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Evaluation of the Effectiveness of Two Alkyl Sulfate based Rigid Gas Permeable Contact Lens Cleaners in a Disinfection System when Challenged with Acanthamoeba Trophozoites and Cysts

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Purpose

Acanthamoeba keratitis (Ak) is a serious sight threatening infection that has been associated with contact lens wear in recent global outbreaks.[1,2] This association is not limited to the predominant soft contact lens population, with similar risk factors observed in rigid gas permeable (RGP) lens wearers.[3] Little information is available regarding the effectiveness of RGP cleaners in regimen disinfection systems for Acanthamoeba species.

There is no current industry standard for Acanthamoeba disinfection of contact lenses in a regimen disinfection system. A regimen procedure for RGP contact lens disinfection was developed utilizing the principles of ISO 14729:2001/AMD 1:2010 methodology[4] adapted for Acanthamoeba and current recovery methods for these organisms found in the literature.[5]

This study determined the effectiveness of two alkyl sulfate based cleaners formulated with different excipients to clean RGP lens materials in regimen against Acanthamoeba species.

Methods

Test Procedure: Silicone acrylate and fluoro silicone acrylate RGP lenses (n=4 each) were inoculated on each side with *A. castellanii* (ATCC 50370) or *A. polyphaga* (ATCC 30461) trophozoites or cysts prepared in organic soil at $1x10^7$ organisms/ml. After 5 minutes of adsorption, each RGP lens was rubbed for 10 seconds per side with 2–4 drops of cleaning solution (See Table 1) and rinsed for 5 seconds with sterile saline solution. The treated lens was placed in a lens case well with 3 ml conditioning and disinfecting solution preserved with chlorhexidine (CHG) and polyaminopropyl biguanide (PAPB) and soaked for 4 hours.

Assay Controls: Inoculum, neutralizer efficacy, and lens controls were inoculated and assessed for recovery, as described to confirm the test validity.

Table 1: Ingredients of two alkyl sulfate based cleaning solutions used prior to disinfection with a RGP conditioning solution

Cleaning and Conditioning Solutions	
Cleaning Solution 1	Surfactant solution containing alkyl ether sulfate, ethoxylated alkyl phenol, tri-quarternary cocoa-based phospholipid, silica gel, and titanium dioxide
Cleaning Solution 2	Surfactant solution containing alkyl ether sulfate, tri-quarternary cocoa-based phospholipid, silica gel, and titanium dioxide
Conditioning and Disinfecting Solution	Buffered hypertonic solution containing cationic cellulose derivative polymer, cellulosic viscosifier, polyvinyl alcohol, derivatized polyethylene glycol, chlorhexidine gluconate, polyaminopropyl biguanide, and edetate disodium

Acanthamoeba Recovery: Recovery of Acanthamoeba was performed by placing each lens and soak solution (1ml), separately, into 10 ml neutralizer broth for 10 minutes. Aliquots were added to a 96-well microtitre plate and serially diluted in ¼ Strength Ringer's Solution. *E. coli* were added to each well, incubated for 14 days at 26-30°C, and inspected microscopically for recovery. Acanthamoeba log_{10} recovery values for controls and test samples were determined using the Spearman-Karber method. The Log_{10} Reduction Factor (LRF) was calculated as LRF = Acanthamoeba Inoculum Control (log_{10}) – Acanthamoeba Recovery regimen (log_{10}).

Results

Acanthamoeba inoculum controls ranged from $8.83x10^4$-$1.05x10^5$ organisms/ml when directly counted with a hemocytometer and 3.9 - 4.3 logs after 14 days of recovery (Table 2). Recovery of the neutralizer efficacy controls ranged from 3.8-4.6 logs showing complete neutralization of the preservatives in the conditioning and disinfecting solution (Table 2).

Table 2: Recovery of Inoculum and Neutralizer Efficacy Controls

Organism	Inoculum Control Organisms/ml	Inoculum Control Recovery (Log_{10})	Neutralizer Efficacy Recovery (Log_{10})
A. castellanii Trophozoite	1.05×10^5	4.3	4.2
A. polyphaga Trophozoite	9.67×10^4	4.2	4.6
A. castellanii Cyst	8.83×10^4	3.9	3.8
A. polyphaga Cyst	9.67×10^4	4.3	4.5

Recovery of Acanthamoeba trophozoite and cyst lens controls ranged from 3.8-4.1 logs for silicone acrylate lenses and 3.8-4.3 logs for fluoro silicone acrylate lenses (Table 3). All inoculum, neutralizer efficacy, and lens controls were within +/- 1 log of the inoculum control supporting the validity of the experimental method employed.

Table 3: Recovery of Lens Controls

Lens Control - Acanthamoeba Recovery (Log_{10})		
Organism	**Silicone Acrylate**	**Fluoro Silicone Acrylate**
A. castellanii Trophozoite	4.1	3.9
A. polyphaga Trophozoite	4.1	4.3
A. castellanii Cyst	3.8	3.8
A. polyphaga Cyst	3.8	4.2

Table 4: Regimen Disinfection Results from *A. castellanii* and *A. polyphaga* Trophozoites

Rub/Rinse/4 Hour Soak Regimen Results (LRF)				
Lens Material	***A. castellanii* Trophozoites**		***A. polyphaga* Trophozoites**	
	Cleaning Solution 1	**Cleaning Solution 2**	**Cleaning Solution 1**	**Cleaning Solution 2**
Silicone Acrylate	>3.8	>3.8	>3.7	>3.7
Fluoro Silicone Acrylate	>3.8	>3.8	>3.7	>3.7

Table 5: Regimen Disinfection Results from *A. castellanii* and *A. polyphaga* Cysts

Rub/Rinse/4 Hour Soak Regimen Results (LRF)				
Lens Material	***A. castellanii* Cysts**		***A. polyphaga* Cysts**	
	Cleaning Solution 1	**Cleaning Solution 2**	**Cleaning Solution 1**	**Cleaning Solution 2**
Silicone Acrylate	>3.4	>3.4	>3.8	>3.8
Fluoro Silicone Acrylate	>3.4	>3.4	>3.8	>3.8

The regimen results demonstrated that cleaning solution 1 exhibited a LRF of >3.8 and >3.7 for *A. castellanii* and *A. polyphaga* trophozoites, respectively and cleaning solution 2, a LRF of >3.8 and >3.7 (Table 4). Similarly, cleaning solution 1 exhibited a LRF of >3.4 and >3.8 for *A. castellanii* and *A. polyphaga* cysts, respectively and cleaning solution 2, a LRF of >3.4 and >3.8, respectively after a rub, rinse, and 4-hour regimen using RGP lens materials (Table 5).

Conclusion

- No recovery of viable organisms was observed with either alkyl sulfate based cleaning solution after disinfection with a RGP conditioning solution in an Acanthamoeba RGP regimen system.
- All controls were +/- 1 log from the inoculum showing method validation acceptance criteria.
- These studies demonstrate robust effectiveness against Acanthamoeba, therefore reducing the potential risk of AK in RGP lens wearers.

References

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