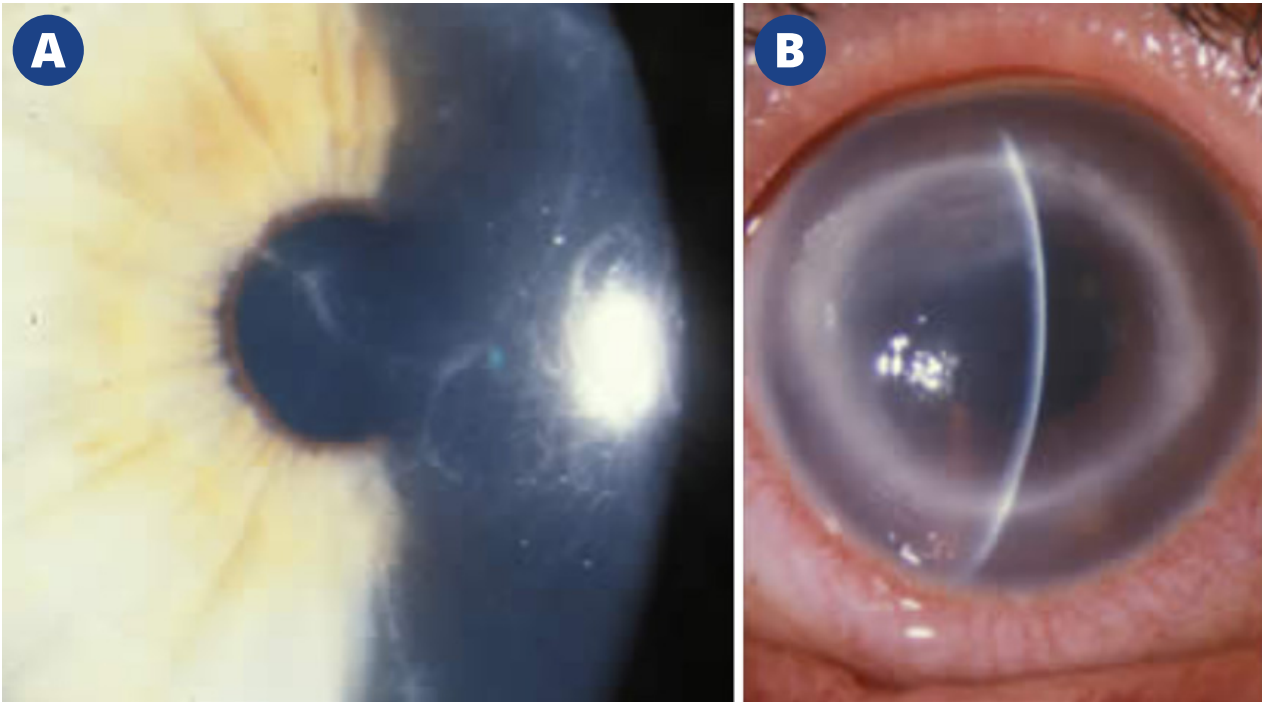


Figure 2 A & B. A) Early stage *Acanthamoeba* keratitis showing pseudodendritic presentation. B) Late stage *Acanthamoeba* keratitis showing ring infiltrate and inflammation. (images from CDC.gov website)⁵

AK is predominantly seen in contact lens wearers (up to 88% of AK cases) with an annual incidence of 1 to 5 per million contact lens users.^{2,3,6} The *Acanthamoeba* spp. typically associated with AK is of the T4 genotype as it is able to bind more strongly to corneal cells than other genotypes and, in turn, has more potential to cause greater damage.²

An identified primary risk factor associated with AK is exposure to water.⁷ Water exposure can occur while showering with lenses on, swimming while wearing lenses, rinsing the lens case with tap water, not drying tap water off of hands prior to handling contact lenses, rinsing lenses in tap water and storing lenses in tap water.^{7,8} Table 1 summarizes findings from a recent (2017) survey study looking at how often water exposure behaviors occurred in soft contact lens wearers.⁷ Unfortunately, most of these water exposure behaviors were perceived by many wearers as having little or no risk indicating that patients need more education regarding risks of water exposure with contact lens wear.⁷ In light of this data, the Center of Disease Control and Prevention and American Academy of Optometry initiated a ‘no water’ campaign to help educate contact lens wearers about the importance of avoiding water during lens wear and lens handling (Figure 3 & 4).^{9,10} Approximately 80-90% of *Acanthamoeba* infections could be prevented by avoiding water exposure, using proper contact lens care techniques and using disinfection systems effective against *Acanthamoeba*.^{6,11}

Lens Exposure	Frequency (%) Reported
Showering	86%
Swimming	62%
Rinsing Lenses	31%
Rinsing Lens Case	37%
Storing Lenses	15%

Table 1: Frequency of Tap Water Exposure Behaviors for Soft Contact Lens Users.⁷



Figure 3. 'No water' campaign produced by the Center of Disease Control and Prevention.

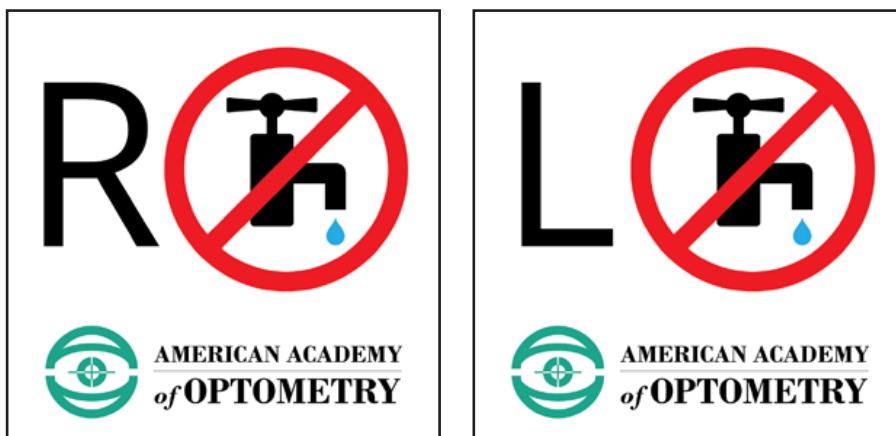


Figure 4. 'No water' stickers produced by the American Academy of Optometry for use on contact lens packaging by manufacturers and ECPs.

International Organization for Standardization (ISO) Testing Requirements for Multipurpose Disinfecting Solutions for Contact Lenses

While manufacturers are required to test contact lens disinfection systems for efficacy against specific microorganisms using defined standardized in vitro testing methods (ISO 14729 stand-alone),¹² they are not required to test against *Acanthamoeba*. The ISO 14729 stand-alone method specifies certain strains of bacteria, fungi and yeast that must be tested against in order to deem the solution efficacious, and it defines the minimum criteria for the amount of log kill required for each microorganism.¹² Although not required, most manufacturers do test for effectiveness of their contact lens solutions against *Acanthamoeba*. However, there is no standardized testing method that has been established, and thus no defined criteria for determining efficacy against *Acanthamoeba*.¹² Given that the ISO 14729 is the only accepted method for testing antimicrobial efficacy, this same method is typically applied for *Acanthamoeba*. However, since this method is not specific to *Acanthamoeba* and there is no defined criteria established for minimum log reduction of *Acanthamoeba*, the test is generally referred to as a “modified ISO 14729 stand-alone testing method”. Other methods of testing for efficacy of contact lens solutions against *Acanthamoeba* are often used in combination with the ISO method.

Further, efficacy against the trophozoite form of *Acanthamoeba* is most important given that it is the active/infectious form. If or when a dormant cyst converts back to the active trophozoite, it is important to have a solution that is efficacious against that trophozoite form during overnight disinfection. Since *Acanthamoeba* species of the T4 genotype are most commonly associated with AK,² these are the strains that manufacturers typically test against. It is important to remember that contact lens disinfecting solutions do not sterilize (eliminate) microbes in the case or on the contact lens. Their purpose is to decrease the microbial load and a solution that more effectively does this can be beneficial by potentially reducing the chance of infection.

Multipurpose Solutions (MPS) for Contact Lenses

MPS on the market use different percentages and combinations of biocides to reduce microbial growth during storage in a contact lens case. Table 2 summarizes the biocide characteristics and recommended soak times for disinfection of the commonly marketed brands of MPS products.

MPS Product	Biocide Composition	Disinfection Time
OPTI-FREE® PureMoist® (Alcon Research LLC)	<ul style="list-style-type: none">• Polyquaternium -1 (0.001%)• Myristamidopropyl dimethylamine (0.0006%)	6 hours
Acuvue RevitaLens (Johnson & Johnson)	<ul style="list-style-type: none">• Polyquaternium -1 (0.0003%)• Alexidine dihydrochloride (0.00016%)	6 hours
Biotrue (Bausch + Lomb)	<ul style="list-style-type: none">• Polyquaternium (0.0001%)• Polyaminopropyl biguanide (0.00013%)	4 hours
renu Advanced Formula (Bausch + Lomb)	<ul style="list-style-type: none">• Polyquaternium (0.00015%)• Alexidine dihydrochloride (0.0002%)• Polyaminopropyl biguanide (0.00005%)	4 hours

Table 2. Biocide Composition and Manufacturer Recommended Disinfection Time of MPS Products.

Modified ISO 14729 Stand-Alone Method for Quantifying MPS Effectiveness against *Acanthamoeba* trophozoites

Antimicrobial efficacy testing of MPS products against *Acanthamoeba* trophozoites was conducted per an accepted modified version of ISO standard 14729 stand-alone test. This method shows the amount of log reduction (kill) of cells based on a known amount of initial microbial load compared to the amount after exposure to MPS.¹²

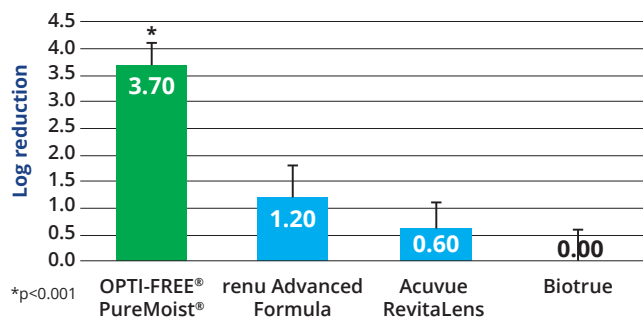
Methods

Acanthamoeba trophozoite strains ATCC 30461 and ATCC 50370 (of the T4 genotype) were tested using an initial concentration of 5.0×10^5 cells/mL (inoculum) in each of the MPS products. Each inoculated MPS was held at room temperature for the manufacturers recommended disinfection time (Table 2). Antimicrobial efficacy was determined by calculating the log reduction between the initial inoculum controls and the cell concentrations recovered from the MPS after disinfection time. Each *Acanthamoeba* strain was tested in two independent trials on different days and the results were averaged. All MPS products were tested simultaneously using the same inoculum stock for direct comparisons. All statistical analyses were conducted using a Student's two tailed t-test assuming equal variance.

Results

OPTI-FREE® PureMoist® showed superior antimicrobial activity against both common strains of *Acanthamoeba* ATCC 30461 (Figure 5) and ATCC 50370 (Figure 6) trophozoites compared to Biotrue, Acuvue RevitaLens and renu Advanced Formula as shown by the log reduction ($p < 0.001$). OPTI-FREE® PureMoist® showed a 3.7 log reduction in *Acanthamoeba* ATCC 30461 trophozoites, whereas Biotrue, Acuvue RevitaLens and renu Advanced Formula showed 0.0, 0.6 and 1.2 log reduction respectively (Figure 5A). For *Acanthamoeba* ATCC 50370 trophozoite activity, OPTI-FREE® PureMoist® showed a 2.2 log reduction as compared to Biotrue, Acuvue RevitaLens and renu Advanced Formula which showed 0.4, 0.6 and 0.9 log reduction respectively (Figure 5B).¹³

A MPS Effectiveness Against *Acanthamoeba* ATCC 30461 Trophozoites



B MPS Effectiveness Against *Acanthamoeba* ATCC 50370 Trophozoites

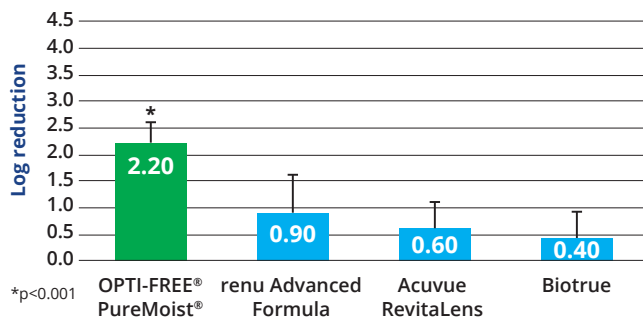


Figure 5 A,B. Evaluation of Antimicrobial activity of MPS products against *Acanthamoeba* ATCC 30461 (A) and ATCC 50370 (B) trophozoites. OPTI-FREE® PureMoist® showed statistically significant antimicrobial activity against *Acanthamoeba* versus comparators.

Propidium Iodide method for Quantifying and Visualizing MPS Effectiveness against *Acanthamoeba* trophozoites

Alcon developed a new testing method using Propidium Iodide (PI), as another method to further quantify the effectiveness of MPS products against *Acanthamoeba* trophozoites. This method not only allows for an alternative method for verifying the amount of *Acanthamoeba* trophozoites killed with MPS products, it also allows for easy visualization of its effectiveness among the products. PI is a fluorescent stain that can only enter a cell whose membrane is compromised, which allows it to identify dead cells by binding to DNA. This fluorescent cellular marking method helps to differentiate MPS product efficacy based on cell death staining. Method development was conducted in order to determine the appropriate initial concentration of *Acanthamoeba* trophozoites needed to differentiate products and obtain an acceptable fluorescent signal. It was also observed that the permeability of cells is impacted by remaining in the MPS during staining, thus the MPS was removed prior to staining in order to eliminate this confounding variable. The PI method showed similar antimicrobial efficacy differences between the MPS products as the modified ISO 14729 testing.

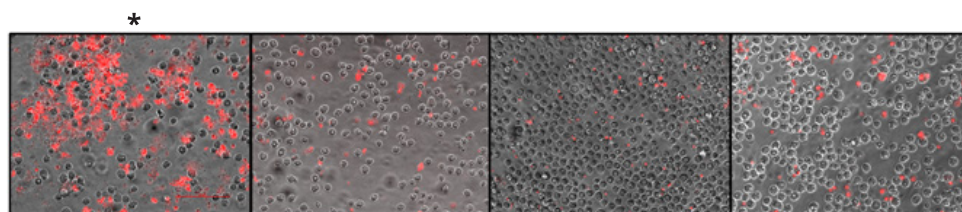
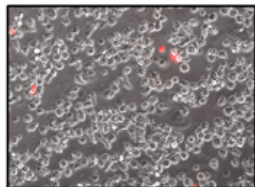
Methods

In a black, clear bottom 96-well plate, *Acanthamoeba* trophozoite strains ATCC 30461 and ATCC 50370 were seeded using an initial concentration of 10^5 cells/well. MPS products were added to the 96 well plate and the plates incubated until disinfection time based on the manufacturers recommended soak time (Table 2). The MPS products were removed, leaving the cells in place, and a PI solution was added. Fluorescence intensity (544nm/620nm) was measured immediately following the addition of PI using a BioTek microplate reader and then cells imaged on a confocal microscope. All statistical analyses were conducted using a Student's two tailed t-test assuming equal variance.

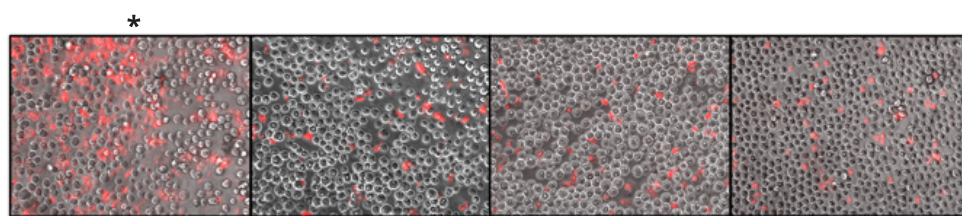
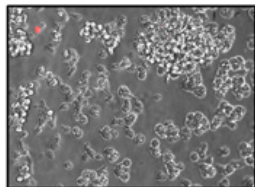
Results

Acanthamoeba ATCC 30461 trophozoites showed significantly higher fluorescent intensity (FIU) of PI staining for OPTI-FREE® PureMoist® (85,854) as compared to Acuvue RevitaLens (36,362), renu Advanced Formula (18,785) and Biotrue (17,873) ($p < 0.001$) (Figures 6A). *Acanthamoeba* ATCC 50370 trophozoites also showed significantly higher fluorescent intensity (FIU) of PI staining for OPTI-FREE® PureMoist® (48,547) as compared to Acuvue RevitaLens (34,884), renu Advanced Formula (35,345) and Biotrue (11,101) ($p < 0.001$) (Figures 6B).¹⁴ Additionally, the fluorescent intensity (cell death) pattern among MPS products closely matched that of the log reduction seen for each MPS in the traditional modified ISO testing method. This further substantiates the different effectiveness of each of the MPS products tested against *Acanthamoeba*.

A. ATCC 30461



B. ATCC 50370



Control - No MPS

OPTI-FREE®
PureMoist®

Acuvue
RevitaLens

Renu Advanced
Formula

Biotrue

Figure 6 A & B. Visualization of *Acanthamoeba* ATCC 30461 (A) and ATCC 50370 (B) trophozoite cell death with Propidium Iodide (PI). PI stains dead *Acanthamoeba* trophozoites cells red. *Acanthamoeba* ATCC 30461 [A] and ATCC 50370 [B] trophozoites showed significantly more PI (cell death) staining for OPTI-FREE® PureMoist® (*) versus the comparators ($p < 0.001$). Images were taken using confocal microscopy at 20X magnification.

Summary

Acanthamoeba can cause a severe corneal infection known as AK and can result in permanent vision loss. Most cases of AK occur in contact lens wearers, and exposure of lenses to water has been identified as a key risk factor. Continual patient education about behavioral habits that put them at risk is required. It is also imperative to recommend contact lens care solutions that have strong efficacy against *Acanthamoeba* in order to help reduce the chance of developing this devastating corneal infection.

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