Efficacy of Contact Lens Systems Against Recent Clinical and Tap Water *Acanthamoeba* Isolates

Megan E. Shoff, MS, Charlotte E. Joslin, OD,†‡ Elmer Y. Tu, MD,†*
Laura Kubatko, PhD,§ and Paul A. Fuerst, PhD**

Purpose: A recent increase in *Acanthamoeba* keratitis (AK) cases has been associated with Complete MoisturePlus, although many cases used other solutions. Complete MoisturePlus contains taurine and hydroxypropyl methylcellulose, unlike other multipurpose solutions (MPSs). The purpose of this study is to (1) determine contact lens solution efficacy against recent clinical and tap water *Acanthamoeaba* isolates and (2) determine whether taurine inclusion increases *Acanthamoeba* survival against contact lens solutions.

Methods: *Acanthamoeba* T4 trophozoites from recent AK clinical and tap water isolates were placed on multiple concentrations of taurine–saline agar for 72 hours with *Enterobacter aerogenes* as prey. Amoebae were exposed for 6 and 24 hours to hydrogen peroxide solutions and MPSs (ReNu Multiplus, Complete MoisturePlus, AMO Trade Name, Opti-free Express, Clear Care, and UltraCare) and tested for survival. Plates were examined over the following week for growth.

Results: Strain type and solution affected survival. MPSs were ineffective, with 100% survival of all strains at 6-hour exposure. Hydrogen peroxide systems were more effective, with survival of 3/5 strains (Clear Care) and 1/5 strains (UltraCare) at 6 hours. The Chicago-area tap water strain was most resistant. Among hydrogen peroxide systems, no statistically significant difference in *Acanthamoeba* survival existed with taurine inclusion.

Conclusions: Recent clinical and tap water *Acanthamoeba* strains, representing proven human pathogens and/or household strains, were highly virulent against contact lens solutions. The Chicago-area tap water strain was most resilient, a concern if tap water is contributing to the AK increase. Results further differentiated resistance among T4 strains, highlighting the importance of multiple strain testing.

Key Words: *Acanthamoeba* keratitis, contact lens solution, taurine, multipurpose lens solution, hydrogen peroxide

(*Cornea* 2008;27:713–719)

Received for publication August 23, 2007; revision received January 3, 2008; accepted January 5, 2008.

From the *Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, Columbus, OH; and the †Department of Ophthalmology/Visual Science, ‡School of Public Health, Division of Epidemiology and Biostatistics, and §Department of Statistics, University of Illinois, Chicago, IL.

Supported by NIH Grant EY 09073.

Reprints: Megan E. Shoff, Department of Evolution, Ecology, and Organismal Biology, The Ohio State University, 300 Aronoff Laboratory, 318 W. 12th Avenue, Columbus, OH 43210 (e-mail: shoff.4@osu.edu).

Acanthamoebae are ubiquitous protozoa most commonly found in freshwater and soil environments. Their ability to encyst during periods of adverse environmental conditions allows them to inhabit a variety of habitats. However, even as trophozoites, these amoebae can tolerate high temperatures, with strains tolerating temperatures up to 39°C and several strains growing at up to 42°C.[1,2] Many strains can grow in salinities up to 32 ppt and are chlorine and biocide resistant,[1–3] which explains their presence in swimming pools, hot tubs, and tap water.[4] Because of the ubiquity of acanthamoebae and the fact that they can be cultured axenically, they have been the subject of much research, with most studies dealing with their opportunistic pathogenicity. However, despite intense research, the factors that make this amoeba an occasional human pathogen are unclear.

Acanthamoeba keratitis (AK) is a serious and rare infection of the cornea that is usually sight threatening. Because AK is rare, the epidemiology is poorly understood. Contact lens wear is generally accepted as the leading risk factor, although exactly how wear increases the risk of infection is unclear. The US annualized incidence has been conservatively estimated to range from 1.65 to 2.01 cases per million contact lens wearers[5]; however, it may be as much as 15 times more common in the United Kingdom, Europe, and Hong Kong.[6–8]

A statistically significant increase in AK cases is occurring in the Chicago area that began in June 2003,[9] with a total of 63 incident cases identified through the end of 2006. Increases in AK have also been observed in Philadelphia,[10,11] Portland,[12] San Francisco,[13] and Boston.[10] Because of the serious nature of AK, the Centers for Disease Control and Prevention (CDC) launched a national outbreak investigation with cases reported in 35 states and Puerto Rico to determine the risk factors associated with AK.[14,15]

Recent results from 2 independent epidemiologic studies by the University of Illinois at Chicago[16] and the CDC[14] found that ~50%–55% of AK cases used Advance Medical Optics Complete MoisturePlus Multi-Purpose Solution (AMO, Santa Ana, CA), resulting in a >15-fold increase in the risk of AK with Complete MoisturePlus use and its voluntary recall by AMO.[17] Unlike other multipurpose solutions (MPSs), Complete MoisturePlus contains the amino acid taurine as an active ingredient, as well as the chemical hydroxypropyl methylcellulose (HPMC), which is used as a lubricant. Taurine, in conjunction with sodium chloride and magnesium chloride, has been shown to induce encystment in *Acanthamoeba*.[18–22] It has been previously hypothesized that the taurine inclusion in

Complete MoisturePlus may provide *Acanthamoeba* a protective benefit, possibly by inducing it to form more resilient cysts, making the amoebae more resistant to the amoebicidal properties of the cleaning solution.[23] In addition, because recent clinical isolates are infrequently used in *Acanthamoeba* disinfectant efficacy testing and the methods used vary greatly among studies, testing conditions may decrease amoeba viability and may overstate apparent solution efficacy. The purpose of this study is (1) to determine the efficacy of commonly available contact lens solutions against recent clinical and tap water *Acanthamoeaba* isolates and (2) to determine whether taurine inclusion increases *Acanthamoeba* survival and resistance against commonly used contact lens disinfectants.

MATERIALS AND METHODS

Selection of Isolates

Isolates used in this study were from various sources, including corneal scrapings from AK patients (courtesy of E.Y.T.), environmental isolates from the Chicago-area water supply (courtesy of C.E.J.), and environmental isolates from the Columbus-area water supply (courtesy of M.E.S.) (Table 1). None of the isolates were axenically grown. Genotyping was performed by using the *Rns* diagnostic fragment 3.[24] All chosen isolates were of the T4 genotype, which is the most common genotype isolated in AK.[25] Corneal isolates were selected according to the patient's clinical presentation, including an early, moderate, and advanced stage of disease. Environmental isolates were selected because they represent potential causative agents of disease in an extremely common and universal exposure, the domestic water supply.

Selection of MPSs

Three MPSs were chosen on the basis of manufacturer dominance in the US market share, including Opti-free Express (Alcon, Ft. Worth, TX), ReNu MultiPlus Multi-Purpose Solution (ReNu; Bausch & Lomb, Rochester, NY), and Complete MoisturePlus Multi-Purpose Solution (Advanced Medical Optics [AMO], Santa Ana, CA).[26] The AMO Trade Name (generic) MPS was also chosen because its formulation contains neither taurine nor HPMC but otherwise uses the same disinfectant as Complete MoisturePlus (polyhexamthylene biguanide, 0.0001%). Hydrogen peroxide systems chosen included 2 one-step peroxide systems commonly available in the United States: Clear Care (CibaVision, Duluth, GA) and UltraCare Disinfecting (AMO). Two-step hydrogen peroxide systems (those that provide a significant exposure time to hydrogen peroxide before peroxide neutralization) are no longer available in the United States or internationally. Hydrogen peroxide systems seem to be more effective against *Acanthamoeba* than the commonly used MPSs.[3,27–29] UltraCare uses a neutralizing tablet that is added at the beginning of treatment and is therefore not a true 2-step system. Contact lens solution formulations and the manufacturer's recommended disinfection time as listed on the bottle labels of tested solutions are shown in Table 2.

Efficacy Tests

Trophozoites, as determined by light microscopy, of the 5 test strains (~100 cells) were placed on either 0.25% taurine–saline agar (levels previously associated with causing encystation of *Acanthamoeba*[20]), 0.05% taurine–saline agar (levels found in Complete MoisturePlus[30]), or nonnutrient amoeba saline agar containing no taurine. These were incubated at room temperature for 72 hours with *Enterobacter aerogenes* as prey. After the 72-hour incubation, the amoebae were tested for 6 and 24 hours in each of the 6 solutions as previously described.[3] However, because we were testing the effects of taurine (and its ability to provide a protective benefit to the *Acanthamoeba*), no consideration was given as to whether cysts or trophozoites were chosen. In brief, blocks (2×2 mm) of agar with amoeba (~50 cells/block) were cut out of the plates and transferred to an aliquot of MPS or hydrogen peroxide solution and held in a 24-well untreated tissue culture plate (MPS and UltraCare) or the container provided with solution (Clear Care). Testing containers (glass, plastic, or untreated polysterene) have previously been shown to result in no significant difference in *Acanthamoeba* survival.[31] For each test solution (ReNu, Complete MoisturePlus, Generic, Opti-free, Clear Care, and UltraCare), 3 trials (ie, replicates) and a control were run. Controls consisted of strains being exposed to amoeba saline. Each strain of *Acanthamoeba* was tested for survival after 6 and 24 hours of exposure to the solution at ~21°C (room temperature). The neutralizing tablet for UltraCare was added immediately in the 6-hour trials and at 12 hours in the 24-hour trials to simulate a 2-step hydrogen peroxide system. After treatment, the agar blocks containing the isolates were transferred to Difco Dey/Engley Broth (Becton Dickinson and Co., Sparks, MD). This broth neutralizes the disinfectant and has been shown to have no toxic effect on amoebae.[32] After neutralizing for 5 minutes, the blocks containing amoebae were rinsed twice (at 5 and 10 minutes) with amoeba saline before reinoculating the amoebae onto a nonnutrient amoeba saline agar plate seeded with live *E. aerogenes* to test for survival. The agar plates were sealed with Parafilm and incubated at 21°C. Plates were examined over the following week to check for growth by using a light microscope. Positive growth (observed as trophic amoebae migrating along the *E. aerogenes* prey streak) was indicative of treatment survival.

It has been suggested that the current method of testing by using agar plugs could lead to the inactivation of the test

TABLE 1. Strain IDs, Sources, Genotypes, and Clinical Presentation of Infections

Strain	Source of Isolate	Genotype	Clinical Presentation of Infection
06-004	UIC-AK	T4	Advanced
06-061	UIC-AK	T4	Moderate
06-035	UIC-AK	T4	Early
06-039	Chicago-area water	T4	NA
C06-038	Columbus water	T4	NA

NA, not applicable.

TABLE 2. Contact Lens Cleaning Solution Brand Names, Including Manufacturer, Recommended Disinfection Times, and Ingredients as Stated on Packages

Brand Name	Manufacturer	Manufacturer-Recommended Disinfection Time (h)	Ingredients
ReNu MultiPlus Multi-Purpose Solution	Bausch & Lomb	4	Hydranate (hydroxyalkylphosphonate), boric acid, edetate disodium, poloxamine, sodium borate and sodium chloride; preserved with DYMED (polyaminopropyl biguanide) 0.0001%
Complete MoisturePlus	AMO	4	Hydroxypropyl methylcellulose, propylene glycol, polyhexamethylene biguanide 0.0001%, phosphate, and taurine, Poloxamer 237, edetate disodium, sodium chloride, potassium chloride, and purified water
Opti-Free Express	Alcon	6	Sodium citrate, sodium chloride, boric acid, sorbitol, AMP-95, Tetronic 1304, with edetate disodium 0.05%, Polyquad (polyquaternium-1) 0.001% and Aldox (myristamidopropyl dimethylamine) 0.0005%
ClearCare	CIBAVision	6	Hydrogen peroxide 3%, sodium chloride 0.79%, stabilized with phosphoric acid, a phosphate-buffered system and Pluronic 17R4 (cleaning agent)
Trade name (generic)	AMO	6	Polyhexamethylene biguanide (0.0001%), phosphate buffer, Poloxamer 237, edetate disodium, sodium chloride, potassium chloride, purified water
UltraCare	AMO	6	Solution contains hydrogen peroxide 3% (stabilized with sodium stannate and sodium nitrate, and buffered with phosphates) and purified water. Neutralizing tablets contain catalase, hydroxypropyl methylcellulose, and cyanocobalamin (vitamin B_{12}) with buffering and tableting agents

Tetronic, registered trademark of BASF; AMP-95, registered trademark of Angus Chemical.

solutions (namely, the binding of the agar acidic polysaccharide to a cationic preservative, such as polyhexamethylene biguanide (PHMB), could lead to loss of biocidal efficacy).[33,34] However, in recent tests,[35] 3 of the strains used in this study (Chicago-area tap water, Columbus-area water, and a corneal isolate) were tested by using Renu, Complete MoisturePlus, and Opti-free, both with the addition of an agar plug and without an agar plug. Tests were run at 27°C for 6 hours, with controls in which strains were exposed to amoeba saline. The results in all cases were the same between the 2 trials; the amoebae survived the treatments both with and without the agar plug.[35]

Statistical Analysis

The presence of any viable *Acanthamoeba* for 1 trial was used as an outcome measure because the quantity of amoeba necessary for corneal infection is unknown. The percentage of trials with *Acanthamoeba* survival in reported logistic regression analyses by using presence or absence of growth as the response were used to assess the effect of solution, strain, and taurine concentration on growth. All analyses were carried out by using SYSTAT version 12.00.08.

RESULTS

Nearly all control trials (178 of 180) resulted in positive growth. The percent survival of the 5 strains when exposed to the different cleaning solutions is shown in Table 3. All 4 MPSs were largely ineffective (Fig. 1); Renu, Complete MoisturePlus, and the AMO generic MPS had 100% survival of all strains after 6 and 24 hours, whereas Opti-free had 100% survival for all strains after 6 hours and for 4 of the 5 strains after 24 hours. The 2 hydrogen peroxide systems fared much better; Clear Care had survival of 3 of the 5 strains after 6 hours and 2 of 5 strains after 24 hours. UltraCare was the most effective, with complete survival of only the Chicago-area tap water strain (06-039) at 6 hours and no survival of any strain at 24 hours. There were significant differences between the effectiveness of the MPSs and the hydrogen peroxide cleaning systems ($P < 0.001$). The 5 strains also showed significant differences in their response to the hydrogen peroxide cleaning solutions ($P < 0.001$). Taurine had no significant effect relating to *Acanthamoeba* survival for the solutions or the strains. This lack of effect is particularly evident when comparing strain survival across taurine levels for the hydrogen peroxide solutions (Table 3).

DISCUSSION

Our results showed that use of recent clinical and tap water *Acanthamoeba* isolates in efficacy testing of common MPSs results in 100% survival and total amoebicidal ineffectiveness at a 6-hour disinfection time (Table 3). These findings are important, because the 6-hour period meets or exceeds manufacturer's recommended disinfection times and approximates overnight disinfection (Table 2). Similarly, *Acanthamoeba* survival was equivalent when comparing AMO Complete MoisturePlus and the AMO generic MPS, which contains neither taurine nor HPMC but otherwise uses the same disinfectant (polyhexamthylene biguanide, 0.0001%). Hydrogen peroxide systems were more effective against most *Acanthamoeba* strains, even at 6 hours (Table 3). Because of complete *Acanthamoeba* survival with all MPSs, we were unable to fully evaluate the effect of taurine addition; however, among hydrogen peroxide systems without complete *Acanthamoeba* survival, there was not a statistically significant difference in *Acanthamoeba* survival with or without taurine present. This finding suggests that the addition of taurine,

TABLE 3. Survival Rates of *Acanthamoeba* Strains

			Survival						
			6 h			24 h			
			Taurine Level			Taurine Level			
Brand	Strain	Source	0%	0.05%	0.25%	0%	0.05%	0.25%	Overall Survival
Renu	06-039	IL TW	100%	100%	100%	100%	100%	100%	100% (90/90)
			3/3	3/3	3/3	3/3	3/3	3/3	
	C06-038	OH TW	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-035	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-061	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-004	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
Complete	06-039	IL TW	100%	100%	100%	100%	100%	100%	100% (90/90)
			3/3	3/3	3/3	3/3	3/3	3/3	
	C06-038	OH TW	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-035	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-061	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-004	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
AMO Generic	06-039	IL TW	100%	100%	100%	100%	100%	100%	100% (90/90)
			3/3	3/3	3/3	3/3	3/3	3/3	
	C06-038	OH TW	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-035	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-061	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-004	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
Opti-Free	06-039	IL TW	100%	100%	100%	100%	100%	100%	94.4% (85/90)
			3/3	3/3	3/3	3/3	3/3	3/3	
	C06-038	OH TW	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-035	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
	06-061	AK	100%	100%	100%	67%	0%	67%	
			3/3	3/3	3/3	2/3	0/3	2/3	
	06-004	AK	100%	100%	100%	100%	100%	100%	
			3/3	3/3	3/3	3/3	3/3	3/3	
Clear Care	06-039	IL TW	100%	100%	100%	100%	100%	100%	54.4% (49/90)
			3/3	3/3	3/3	3/3	3/3	3/3	
	C06-038	OH TW	67%	33%	67%	33%	0%	0%	
			2/3	1/3	2/3	1/3	0/3	0/3	
	06-035	AK	100%	100%	100%	100%	100%	33%	
			3/3	3/3	3/3	3/3	3/3	1/3	
	06-061	AK	100%	67%	67%	0%	0%	0%	
			3/3	2/3	2/3	0/3	0/3	0/3	
	06-004	AK	33%	33%	0%	0%	0%	0%	
			1/3	1/3	0/3	0/3	0/3	0/3	

(*continued on next page*)

TABLE 3. (*continued*) Survival Rates of *Acanthamoeba* Strains

			Survival						
			6 h			24 h			
			Taurine Level			Taurine Level			
Brand	**Strain**	**Source**	**0%**	**0.05%**	**0.25%**	**0%**	**0.05%**	**0.25%**	**Overall Survival**
UltraCare	06-039	IL TW	100%	100%	100%	0%	0%	0%	25.5% (23/90)
			3/3	3/3	3/3	0/3	0/3	0/3	
	C06-038	OH TW	33%	0%	33%	0%	0%	0%	
			1/3	0/3	1/3	0/3	0/3	0/3	
	06-035	AK	0%	100%	100%	0%	0%	0%	
			0/3	3/3	3/3	0/3	0/3	0/3	
	06-061	AK	33%	0%	33%	0%	0%	0%	
			1/3	0/3	1/3	0/3	0/3	0/3	
	06-004	AK	100%	0%	33%	0%	0%	0%	
			3/3	0/3	1/3	0/3	0/3	0/3	

Percent survival and number of trials with surviving amoebae are given.

a component unique to Complete MoisturePlus, may not be responsible for the increased AK risk seen in users of this contact lens disinfectant.

The *Acanthamoeba* T4 strains used in this study are isolates selected from clinical disease and isolates present in the domestic water supply, which is a common, universal exposure. Of all tested strains, the Chicago-area tap water strain (06-039) was the strain most resistant to solution disinfection (Table 3). This finding is concerning, because the Chicago-area tap water strain has the same genetic T4 sequence as one of the corneal *Acanthamoeba* isolates collected in the Chicago AK series, suggesting not only that the water supply could be the source of the organism but also that *Acanthamoeba* strains present in the water supply are pathogenic in causing AK in humans (RAWDON U07410 BCM:0288:37).[36] In previous UK studies, the genetic typing of corneal *Acanthamoeba* isolates matched the *Acanthamoeba* isolates cultured from the water supply within the patient's home.[37]

No standard protocols exist when testing the efficacy of contact lens solutions against *Acanthamoeba*, and neither the US Food and Drug Administration nor the International Organization for Standardization require *Acanthamoeba* inclusion as a challenge organism when testing solution efficacy.[32] Therefore, methods used in testing *Acanthamoeba* disinfectant efficacy vary greatly, and results among different studies are frequently contradictory.[21,32] We chose a dichotomous outcome measure from the presence or absence of viable *Acanthamoeba* cysts and trophozoites. Ideally, if *Acanthamoeba* pathogenesis were better understood, particularly if the threshold for allowable organisms under which clinical disease does not occur could be established, quantitative methods would be preferred. Unfortunately, the quantity of amoebae necessary for promoting corneal infection remains undetermined.[38] However, in the presence of an increase of AK both in Chicago and nationally,[14,16] changes in the water supply may be promoting biofilm growth and consequently increasing the *Acanthamoeba* load in the water supply to undetermined and potentially pathogenic levels.[9] It is important, therefore, to consider that the dichotomous outcome measure may be more appropriate if an increase in the overall load of *Acanthamoeba* organisms, overwhelming the marginal antiacanthamoebal properties of current MPSs, is a mechanism of the current outbreak. This factor may be especially relevant because ~40% of AK cases in both the University of Illinois at Chicago and CDC studies were using solutions other than the AMO Complete MoisturePlus product strongly associated with disease.[14,16]

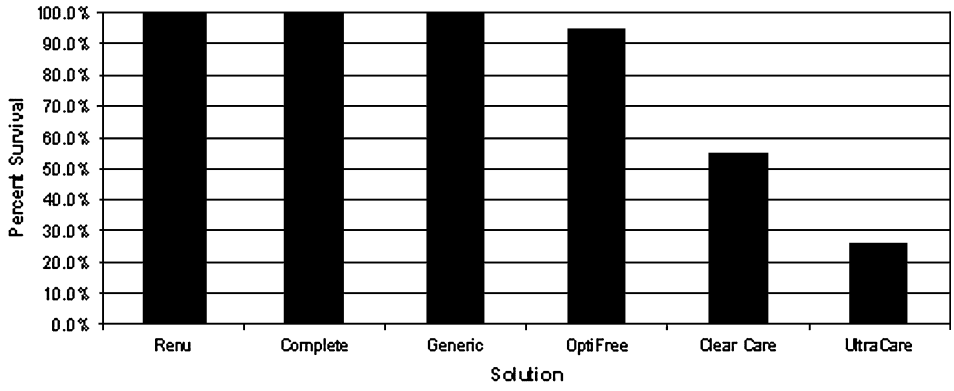

FIGURE 1. Percent survival of trials for each cleaning solution tested. Survival percentages are for all times, strains, and taurine levels combined.

Our results suggesting poor MPS efficacy are generally consistent with those of previous studies that use various methods in the evaluation of disinfectant efficacy[3,28,39–41]; however, we had a high amoeba survival rate, which may be a function of the virulence of the organisms used. Several factors have been identified that affect the viability of *Acanthamoeba* in vitro. Because extensive laboratory cycling may significantly reduce the viability of *Acanthamoeba* isolates,[21] our using recent clinical and tap water isolates may be a factor in our success in cultivating the amoeba and its comparatively higher virulence. Our MPS testing with recent clinical and tap water T4 isolates resulted in even higher *Acanthamoeba* survival than previous MPS testing with primarily environmental T3, T4, and T5 isolates by using our same methods,[42] suggesting that recent clinical and tap water T4 isolates may indeed have increased virulence. In microbiologic methods, however, there is little argument that the use of recent clinical isolates is preferred when available and would probably be the most valid reflection of true environmental virulence and pathogenicity.

Alternative explanations for increased *Acanthamoeba* survival could be that our method of amoebicidal testing uniquely uses attached cells grown on bacteria agar cubes to mimic growing conditions on a biofilm-coated contact lens or lens case surface, as opposed to using trophozoites or cysts suspended in liquid media or test solution. Because cell attachment could protect *Acanthamoeba* from amoebicidal effects of lens solutions, MPS testing by using less resistant suspended trophozoite and cyst techniques may suggest improved solution *Acanthamoeba* efficacy than our methods of real-life simulation. Similarly, many studies use axenic cultures, which decrease organism virulence,[21,43] potentially decreasing *Acanthamoeba* survival with disinfection. In contrast, the wild-type strains from recent corneal and tap water isolates were grown on nonnutrient amoeba saline agar streaked with live *E. aerogenes*, which best simulates the amoeba food source and growth in nonlaboratory settings. Furthermore, our method of amoebicidal testing induces minimal artifactual damage to the *Acanthamoeba* during testing—cells are never dislodged from the agar surface, centrifuged, or pipetted—which is more realistic with the environment of a contact lens or lens case. These various stressors, all of which may decrease amoeba viability, are frequently imposed in other studies and could overstate apparent solution efficacy. Therefore, our results with nearly complete *Acanthamoeba* survival may more accurately reflect actual amoeba survival because we have more closely simulated the natural environment of the *Acanthamoeba*.

Our findings are important for 2 reasons. First, they suggest that MPSs in general, when challenged against *Acanthamoeba* organisms under conditions that closely simulate a natural environment, are ineffective. Second, we found considerable survival differences among T4 strains with different genetic subtypes, with the strain most resistant to disinfectants originating from the Chicago-area tap water, which emphasizes the importance of testing with multiple strains. Results strongly suggest that additional study of amoebicidal properties of contact lens solutions and the virulence of tap water isolates from the water supply is warranted. Furthermore, consideration of these findings should be strongly weighed in clinical settings, including the possibility of daily disposable contact lenses and/or hydrogen peroxide systems use to minimize potential *Acanthamoeba* exposure resulting from inadequate MPS disinfection.

REFERENCES

1. Shoff M. *The Importance of Strain Differences in the Opportunistic Pathogen Acanthamoeba*. Dania Beach, FL: Oceanographic Center, Nova Southeastern University; 2006.
2. Booton GC, Rogerson A, Bonilla TD, et al. Molecular and physiological evaluation of subtropical environmental isolates of *Acanthamoeba* spp., causal agent of *Acanthamoeba* keratitis. *J Eukaryot Microbiol*. 2004;51: 192–200.
3. Shoff M, Rogerson A, Schatz S, et al. Variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. *Optom Vis Sci*. 2007; 84:202–207.
4. Khan NA. Pathogenesis of *Acanthamoeba* infections. *Microb Pathog*. 2003;34:277–285.
5. Schaumberg D, Snow K, Dana M. The epidemic of *Acanthamoeba* keratitis: where do we stand? *Cornea*. 1998;17:3–10.
6. Seal DV, Kirkness CM, Bennett HG, et al. Population-based cohort study of microbial keratitis in Scotland: incidence and features. *Cont Lens Anterior Eye*. 1999;22:49–57.
7. Lam DS, Houang E, Fan DS, et al. Incidence and risk factors for microbial keratitis in Hong Kong: comparison with Europe and North America. *Eye*. 2002;16:608–618.
8. Radford CF, Minassian DC, Dart JK. Acanthamoeba keratitis in England and Wales: incidence, outcome, and risk factors. *Br J Ophthalmol*. 2002; 86:536–542.
9. Joslin CE, Tu EY, McMahon TT, et al. Epidemiological characteristics of a Chicago-area *Acanthamoeba* keratitis outbreak. *Am J Ophthalmol*. 2006;142:212–217.
10. Thebpatiphat NM, Hammersmith KMM, Rocha FNM, et al. *Acanthamoeba* keratitis: a parasite on the rise. *Cornea*. 2007;26:701–706.
11. Hammersmith K. Diagnosis and management of *Acanthamoeba* keratitis. *Curr Opin Ophthalmol*. 2006;17:327–331.
12. Mathers W. Use of higher medication concentrations in the treatment of *Acanthamoeba* keratitis. *Arch Ophthalmol*. 2006;124:923.
13. Sansanayudh W, Cevallos V, Ou J, et al. Trends in the etiology of infectious corneal ulcers at the F.I. Proctor Foundation from 1976 to 2006. *Invest Opthalmol Vis Sci*. 2007;48:ARVO E-Abstract 756.
14. Centers for Disease Control and Prevention. Acanthamoeba keratitis multiple states, 2005-2007. *MMWR Morb Mortal Wkly Rep*. 2007;56:532–534.
15. Deleted in proof.
16. Joslin C, Tu EY, Shoff M, et al. The association of contact lens solution use and *Acanthamoeba* keratitis. *Am J Ophthalmol*. 2007;144:169–180.
17. U.S. Food and Drug Administration. Advanced medical optics voluntarily recalls Complete MoisturePlus contact lens solution. Available at http://www.fda.gov/bbs/topics/NEWS/2007/NEW01641.html. Accessed July 18, 2007.
18. Singh BN. *Pathogenic and Non-Pathogenic Amoeba*. London: Macmillan; 1975.
19. Weisman RA. Differentiation in *Acanthamoeba castellanii. Annu Rev Microbiol*. 1976;30:189–219.
20. Murti CRK, Shukla OP. Differentiation of pathogenic amoebae: encystation and excystation of *Acanthamoeba culbertsoni*—a model. *J Biosci*. 1984;6:475–489.
21. Hughes R, Heaselgrave W, Kilvington S. *Acanthamoeba polyphaga* strain age and method of cyst production influence the observed efficacy of therapeutic agents and contact lens disinfectants. *Antimicrob Agents Chemother*. 2003;47:3080–3084.
22. Hughes R, Andrew P, Kilvington S. Enhanced killing of *Acanthamoeba* cysts with a plain peroxidase-hydrogen peroxide-halide antimicrobial system. *Appl Environ Microbiol*. 2003;69:2563–2567.
23. Moskowitz A. Task force report offers contact lens solution safety warnings. Available at http://www.osnsupersite.com/view.asp?rID=22776. Accessed Dec 2, 2007.
24. Booton GC, Kelly DJ, Chu Y-W, et al. 18S ribosomal DNA typing and tracking of *Acanthamoeba* species isolates from corneal scrape specimens,

contact lenses, lens cases, and home water supplies of *Acanthamoeba* keratitis patients in Hong Kong. *J Clin Microbiol*. 2002;40:1621–1625.
25. Stothard DR, Schroeder-Diedrich JM, Awwad MH, et al. The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. *J Eukaryot Microbiol*. 1998;45:45–54.
26. Westphal SP. Bausch & Lomb solution recall exposes risks for eye infections. *Wall Street J*. July 26, 2006.
27. Hiti K, Walochnik J, Faschinger C, et al. One- and two-step hydrogen peroxide contact lens disinfection solutions against *Acanthamoeba*: how effective are they? *Eye*. 2005;19:1301–1305.
28. Hughes R, Kilvington S. Comparison of hydrogen peroxide contact lens disinfection systems and solutions against *Acanthamoeba polyphaga*. *Antimicrob Agents Chemother*. 2001;45:2038–2043.
29. Beattie T, Seal D, Tomlinson A, et al. Determination of amoebicidal activities of multipurpose contact lens solutions by using a most probable number enumeration technique. *J Clin Microbiol*. 2003;41:2992–3000.
30. Thimons JJ. Examining taurine's role in ocular biochemistry and in CL wear. Available at http://www.clspectrum.com/article.aspx?article=&loc=archive\2004\april\0404038.htm. Accessed May 29, 2007.
31. Deleted in proof.
32. Buck SL, Rosenthal RA, Schlech BA. Methods used to evaluate the effectiveness of contact lens solutions and other compounds against *Acanthamoeba*: a review of the literature. *Contact Lens Assoc Ophthalmol J*. 2000;26:72–84.
33. Kennedy J, Bradshaw I. The assay of acidic polysaccharides in solution with poly(hexamethylenebiguanidinium chloride). *Carbohydr Res*. 1986;156:79–85.
34. Groemminger S, Norton S. Letter to the editor: variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. *Optom Vis Sci*. 2007;84:996.
35. Shoff M, Seal D, Rogerson A. Authors' response: variable responses of *Acanthamoeba* strains to three multipurpose lens cleaning solutions. *Optom Vis Sci*. 2007;84:996–997.
36. Booton GC, Joslin CE, Shoff M, et al. Genotypic identification of *Acanthamoeba* sp. isolates associated with an outbreak of *Acanthamoeba* keratitis (AK). *Invest Opthalmol Vis Sci*. 2007;48:ARVO E-Abstract 3234.
37. Kilvington S, Gray T, Dart J, et al. *Acanthamoeba* keratitis: the role of domestic tap water contamination in the United Kingdom. *Invest Ophthalmol Vis Sci*. 2004;45:165–169.
38. Niederkorn JY, Alizadeh H, Leher H, et al. The pathogenesis of *Acanthamoeba* keratitis. *Microbes Infect*. 1999;1:437–443.
39. Harf C. Efficacy of contact lens disinfecting solutions for prevention of *Acanthamoeba* keratitis. *Clin Infect Dis*. 1991;13(Suppl):S413.
40. Liedel KK, Begley GC. The effectiveness of soft contact lens disinfection systems against *Acanthamoeba* on the lens surface. *J Am Optom Assoc*. 1996;67:135–142.
41. Ludwig I, Meisler D, Rutherford I, et al. Susceptibility of *Acanthamoeba* to soft contact lens disinfection systems. *Invest Ophthalmol Vis Sci*. 1986;27:626–628.
42. Shoff M, Rogerson A, Schatz S, et al. Variable responses of Acanthamoeba strains to three multipurpose lens cleaning solutions. *Optom Vis Sci*. 2007;84:202–207.
43. Mazur T, Hadas E, Iwanicka I. The duration of the cyst stage and the viability and virulence of *Acanthamoeba* isolates. *Trop Med Parasitol*. 1995;46:102–108.

