

RESEARCH PAPER

Biocidal efficacy of multipurpose solutions against Gram-negative organisms associated with corneal infiltrative events

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Background: Because corneal infiltrative events (CIEs) may result from bacterial components on contact lenses, which can come from contaminated lens cases, we evaluated the biocidal efficacy of five multipurpose solutions against Gram-negative commonly isolated and CIE-associated organisms.

Methods: Of the multipurpose solutions tested, one contained polyhexamethylene biguanide (PHMB)/polyquatarnium-1 (PQ-1; Bausch & Lomb Incorporated: Biotrue), one contained alexidine dihydrochloride (alexidine)/PQ-1 (AMO: RevitaLens OcuTec) and three contained PQ-1/myristamidopropyl dimethylamine (MAPD; Alcon: Opti-Free PureMoist, PQ-1/MAPD-1; Opti-Free RepleniSH, PQ-1/MAPD-2; Opti-Free Express, PQ-1/MAPD-3). Challenge organisms were CIE-associated *Achromobacter xylosoxidans*, *Delftia acidovorans* and *Stenotrophomonas maltophilia* at manufacturer-recommended durations (stand-alone), in lens cases without lenses (up to seven days) and in lens cases with etafilcon A lenses (up to 30 days).

Results: In stand-alone testing against CIE-associated organisms, PHMB/PQ-1 and alexidine/PQ-1 were significantly superior versus MAPD-based multipurpose solutions against *A. xylosoxidans* (all $p \leq 0.01$), *D. acidovorans* (all $p \leq 0.001$) and *S. maltophilia* (all $p \leq 0.05$). In lens cases, PHMB/PQ-1 and alexidine/PQ-1 achieved greater than 3-log reductions against all challenge organisms at all times evaluated. PQ-1/MAPD-1 achieved a greater than 3-log reduction against *D. acidovorans* at 24 hours; PQ-1/MAPD-1 and PQ-1/MAPD-3 achieved greater than 3-log reductions at seven days against all organisms. In lens cases with lenses, PHMB/PQ-1 and alexidine/PQ-1 achieved greater than 3-log reductions against all organisms at all times. PQ-1/MAPD-1 and PQ-1/MAPD-3 achieved greater than 3-log reductions at seven or more days against all organisms. PQ-1/MAPD-2 did not achieve a greater than 3-log reduction at any time; some regrowth was observed.

Conclusions: PHMB- and alexidine-based multipurpose solutions demonstrated significantly greater biocidal activity compared with PQ-1/MAPD-based agents against Gram-negative organisms commonly isolated and CIE-associated pathogens.

Key words: gram-negative organisms, multipurpose solution, myristamidopropyl dimethylamine, polyhexamethylene biguanide

Corneal infiltrative events (CIEs) result from single or multiple clusters of immune cells (primarily neutrophils but also lymphocytes and macrophages) that have migrated from the limbal vasculature to the cornea.^{1,2}

The presentation of contact lens-related CIEs is variable and ranges from small, generally asymptomatic, peripheral lesions limited to the corneal epithelium to central stromal ulcers that may result in pain and loss of vision.³ Both asymptomatic and symptomatic CIEs are relatively common⁴ and can be costly: in the United States in 2010,⁵ there were 32,031 non-severe (score up to eight out of 10 relevant signs and

symptoms, based on the Aasuri, Venkata and Kumar⁶ matrix) and 17,248 severe (score more than eight out of 10 relevant signs and symptoms and typically requiring intervention; also based on the Aasuri, Venkata and Kumar⁶ matrix) contact lens-related CIEs, with an overall associated cost of \$58 million.^{5,6} Symptomatic CIEs can result in substantial patient burden, including interruption of contact lens wear, visits to an optometrist, ophthalmologist and/or emergency department and loss of work time.⁵

Several factors are associated with increased risk for CIEs, including male gender,^{7,8} ametropia of 5.00 D or more,³ poor hand

hygiene,⁹ prior corneal vascularisation,¹⁰ smoking,^{8,11} age up to 25 years or greater than 50 years,^{3,10} overnight contact lens wear,^{3,8,9,11,12} contact lens wear six days or more per week^{9,12} and bacterial adhesion to contact lenses (lens bioburden).^{8,12–15} These risk factors have repeatedly been shown to have a high degree of correlation with CIEs.

Studies reveal that lens cases can act as conduits for lens contamination, with the lens acting as a vector, transferring bacteria from the lens case to the eye.^{16–19} A recent review of the literature (11 studies over the past two decades) listed a range of 24 to 81 per cent positive bacterial bioburden in lens cases.¹⁷

Lens care solution (brand; manufacturer)	Formulation	Manufacturer-recommended soak time
PHMB*/PQ-1 (Biotrue; Bausch + Lomb)	Hyaluronan, sulfobetaine, poloxamine, boric acid, sodium borate, edetate disodium and sodium chloride and preserved with a dual disinfection system polyaminopropyl biguanide* 0.00013 per cent and polyquaternium 0.0001 per cent	Four hours
Alexidine/PQ-1 (RevitaLens OcuTec; Abbott Medical Optics)	Alexidine dihydrochloride 0.00016 per cent and polyquaternium-1 0.00030 per cent as preservatives/disinfectants, boric acid, sodium borate decahydrate, Tetric 904, edetate disodium, trisodium citrate dihydrate, sodium chloride and purified water	Six hours
PQ-1/MAPD-1 (Opti-Free PureMoist; Alcon)	Sodium citrate, sodium chloride, boric acid, sorbitol, aminomethylpropanol, disodium ethylenediaminetetraacetic acid, two wetting agents (Tetric 1304 and Hydraglyde Moisture Matrix [EOBO-41- polyoxyethylene-polyoxybutylene]) with Polyquad (polyquaternium-1) 0.001 per cent and Aldox (myristamidopropyl dimethylamine) 0.0006 per cent preservatives	Six hours
PQ-1/MAPD-2 (Opti-Free RepleniSH; Alcon)	Sodium citrate, sodium chloride, sodium borate, propylene glycol, Tearglyde proprietary dual action reconditioning system (Tetric 1304 nonanoyl ethylenediaminetriacetic acid) with Polyquad (polyquaternium-1) 0.001 per cent and Aldox (myristamidopropyl dimethylamine) 0.0005 per cent preservatives	Six hours
PQ-1/MAPD-3 (Opti-Free Express; Alcon)	Sodium citrate, sodium chloride, boric acid, sorbitol, aminomethylpropanol, Tetric 1304, with edetate disodium 0.05 per cent, Polyquad (polyquaternium-1) 0.001 per cent and Aldox (myristamidopropyl dimethylamine) 0.0005 per cent preservatives	Six hours

*The disinfectant polyaminopropyl biguanide is a type of polyhexamethylene biguanide (PHMB).

Table 1. Multipurpose solutions evaluated for biocidal activity²³⁻²⁷

Multipurpose solution formulation	<i>Achromobacter xylosoxidans</i> (mean log reduction)	<i>Delftia acidovorans</i> (mean log reduction)	<i>Stenotrophomonas maltophilia</i> (mean log reduction)
PHMB/PQ-1	2.90[‡]	4.60[‡]	3.50*
Alexidine/PQ-1	3.67[†]	4.80[‡]	5.00^{NA}
PQ-1/MAPD-1	0.13	2.97	1.23
PQ-1/MAPD-2	-0.03	1.40	1.33
PQ-1/MAPD-3	0.20	2.93	1.20

PHMB/PQ-1 manufacturer recommended soak time = four hours; all others = six hours.
 Mean log reductions in bold indicate statistical significance.
 NA = As alexidine results had no variation, comparisons with this formulation used median results. Median p ≤ 0.05 versus PQ-1/MAPD-1, -2, -3.
 *p ≤ 0.05 versus PQ-1/MAPD-1, -2, -3.
 †p ≤ 0.01 versus PQ-1/MAPD-1, -2, -3.
 ‡p ≤ 0.001 versus PQ-1/MAPD-1, -2, -3.

Table 2. Stand-alone testing against corneal infiltrative event-associated bacterial strains

Biofilm, the protective medium that functions to enhance the antibiotic and antiseptic resistance of bacteria, serves to improve their adherence to lens case surfaces. Biofilm is also a frequent factor in the failure of lens care products to effectively protect against lens case contamination.^{17,18} After becoming established in a lens case, biofilm can become resistant to the biocidal activity of lens care products; this can result in colonisation on the lens of resistant bacteria in the case, which can then be transferred onward to the ocular surface.^{1,2} In particular, Gram-negative bacteria such as *Delftia*, *Stenotrophomonas* and *Achromobacter* are prone to forming biofilms.¹⁸ Assessment using 16S ribosomal RNA gene sequencing of lens bioburden in lens cases from patients with contact lens-related corneal disease, found *Delftia*,

Stenotrophomonas and *Achromobacter* in significant amounts in contact lens cases from patients with CIEs compared with cases from control patients.¹⁸

Studies have found that Gram-negative isolates have demonstrated resistance to multiple polyquaterrum-1 (PQ-1)/myristamidopropyl dimethylamine (MAPD) multipurpose solutions.^{20–22} The first of these studies to demonstrate this resistance was Willcox and colleagues²² in lens cases exposed to a PQ-1/MAPD solution. Additionally, comparing PQ-1/MAPD solutions (PQ-1/MAPD-1, -2 and -3; Table 1), a polyhexamethylene biguanide (PHMB)-based and a hydrogen peroxide-based solution system showed that lens cases containing PQ-1/MAPD-2 had significantly higher mean colony-forming units (CFUs) of Gram-negative bacteria (*D. acidovorans*, *Stenotrophomonas maltophilia* and *Achromobacter* Group A) than did lens cases containing any other solution.²²

Multipurpose solutions are formulated to reduce the potential for microbial insult to the ocular surface and are successful insofar as they effectively reduce the vectored transmission of microbes from a lens case via a contact lens to the wearer's eye. The present study assessed the biocidal efficacy of different multipurpose solutions, using International Organization for Standardization (ISO) 14729 as a guideline in stand-alone conditions against three Gram-negative bacterial strains closely associated with CIEs, as well as in lens cases and in lens cases with a lens.

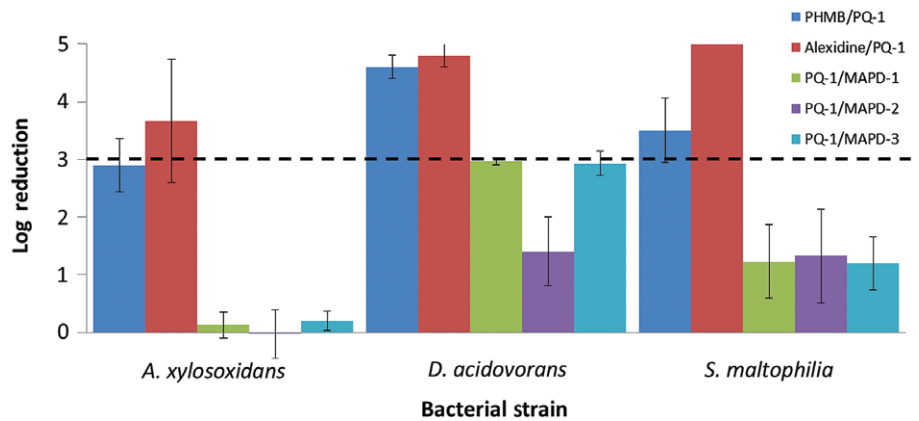


Figure 1. Gram-negative organisms associated with corneal infiltrative events, following incubation with multipurpose solutions at manufacturers' recommended soak times (dashed line indicates minimum acceptable log reduction for bacteria according to ISO 14729). Polyhexamethylene biguanide (PHMB)/PQ-1 manufacturer-recommended soak time = four hours; all others = six hours.

METHODS

Materials

MULTIPURPOSE SOLUTIONS

Three unique formulations were tested: one multipurpose solution formulation containing preservative agents PHMB and PQ-1 (Bausch & Lomb Incorporated: Biotrue), one containing alexidine dihydrochloride (alexidine) and PQ-1 (AMO: RevitaLens OcuTec) and three multipurpose solutions based on PQ-1/MAPD combinations (Alcon: Opti-Free PureMoist, PQ-1/MAPD-1; Opti-

Free RepleniSH, PQ-1/MAPD-2; Opti-Free Express, PQ-1/MAPD-3) (Table 1).

BACTERIAL STRAINS

The challenge organisms, *Achromobacter xylosoxidans* (ATCC 27061), *Delftia acidovorans* (ATCC 17438) and *Stenotrophomonas maltophilia* (clinical isolate) were used for stand-alone testing as well as testing of the multipurpose solutions in lens cases and in lens cases with a lens, as they are increasingly identified in CIEs diagnosed from contact lens wearers.¹⁸ Each organism was prepared according to ISO 14729. Organic

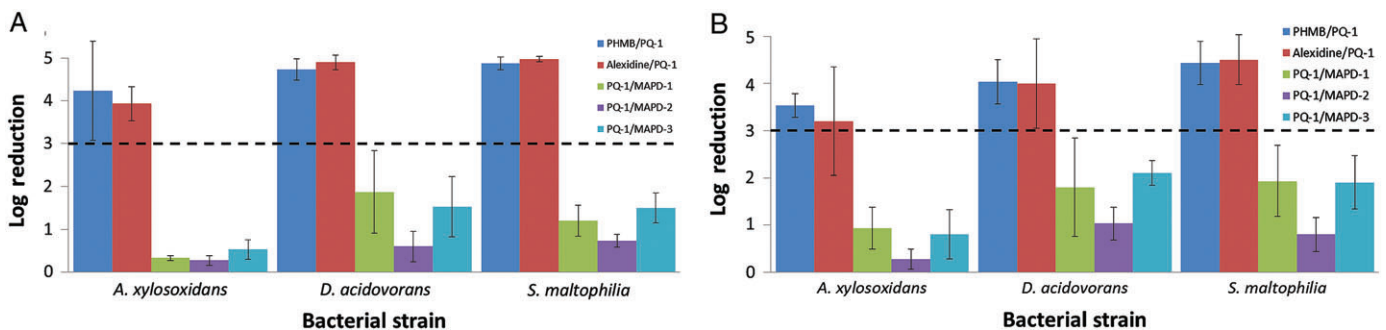


Figure 2. A. Log reductions for *Achromobacter xylosoxidans*, *Delftia acidovorans* and *Stenotrophomonas maltophilia* following incubation with multipurpose solutions in a lens case at manufacturer-recommended soak times (dashed line indicates minimum acceptable log reduction for bacteria according to ISO 14729). B. Log reductions for *A. xylosoxidans*, *D. acidovorans* and *S. maltophilia* following incubation with multipurpose solutions and etafilcon A daily wear lens in a lens case for manufacturer-recommended soak times (dashed line indicates minimum acceptable log reduction for bacteria according to ISO 14729). Polyhexamethylene biguanide (PHMB)/PQ-1 manufacturer-recommended soak time = four hours; all others = six hours.

Multipurpose solution formulation	<i>Achromobacter xylosoxidans</i> (mean log reduction)	<i>Delftia acidovorans</i> (mean log reduction)	<i>Stenotrophomonas maltophilia</i> (mean log reduction)
Four-hour soak			
PHMB/PQ-1	4.23*	4.73*	4.87†
Alexidine/PQ-1	4.50†	4.33*	4.97‡
PQ-1/MAPD-1	0.57	1.80	0.93§
PQ-1/MAPD-2	0.40	0.33	0.70
PQ-1/MAPD-3	0.73	1.77	1.07
Six-hour soak			
PHMB/PQ-1	4.93‡	4.90†	4.97†
Alexidine/PQ-1	3.93†	4.90†	4.97†
PQ-1/MAPD-1	0.33	1.87	1.20
PQ-1/MAPD-2	0.27	0.60	0.73
PQ-1/MAPD-3	0.53	1.53	1.50§
24-hour soak			
PHMB/PQ-1	4.93†	4.90#	4.97*
Alexidine/PQ-1	4.93†	4.90#	4.97*
PQ-1/MAPD-1	1.70 	3.10§	2.53§
PQ-1/MAPD-2	0.13	0.07	0.67
PQ-1/MAPD-3	1.83 	2.37 	2.47§
7-day soak			
PHMB/PQ-1	4.93**	4.90 	4.97**
Alexidine/PQ-1	4.93**	4.90 	4.97**
PQ-1/MAPD-1	4.93**	3.83**	4.97**
PQ-1/MAPD-2	-0.1	-0.87	-0.27
PQ-1/MAPD-3	4.93**	4.07**	4.97**

Mean log reductions in bold indicate statistical significance.
 *p ≤ 0.05 versus PQ-1/MAPD-1, -2, -3.
 †p ≤ 0.01 versus PQ-1/MAPD-1, -2, -3.
 ‡p ≤ 0.001 versus PQ-1/MAPD-1, -2, -3.
 §p ≤ 0.05 versus PQ-1/MAPD-2.
 ||p ≤ 0.05 versus PQ-1/MAPD-1, -2.
 ¶p ≤ 0.01 versus PQ-1/MAPD-2.
 #p ≤ 0.01 versus PQ-1/MAPD-2, -3.
 **p ≤ 0.001 versus PQ-1/MAPD-2.

Table 3. Testing against corneal infiltrative event-associated bacterial strains in a lens case

soil was used following the ISO organism preparation guidelines.²⁸

CONTACT LENS CASES

The contact lens cases used were those that accompanied each manufacturer’s multipurpose solutions and were used with that multipurpose solution formulation only.

CONTACT LENSES

Etafilcon A daily wear hydrogel lenses (J & J Vision Care, Jacksonville, Florida, USA)

were used, as they have been shown to take up appreciable amounts of PHMB and MAPD,²⁹ which allowed testing of whether, under these conditions, uptake of PHMB or MAPD affected the biocidal efficacy of solutions against the challenge organisms.

Procedures

STAND-ALONE BIOCIDAL EFFICACY TESTING OF MULTIPURPOSE SOLUTIONS

ISO 14729 was used as the guideline (3-log reduction or more) for testing

biocidal efficacy in test tubes of the five multipurpose solutions against the three Gram-negative bacterial strains associated with CIEs (*A. xylosoxidans*, *D. acidovorans* and *S. maltophilia*). Test organisms were resuspended in 10 per cent organic soil to yield a final concentration of approximately 5.0×10^5 CFUs/ml. Efficacy was evaluated at manufacturers’ recommended soak times: PHMB/PQ-1 for four hours and alexidine/PQ-1, PQ-1/MAPD-1, PQ-1/MAPD-2, PQ-1/MAPD-3 for six hours.^{23–27}

BIOCIDAL EFFICACY TESTING OF MULTIPURPOSE SOLUTIONS IN A LENS CASE

Biocidal testing (three separate assays) was performed on each multipurpose solution directly in the manufacturer-provided polypropylene lens cases, each of which was aseptically filled with 3.0 ml of the respective manufacturer’s multipurpose solution. Each solution was inoculated with the challenge organisms, *A. xylosoxidans*, *D. acidovorans* or *S. maltophilia*, resuspended in 10 per cent organic soil at a final concentration of approximately 5.0×10^5 CFU/ml. Cases were vortexed and 1.0 ml of test solution was aliquoted from the lens case well; test solutions were neutralised with Dey Engley Broth (Remel, a division of Thermo Fisher Scientific, Lenexa, Kansas, USA) and plated with trypticase soy agar (Becton Dickinson and Co, Sparks, Maryland, USA) at four, six and 24 hours, as well as at seven days for recovery of organisms. Surviving microbes were enumerated and log reduction values relative to the inoculum control were calculated. Three lots of each multipurpose solution were tested on three separate days.

BIOCIDAL EFFICACY TESTING OF MULTIPURPOSE SOLUTIONS IN A LENS CASE IN THE PRESENCE OF A LENS

Testing with etafilcon A contact lenses was based on ISO 18259 (system biocidal).³ Lenses were placed in the manufacturer-provided polypropylene lens cases and were inoculated with 0.1 ml of test inoculum of the challenge organisms, *A. xylosoxidans*, *D. acidovorans* or *S. maltophilia*, resuspended in 10 per cent organic soil at a final concentration of approximately 5.0×10^5 CFU/ml and left in contact with the test lens for five to 10 minutes. Only one side of the lens case was used and a separate case was used at each time (four, six and 24 hours,

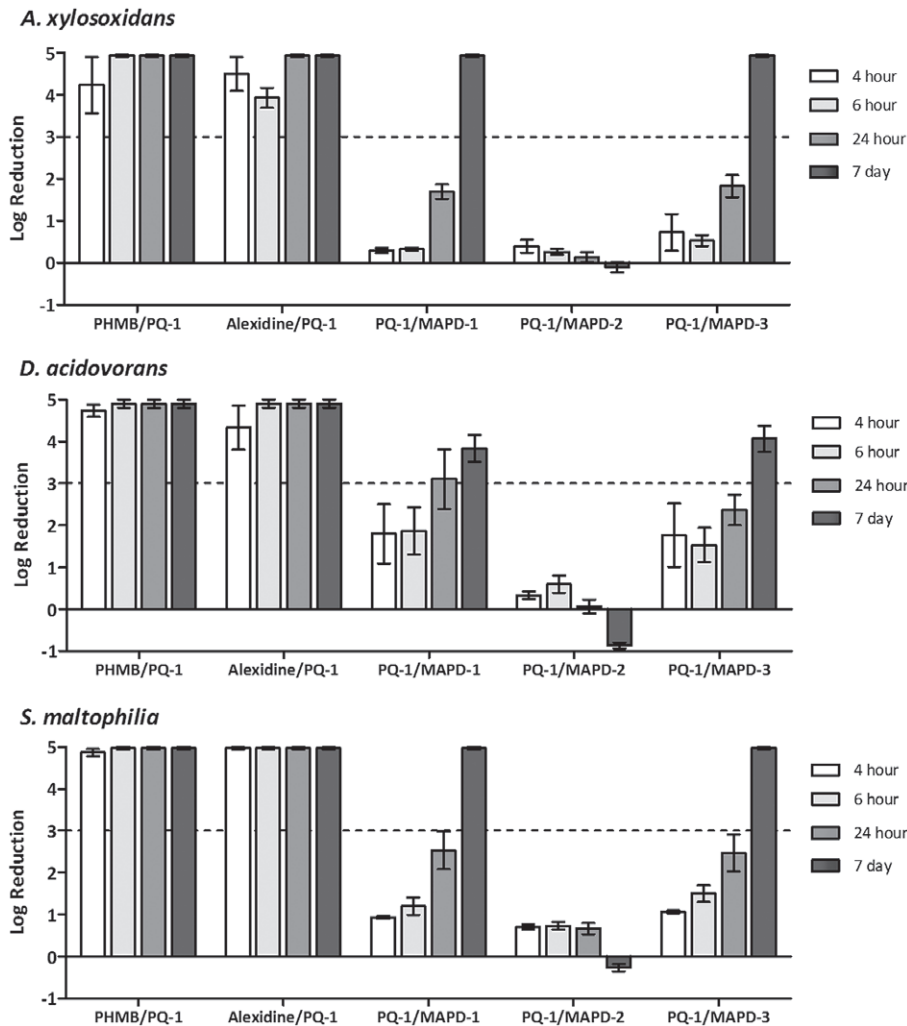


Figure 3. Log reductions for *Achromobacter xylosoxidans*, *Delftia acidovorans* and *Stenotrophomonas maltophilia* following incubation with multipurpose solutions in a lens case for indicated time points (dashed line indicates minimum acceptable log reduction for bacteria according to ISO 14729)

as well as at seven and 30 days). Each manufacturer-provided polypropylene lens case was aseptically filled with 3.0 ml of the respective manufacturer's multipurpose solution. The lens was completely immersed in the solution and the lens case was capped, taking care not to agitate the contents of the case to eliminate potential contamination of the lens cap. Prior to each time point, lens cases were vortexed, after which the lens was removed and one millilitre was aliquoted from the lens case well. Test solutions were neutralised with Dey Engle Broth and plated with trypticase soy agar for recovery of organisms. Surviving microbes were enumerated and log reduction

values relative to the inoculum control were calculated. All assays were performed in triplicate.

Calculations and statistical analysis

Log reduction values were calculated by determining CFU counts, after which the mean log value for each lens care solution was subtracted from its mean log value at baseline to obtain the log reduction, as follows:

$$\text{Log reduction} = \text{Log}_{10}(\text{mean baseline CFU}) - \text{Log}_{10}(\text{mean lens care solution CFU})$$

Log reductions among PHMB/PQ-1, alexidine/PQ-1 and each PQ-1/MAPD solution

were compared using an analysis of variance. Statistical significance between solutions was determined using a two-sided, two-sample t-test with a type I error rate of 0.05.

RESULTS

For most of the test conditions and times, the PHMB/PQ-1 and alexidine/PQ-1 solutions showed statistically greater biocidal activity compared with the three PQ-1/MAPD formulations. Overall, log reductions against challenge organisms ranged from 4.97 (no microbial recovery) to -0.87 (regrowth).

Stand-alone efficacy testing of multipurpose solutions at manufacturer-recommended soak times

When tested against Gram-negative bacterial strains associated with CIEs at manufacturer-recommended soak times, PHMB/PQ-1 achieved a greater than 3-log reduction against *D. acidovorans* and *S. maltophilia* and a 2.9-log reduction against *A. xylosoxidans*, while alexidine/PQ-1 achieved greater than 3-log reductions against all three organisms. Biocidal activity for each of the three PQ-1/MAPD formulations at manufacturer-recommended soak times was less than 3-log reduction against these bacterial strains; for PQ-1/MAPD-2 against *A. xylosoxidans*, there was regrowth (Table 2 and Figure 1).

Biocidal efficacy of multipurpose solutions in a lens case at manufacturer-recommended and extended soak times

Multipurpose solutions tested against Gram-negative bacterial strains associated with CIEs in a lens case at their respective manufacturer-recommended soak times showed PHMB/PQ-1 and alexidine/PQ-1 achieving greater than 3-log reductions against all three organisms. PQ-1/MAPD-1, -2 and -3 did not achieve a greater than 3-log reduction against any bacterial strain at their manufacturer-recommended soak time (Figures 2A and 2B). At extended soak times (24 hours and seven days), multipurpose solutions also demonstrated the efficacy seen at manufacturer-recommended soak times for PHMB/PQ-1 and alexidine/PQ-1 and showed improved efficacy of PQ-

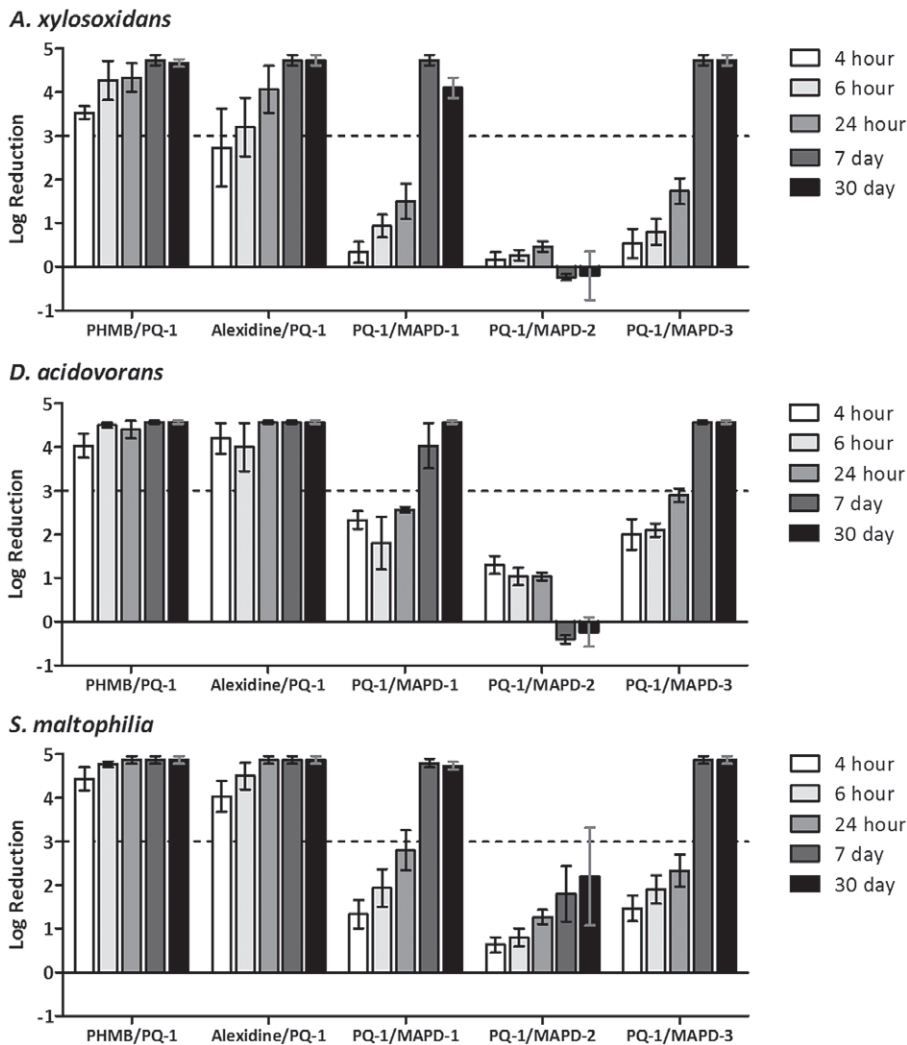


Figure 4. Log reductions for *Achromobacter xylosoxidans*, *Delftia acidovorans* and *Stenotrophomonas maltophilia* following incubation with multipurpose solutions and etafilcon A lens in a lens case for indicated time points (dashed line indicates minimum acceptable log reduction for bacteria according to ISO 14729)

1/MAPD-1 and PQ-1/MAPD-3 compared with PQ-1/MAPD-2 (Table 3 and Figure 3).

Biocidal efficacy of multipurpose solutions in a lens case in the presence of a lens at manufacturer-recommended and extended soak times

For multipurpose solution testing in a lens case in the presence of an etafilcon A lens, both PHMB/PQ-1 and alexidine/PQ-1 demonstrated greater than 3-log reductions against the Gram-negative bacterial strains associated with CIEs at nearly all times (Figure 4). The one exception was

alexidine/PQ-1 against *A. xylosoxidans* (2.73 logs) at four hours (less than the recommended soak time for this solution). The three PQ-1/MAPD-based multipurpose solutions demonstrated lower biocidal efficacy, particularly at the early (up to 24 hours) times (Table 4).

DISCUSSION

Despite increasing evidence that CIEs are an acute inflammatory response to bacterial toxins, enzymes and/or metabolic byproducts accumulated on the contact lens surface,^{8,30} our understanding of the role of these organisms in the aetiology of

CIEs is far from complete. In fact, no microorganisms are found on lenses of about 22 per cent of patients experiencing CIEs.⁴

A study by Cheung and colleagues³¹ showed that both *S. maltophilia* and *D. acidovorans* were resistant to PQ-1/MAPD-based multipurpose solutions but not to PHMB/PQ-1, PHMB, peroxide or alexidine/PQ-1 multipurpose solution formulations. In the Cheung and colleagues study,³¹ three strains each of *S. maltophilia* and *D. acidovorans* were tested with frequently used multipurpose solutions and peroxide care systems. A six-hour soak in PQ-1/MAPD-2 resulted in a less than 1-log kill for all *S. maltophilia* strains and substantial regrowth for two strains by seven days. There was also a less than 1-log kill for *D. acidovorans* at 24 hours and substantial regrowth at seven to 14 days. In contrast, four hours of exposure to alexidine/PQ-1, PHMB/PQ-1, PHMB- or peroxide-based solutions resulted in 4.0 or more log reductions in these organisms, with no regrowth for up to 21 days. The ability of these bacteria to survive and replicate in PQ-1/MAPD-based multipurpose solutions may result in biofilm in contact lens cases.³¹

For stand-alone testing against *A. xylosoxidans*, *D. acidovorans* and *S. maltophilia* at manufacturer-recommended soak times, the five multipurpose solutions showed varying degrees of biocidal efficacy. PHMB/PQ-1 achieved greater than 3-log reductions for *D. acidovorans* and *S. maltophili*, but achieved only a 2.90 log reduction for *A. xylosoxidans*, while alexidine/PQ-1 achieved greater than 3-log reductions against all three organisms. None of the MAPD-based formulations achieved more than 3-log reductions. Interestingly, PHMB/PQ-1 and alexidine/PQ-1 both achieved greater than 3-log reductions against all three CIE-associated Gram-negative organisms, when tested in lens cases and in lens cases with a lens at their respective manufacturer-recommended soak times; however, results were not significant compared with results of stand-alone testing for these two multipurpose solutions. In lens cases and in lens cases with a lens at their manufacturer-recommended soak time, none of the three MAPD-based multipurpose solutions achieved a greater than 3-log reduction (Tables 3 and 4).

For the extended soak periods (24 hours or more) against the bacterial strains in lens cases without a lens present (up to seven days) and in lens cases with a lens present (up to 30 days), PHMB/PQ-1 and

Multipurpose solution formulation	<i>Achromobacter xylosoxidans</i> (mean log reduction)	<i>Delftia acidovorans</i> (mean log reduction)	<i>Stenotrophomonas maltophilia</i> (mean log reduction)
Four-hour soak			
PHMB/PQ-1	3.53[†]	4.03[†]	4.43[†]
Alexidine/PQ-1	2.73[§]	4.57[*]	4.03[†]
PQ-1/MAPD-1	0.33	2.33[§]	1.33
PQ-1/MAPD-2	0.17	1.30	0.63
PQ-1/MAPD-3	0.53	2.00	1.47
Six-hour soak			
PHMB/PQ-1	4.27[†]	4.50[*]	4.77[*]
Alexidine/PQ-1	3.20[*]	4.00^{††}	4.50[†]
PQ-1/MAPD-1	0.93	1.80	1.93
PQ-1/MAPD-2	0.27	1.03	0.80
PQ-1/MAPD-3	0.80	2.10[§]	1.90[§]
24-hour soak			
PHMB/PQ-1	4.33[†]	4.40[†]	4.87[*]
Alexidine/PQ-1	4.07[*]	4.57[†]	4.87[*]
PQ-1/MAPD-1	1.50	2.57^{**}	2.80[§]
PQ-1/MAPD-2	0.47	1.03	1.27
PQ-1/MAPD-3	1.73[§]	2.90^{**}	2.33
7-day soak			
PHMB/PQ-1	4.73^{**}	4.57^{**}	4.87[§]
Alexidine/PQ-1	4.73^{**}	4.57^{**}	4.87[§]
PQ-1/MAPD-1	4.73^{**}	4.17^{**}	4.80[§]
PQ-1/MAPD-2	-0.23	-0.40	1.80
PQ-1/MAPD-3	4.73^{**}	4.57^{**}	4.87[§]
30-day soak			
PHMB/PQ-1	4.67[§]	4.57[¶]	4.87
Alexidine/PQ-1	4.73^{**}	4.57[¶]	4.87
PQ-1/MAPD-1	4.10[¶]	4.57[¶]	4.73
PQ-1/MAPD-2	-0.20	-0.23	2.20
PQ-1/MAPD-3	4.73^{**}	4.57[¶]	4.87

Mean log reductions in bold indicate statistical significance.
^{*}p ≤ 0.05 versus PQ-1/MAPD-1, -2, -3.
[†]p ≤ 0.01 versus PQ-1/MAPD-1, -2, -3.
^{††}p ≤ 0.001 versus PQ-1/MAPD-1, -2, -3.
[‡]p ≤ 0.05 versus PQ-1/MAPD-2.
[§]p ≤ 0.05 versus PQ-1/MAPD-2.
[¶]p ≤ 0.05 versus PQ-1/MAPD-1, -2.
^{¶¶}p ≤ 0.01 versus PQ-1/MAPD-2.
[#]p ≤ 0.01 versus PQ-1/MAPD-2, -3.
^{**}p ≤ 0.001 versus PQ-1/MAPD-2.
^{†††}p ≤ 0.05 versus PQ-1/MAPD-2, -3.

Table 4. Testing against corneal infiltrative event-associated bacterial strains in a lens case with a lens

alexidine/PQ-1 achieved greater than 3-log reductions against each organism at each time. Despite the ability of the etafilcon A lens to take up PHMB and MAPD, results

of this study showed that the overall trend, across all times and organisms tested, was one of minimal impact on biocidal efficacy of the multipurpose solutions analysed,

when compared with the results of testing in lens cases without a lens. With a lens in the case, alexidine/PQ-1 showed lower biocidal activity against *A. xylosoxidans* at four hours, although it did achieve a greater than 3-log reduction at its manufacturer-recommended soak time of six hours. This may suggest the presence of a lens delay in the kinetics of the biocidal effect, perhaps related to biocide uptake by the contact lens, as seen in other studies.³²

Our results may reflect the fact that all five multipurpose solutions tested were formulated with two biocides and given that all five contain PQ-1 at concentrations of 0.001 per cent (all three PQ-1/MAPD formulations) or lower (alexidine/PQ-1, 0.0003 per cent; PHMB/PQ-1, 0.0001 per cent) (Table 1), our data could suggest that the differences in efficacy may be related to the properties of the second biocide in the multipurpose solution formulation, as has been found in other studies.³² Both the PHMB- and alexidine-containing multipurpose solutions showed superior biocidal efficacy compared with MAPD-based multipurpose solutions, especially against the CIE-associated Gram-negative *A. xylosoxidans*, *D. acidovorans* and *S. maltophilia*. The lack of biocidal efficacy of MAPD-based multipurpose solutions at all but the lengthier soak times in our study suggests the strains we tested may be more resistant to the biocidal effects of MAPD-based multipurpose solutions.

Our study did not account for such patient behaviour as 'topping off' or for introduction of additional bacteria, as might be seen when patients repeatedly remove and reinsert contact lenses into the same lens case well containing old or topped-off solution. Because of our testing methods (aliquot), we also cannot account for the possibility that the respective manufacturers' cases used in this study may have been susceptible in varying degrees to bacterial colonisation, which may have played a role in the results achieved in our study.³³ For testing with the lens in the lens case, etafilcon A daily wear hydrogel lenses (J & J Vision Care) were used, as they have been shown to take up appreciable amounts of PHMB and MAPD.²⁹ Further biocidal efficacy testing with silicone hydrogel lenses having different uptake and release profiles of antimicrobial agents may be of interest.

In conclusion, our results show that the biocidal efficacies of PHMB/PQ-1 and alexidine/PQ-1 were quicker in onset and more consistent against organisms tested, across times. PHMB/PQ-1 and alexidine/PQ-1 had significantly greater log reductions of organisms, when compared to the three PQ-1/MAPD solutions at their respective manufacturer-recommended soak times (for the lens care products in our study, four hours and six hours), whether in stand-alone conditions in test tubes, in lens cases or in lens cases with an etafilcon A lens. Only at extended soak durations (seven days or more soak time, without or with a lens in the case), were two of the three MAPD-based formulations able to achieve consistent and similar log reductions as seen with PHMB/PQ-1 and alexidine/PQ-1.

Patients should be advised to develop and maintain a lens-care regimen in accordance with manufacturer guidelines for the products they use and it remains vital for optometrists, ophthalmologists and allied health-care providers to continue to counsel patients on the most appropriate lens care products for their lenses, as well as best practices regarding contact lens wear and hygiene.

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2. Merchea MM, David B, Callahan D, Bair J. Efficacy of multi-purpose solutions against gram-negative clinical isolates associated with infiltrative keratitis. Poster presented at: American Academy of Optometry, 2012, Phoenix, Arizona, USA.
3. Walsh PA, David BC, Callahan DE, Bair JA, Norton SE. Biocidal efficacy of multi-purpose solutions against gram-negative organisms in the presence of a lens. Poster presented at: American Academy of Optometry; October 23–26, 2013; Seattle, Washington, USA.

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