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ORIGINAL RESEARCH

Biocidal Efficacies of Contact Lens Disinfecting Solutions Against Gram-Negative Organisms Associated with Lens Case-Associated Corneal Infiltrative Events

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Background: Multi-purpose solutions (MPSs) are designed to clean, disinfect, and condition contact lenses (CLs) to reduce the risk of contact lens-related adverse events, such as corneal infiltrative events (CIEs) that are often associated with opportunistic bacteria from contaminated CLs or CL cases. This study evaluated the disinfection efficacies of six MPSs and one hydrogen peroxide solution (HPS) comparator against three lens case CIE-associated organisms.

Methods: The solutions in this study were evaluated against commonly isolated Gram-negative lens case CIE-associated organisms in the presence of organic soil, according to the International Organization for Standardization 14729 (ISO) stand-alone test protocol. Challenge organisms (*Achromobacter xylosoxidans, Delftia acidovorans* and *Stenotrophomonas maltophilia*) were inoculated into the solutions in conical tubes (for MPS) or CL cases (for HPS), and plated for organism quantification after the manufacturer-recommended minimum soak times. Disinfection efficacy for each solution is presented as mean log reduction per organism and overall disinfection across three challenge organisms (post hoc analysis).

Results: Stand-alone testing against the challenge organisms demonstrated that PAPB/Alexidine/PQ-1, Alexidine/PQ-1, and PHMB-containing MPSs, as well as HPS were significantly superior versus MAPD/PQ-1-based MPSs (all $p < 0.05$). While there is no ISO criteria for reduction of CIE-associated organisms, all solutions containing PAPB/Alexidine/PQ-1, Alexidine/PQ-1, and PHMB, as well as HPS, achieved greater than 3-log reductions (the primary criteria for ISO 14729 compendial bacterial organisms) for all challenge organisms. The three MAPD/PQ-1-containing MPSs achieved between 0.6 and 1.7 log reduction across the MPS/test organism combinations.

Conclusion: The PAPB, PHMB, and Alexidine-based MPSs demonstrated significantly greater disinfection efficacy than MAPD-based MPSs, and comparable disinfection efficacy to HPS, against commonly isolated Gram-negative CL case CIE-associated organisms.

Keywords: multi-purpose solution, MPS, hydrogen peroxide solution, HPS, disinfection, contact lens case contamination, gram-negative organisms, corneal infiltrates, ISO 14729

Introduction

A proper lens care routine is very important for successful contact lens (CL) wear, and non-compliance can lead to adverse events including corneal infiltrative events (CIEs) and microbial keratitis (MK).[1–3]

CIE can be common with CL wear and are classified as either sterile or infectious.[4] MK may be referred to as a type of CIE, although CIEs may also be considered as self-limiting inflammatory responses, and MK as an active pathogenic infection requiring antimicrobial treatment.[4–6] CL wear is responsible for 52–65% of new MK cases,[2] which can be distinguished as clinically severe keratitis (MK requiring antimicrobial treatment) or non-severe keratitis (typically CIEs requiring no medical treatment).[2,4,5]

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When stimulated by live pathogens or other bacterial-derived antigens, corneal epithelial cells release inflammatory cytokines and chemokines, which effect infiltration of inflammatory cells (leukocyte, neutrophils, macrophage, etc) into the cornea.[1,7] MK involves persistent pathogens that elicit an intense secondary immune response,[2,3] and is serious and potentially sight threatening.[1,2,4] Most CIEs do not typically adversely affect long-term eye health and may be resolved with temporary discontinuation of CL wear, although serious cases can cause scarring, which can negatively impact visual acuity. Severity of CIEs has also been linked to corneal tissue damage. Lifestyle and CL hygiene can increase the risk and impact of CIE.[2–4]

CL wear is one of the main risk factors for MK and CIEs, with modifiable risk factors that include wear of planned replacement CLs, use of CL multi-purpose solutions (MPSs), overuse of CL storage cases, high eyelid bioburden and microbial colonization, and smoking.[1,3,4] Poor hygiene and overnight CL wear are the biggest risk factors for contact lens-induced infectious MK.[1,2] CIEs are reported to occur more frequently in wearers of silicone hydrogel (SH) CLs than hydrogel CLs, with incidence reported to be dependent on both CL material and design, and CL care solution.[1,4,8]

CL storage cases can be a source of microbial growth and case hygiene and replacement time are modifiable risk factors for MK.[1] The case can act as a reservoir for MK-causing organisms and their byproducts and enable their transfer from case to CL to corneal surface.[1,9] Bacterial biofilms protect bacteria from the effects of disinfecting CL care solutions, making biofilm bacteria difficult to kill, and they account for many of the bacteria isolated from the conjunctiva, CL, and CL cases.[10–12] In addition, although not normally reported as causative of CIEs or MK, the Gram-negative bacteria *Delftia, Stenotrophomonas*, and *Achromobacter* are recovered in significantly greater amounts from CL cases of patients diagnosed with CIEs, implicating these bacteria in CIE associated with contaminated CL cases.[13–15]

MPSs are formulated to clean, rinse, disinfect, and store soft CLs through a multifunctional regimen (rub, rinse, soak).[16,17] To minimize the risk of CIE and MK, these solutions should be effective at inhibiting the growth of the pathogenic microbes commonly reported to cause MK,[16,17] as well as those implicated in contaminated CL case-associated CIEs. Modern MPS disinfectant components include polyquaternium-1 (PQ-1), myristamidopropyl dimethylamine (MAPD), polyaminopropyl biguanide (PAPB), alexidine dihydrochloride (Alexidine), and polyhexamethylene biguanide (PHMB), which have a large chemical structure that reduces risk of uptake into CL materials, thereby minimizing risk of damage to the ocular surface following release.[17] PQ-1 is a bactericidal quaternary ammonium compound that denatures proteins in bacterial cell walls, myristamidopropyl dimethylamine is an amidoamine that cationically disrupts the cell membranes of fungi, and biguanides such as PAPB, Alexidine, and PHMB interact with intracellular microbial DNA to disrupt DNA function and/or cause its precipitation, leading to cell death.[17] Hydrogen peroxide is an oxidizing agent that acts as a broad-spectrum disinfectant by altering the microbial membrane permeability; distinct from MPSs, hydrogen peroxide-based solutions (HPS) chemically clean and disinfect CLs during incubation with a neutralizing platinum or enzyme catalyst.[17]

This study evaluated the disinfection efficacies of six commercially available MPSs (Table 1) against the *Delftia, Stenotrophomonas*, and *Achromobacter* bacterial strains, which have been reported as recovered from contaminated CL cases of patients diagnosed with CIEs.[15] Mean log reductions for each MPS were compared against each other for each organism, and overall disinfection; an HPS was included as a comparator, based on the established disinfection efficacy, and distinct catalyst-driven mechanism of action.[17]

Materials and Methods

Multi-Purpose Solutions

The biocidal efficacies of the six MPSs and one hydrogen peroxide solution (HPS) summarized in Table 1 were evaluated. Listed solution components, including surfactants/cleaners, disinfectants, and additional ingredients such as comfort agents are based upon product package inserts.[18–24] HPS was used as a comparator, based on the historical demonstration of disinfection efficacy, and distinct mechanism of action based on a catalyst-driven system.[17]

Challenge Organisms

The challenge organisms, *Achromobacter xylosoxidans* (American Type Culture Collection (ATCC 27061, Manassas, VA), *Delftia acidovorans* (ATCC 17438), and *Stenotrophomonas maltophilia* (clinical isolate) were used for stand-alone

Table 1 Multi-Purpose (MPS) and Hydrogen Peroxide (HPS) Test Solutions

Disinfecting Solution	Manufacturer	Buffer	Surfactants, Cleaners	Additional Ingredients	Disinfectant(s)	Manufacturer-Recommended Minimum Soak Time (hours)
OPTI-FREE pure*moist* (MPS-1)[18]	Alcon Fort Worth, TX	Borate/ citrate	Tetronic 1304 EOBO-41 EDTA	Sorbitol	PQ-1 (0.001%) MAPD (0.0006%)	6
OPTI-FREE Replenish (MPS-2)[19]		Borate/ citrate	Tetronic 1304 C9-ED3A	Propylene glycol	PQ-1 (0.001%) MAPD (0.0005%)	6
OPTI-FREE Express (MPS-6)[20]		Borate/ citrate	Tetronic 1304 EDTA	Sorbitol	PQ-1 (0.001%) MAPD (0.0005%)	6
CLEAR CARE PLUS (HPS)[21]		Phosphate	Pluronic 17R4 EOBO-21		Hydrogen Peroxide (3%)	6
Biotrue Hydration Plus (MPS-3)[22]	Bausch & Lomb Incorporated Rochester, NY	Borate	Poloxamine 1107 Poloxamer 181 EDTA	Hyaluronan, erythritol, potassium	PAPB (0.00005%) Alexidine (0.00025%) PQ-1 (0.00015%)	4
ACUVUE RevitaLens (MPS-4)[23]	Johnson & Johnson Vision, Jacksonville, FL	Borate/ citrate	Tetronic 904 EDTA		PQ-1 (0.0003%) Alexidine (0.00016%)	6
COMPLETE (MPS-5)[24]		Phosphate	Poloxamer 237 EDTA	Potassium	PHMB (0.0001%)	6

Abbreviations. *Surfactants and Cleaners*. EOBO, block copolymer of polyoxyethylene and polyoxybutylene; Tetronic, poloxamine; Pluronic, poloxamer; EDTA, edetate disodium; C9-ED3A, Nonanoyl ethylenediaminetriacetic acid. *Disinfectants*. MAPD (Aldox), myristamidopropyl dimethylamine; Alexidine: alexidine dihydrochloride; PHMB, polyhexamethylene biguanide; PAPB, Polyaminopropyl biguanide, a type of PHMB, PQ-1, Polyquaternium-1.

testing due to their association with contaminated CL case CIEs. Organisms were cultured, harvested, and adjusted using a spectrophotometer and Dulbecco's Phosphate Buffered Saline plus 0.05% m/v polysorbate 80 (DPBST; Gibco™ DPBS, Catalog number 14190–136, ThermoFisher Scientific, Waltham, MA; Tween™ 80, CAS No. 9005–65-6, Fisher Scientific, Waltham, MA).

Organic soil was prepared according to Annex E of the International Organization for Standardization (ISO, Geneva, Switzerland) 14729,[25] and incorporated into each challenge organism suspension, achieving a final concentration of 1.0×10^7 to 1.0×10^8 colony forming units (cfu)/mL.

Experimental Procedure

Disinfection efficacy was evaluated with three individual replicates of each organism in each solution according to the ISO 14729 stand-alone test protocol.[25] For each replicate, the six MPSs (10 mL) were dispensed into conical tubes (3 tubes per solution for the 3 organisms tested), and the HPS (10 mL) was dispensed into the accompanying neutralizing CL cases provided by the manufacturer. Full details of the procedures used have been previously reported.[26] Conical tubes and neutralizing CL cases were inoculated with 0.1 mL of challenge organism with organic soil to achieve a final concentration of 1.0×10^5 to 1.0×10^6 cfu/mL.

Stand-alone disinfection efficacy was assessed at manufacturer-recommended minimum soak time (4 or 6 hours, Table 1). Each MPS was neutralized with Remel™ D/E (Dey-Engley) Neutralizing Broth (ThermoFisher Scientific, catalog number R453042,), and the HPS was neutralized with 0.1% Catalase solution (Catalase from bovine liver, CAS No. 9001–05-02, Sigma Aldrich, St. Louis, MO). Neutralized test solutions were plated and poured with Trypticase Soy

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Agar (BD Trypticase™ soy agar, SKU 299099, BD, Franklin Lakes, NJ). Plates were incubated for recovery at 30°C to 35°C, for the same amount of time for each test replicate. An inoculum control was plated to determine the initial concentration of each challenge organism.

Following incubation, plates were enumerated for the recovery of challenge organisms. Log reduction values were calculated, based upon the mean log recovery of the innoculum control, and mean values and standard deviation determined for each solution/organism combination.

Statistical Analysis

SAS software version 9.4 was used for data analysis.

Stand-alone disinfection efficacy was calculated as mean log reduction of each microorganism in each solution based upon triplicate observations. Overall stand-alone disinfection efficacy of each solution was calculated as the efficacy averaged over all three challenge organisms (mean ± standard deviation).

A post hoc analysis of the data comparing each solution to the others was performed for overall efficacy. Differences in overall disinfection efficacy, as well as efficacy against each organism individually were compared using Analysis of Variance (ANOVA). Multiple comparisons were made using Tukey's procedure with an overall α level of 0.05.

Log reductions of the six MPS solutions were compared to one another for individual organisms and overall disinfection. HPS was within the same statistical grouping and log reductions were compared against each other.

Results

Stand-alone disinfection efficacy (mean log reduction of 3 replicates of each organism/disinfectant solution combination) is shown in Figure 1 (average ± standard deviation). While the ISO 14729 criteria apply only to the compendial organisms, MPSs containing PHMB alone (MPS-4) or PAPB in combination with Alexidine and PQ-1 (MPS-3), as well as MPS containing Alexidine in combination with PQ-1 (MPS-4) and HPS all achieved greater than 3-log reduction for the three CL case CIE-associated challenge organisms in this study. In contrast, MPSs containing the combination of MAPD and PQ-1 (MPS-1, MPS-2, MPS-6) achieved only between 0.6 to 1.7-log reduction across all organisms. The

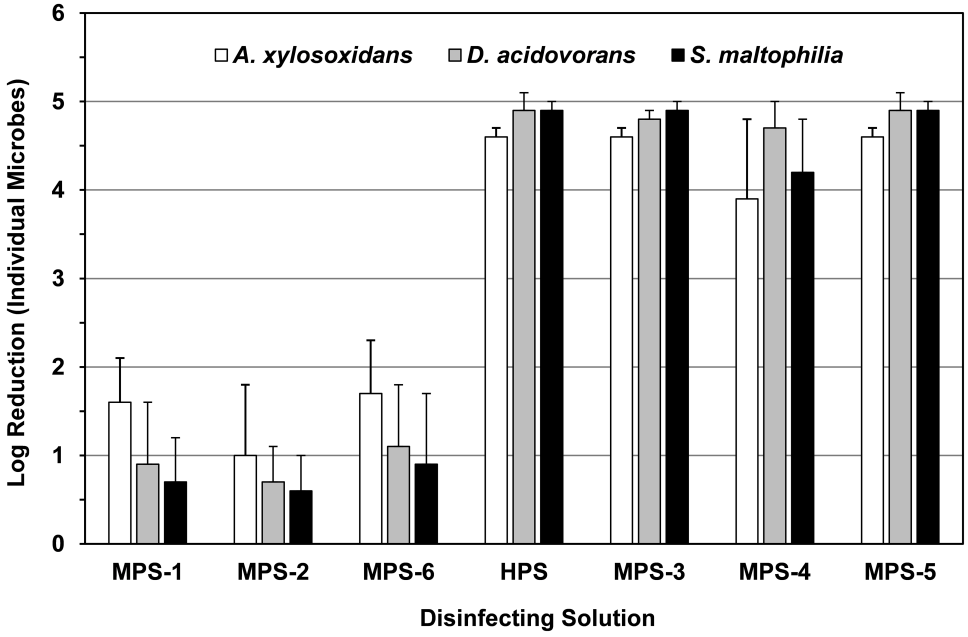

Figure 1 Disinfection efficacy of multi-purpose (MPS) and hydrogen peroxide (HPS) solutions against lens case CIE-associated organisms (mean log reduction ± standard deviation, n=3 for each solution/organism combination). Solutions tested: MPS-1 = OPTI-FREE pure*moist*; MPS-2 = OPTI-FREE Replenish; HPS = CLEAR CARE PLUS; MPS-3 = Biotrue Hydration Plus; MPS-4 = ACUVUE RevitaLens; MPS-5 = COMPLETE; MPS-6 = OPTI-FREE Express.

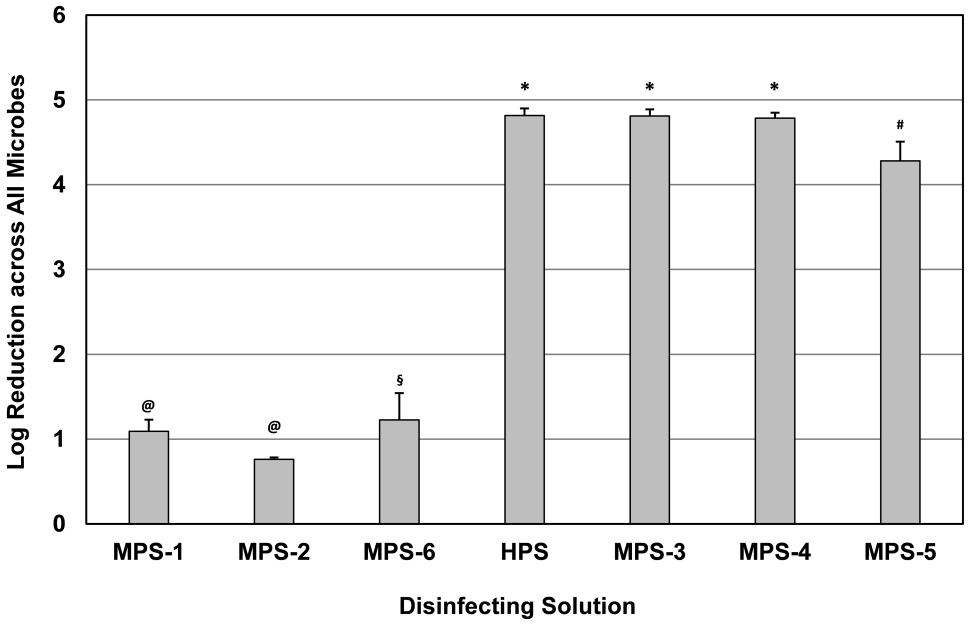

Figure 2 Overall disinfection efficacy of multi-purpose (MPS) and hydrogen peroxide (HPS) solutions across three lens case CIE-associated organisms (mean log reduction ± standard deviation). Solutions tested: MPS-1 = OPTI-FREE puremoist; MPS-2 = OPTI-FREE Replenish; HPS = CLEAR CARE PLUS; MPS-3 = Biotrue Hydration Plus; MPS-4 = ACUVUE RevitaLens; MPS-5 = COMPLETE; MPS-6 = OPTI-FREE Express. Key to statistical comparisons, all at $\alpha = 0.05$: *Not significantly different from each other, but greater than all other solutions; #Significantly greater than all remaining solutions; §Significantly greater than MPS-2 but not MPS-1; @Not significantly different from each other.

difference in efficacies between each of the solutions in the former group and each of the solutions in the latter group was significant for all three organisms (all $p < 0.05$).

Overall stand-alone disinfection efficacy (average over all three challenge organisms) is shown for all disinfectant solutions in Figure 2. MPS-3 and MPS-4 performed comparable to HPS (both $p \geq 0.05$). Each of the solutions in this group performed significantly better than did MPS-5 (all $p < 0.05$), which performed better than the three MAPD/PQ-1-containing solutions (all $p < 0.05$).

Discussion

While still not completely understood a half-century after their discovery, CIEs are believed the result of an acute inflammatory response to bacteria, bacterial endotoxin, enzymes, and/or metabolic byproducts carried from a contaminated CL case to the ocular surface via the CL.[1–4,9] As 25–50% of all CL cases are estimated to be contaminated,[5,9] CL care solutions that are effective at reducing populations of CL case CIE-associated organisms, including *Delftia, Stenotrophomonas*, and *Achromobacter* species, should benefit reusable CL wearers.[9,13–15]

In this study, disinfection efficacies of six MPSs and one HPS against three CL case CIE-associated organisms was found to be solution- and microbe-dependent. Solutions containing PHMB (MPS-5), PAPB/Alexidine/PQ-1 (MPS-3) or Alexidine/PQ-1 (MPS-4) were more effective than those containing MAPD/PQ-1 (MPS-1, MPS-2, and MPS-6; Figure 1), with relative efficacies reflecting the different actions of modern MPS disinfectant components.[17] In a previous study,[26] each of these disinfectant solutions achieved the ISO 14729 standard primary criteria for ISO compendial organisms (3-log reduction in each of three bacteria (*Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Serratia marcescens* (ATCC 13880)), and a 1-log reduction in each of two fungi (*Candida albicans* (ATCC 10231) and *Fusarium solani* (ATCC 36031)); MPS-5 only achieved a 0.4 log reduction for *C. albicans*.[26] It should be noted that ISO 14729 also specifies a second test method (Regimen test) for evaluating CL disinfection as part of a cleaning/disinfecting regimen for solutions that meet a secondary criteria (stasis for yeast and mold), which MPS-5 did in the previous study.[25]

Lower biocidal efficacy of MAPD-containing solutions against these three CL cases CIE-associated organisms was also reported previously.[14,27] Examination of CL storage cases of CIE patients, all of whom reported using the same MPS

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containing MAPD and PQ-1 (MPS-2 in this study), revealed the bacterial species present in those cases as *Achromobacter* spp. (61% of cases), *Stenotrophomonas maltophilia* (22%), *Serratia marcescens* (17%), *Delftia* spp. (17%), and *Elizabethkingia* spp. (11%).[14] Subsequent in vitro testing found this MPS to achieve less than one-log reduction for all of the recovered *Achromobacter* strains after incubation between 6 hours and 14 days, and two recovered strains of *S. maltophilia* and *Delftia* spp. were found to grow in the MPS over 14 days. In contrast, two other MPSs containing MAPD/PQ-1 performed better overall after 6 hours incubation (MPS-1 and MPS-6 in this study), as did an MPS containing PAPB/PQ-1 (Biotrue Multi-Purpose Solution, Bausch and Lomb Incorporated).[14] A different study found that MPSs containing either PAPB/PQ-1 or Alexidine/PQ-1 (Biotrue Multi-Purpose Solution and MPS-4 in this study, respectively) achieved 2.90 to 5.00-log reduction in the same CL case CIE-associated microbes evaluated in this study after incubation for the manufacturer-recommended minimum soak times, while the three solutions containing MAPD/PQ-1 (MPS-1, MPS-2, MPS-6 in this study) achieved less than 3-log reduction for all microbes (range −0.03 to 2.97).[27]

The results of this study provide insight into the relative efficacy of MPSs in an in vitro setting, but should be considered in the context of the limitations inherent with a small study in an in vitro setting. The findings presented here do not account for the impact of patient behavior on MPS efficacy in a real-world context,[28,29] including additional microbial contamination after repeated removal or insertion of CLs, into cases containing old or 'topped off' solution. In addition, while we have shown the relative efficacy of MPSs tested, determining the impact of additives (surfactants, wetting agents, etc.) and each individual disinfectant component during cleaning and lens wear is beyond the scope of this study.

Eye care practitioners should consider disinfection efficacy against organisms frequently associated with CL storage case contamination when recommending CL disinfecting solutions to their patients, and continue to counsel patients on the most appropriate CLs and CL care products to fit their needs. In addition, emphasis should be placed on the importance of good hand, CL, and CL case hygiene, as they remain important components for minimizing the risk of CIEs and MK.[1,2,17] Patients should also be encouraged to follow manufacturer recommendations regarding use of care solutions for CL and CL case cleaning and disinfection, as well as CL and CL case replacement schedules.[1,2]

Conclusion

MPSs differ in their antimicrobial efficacies. With respect to the three Gram-negative CL case CIE-associated organisms evaluated in this study, disinfectants PAPB, PHMB, and Alexidine appear to be more effective than MAPD at the concentrations studied. In addition, our findings suggest that MPS formulations containing PAPB and Alexidine, or PQ-1 and Alexidine, were as effective as HPS in this study against the three Gram-negative organisms evaluated.

Data Sharing Statement

All relevant data are within the manuscript. Clarification requests around the manuscript and its data can be made to the corresponding author.

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Disclosure

All the authors are employees of Bausch & Lomb Incorporated. All authors attest that they meet the current ICMJE criteria for authorship.

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