


Biocidal Efficacies of Contact Lens Disinfecting Solutions Against International Organization for Standardization (ISO) Compendial Organisms

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Purpose: This study was conducted to evaluate and compare the in vitro disinfection efficacies of six commercial lens cleaning and disinfecting products for planned replacement soft contact lenses.

Methods: Disinfection efficacies of five multi-purpose solutions (MPSs) and one hydrogen peroxide solution (HPS) as control were evaluated in the presence of organic soil according to the International Organization for Standardization (ISO, Geneva, Switzerland) ISO 14729 stand-alone test protocol. The five specified compendial organisms, three bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Serratia marcescens*) and two fungi (*Candida albicans* and *Fusarium solani*) were incubated with each solution under standard conditions, after which microbes were recovered and quantified.

Results: Each of the solutions evaluated met or exceeded the standard's primary criteria (3-log reduction of bacteria and 1-log reduction of fungi) after incubation for the manufacturer-recommended soaking time, except for COMPLETE MPS, which achieved only 0.4 ± 0.1 average log reduction for *C. albicans*. However, differences in efficacy between the solutions were noted. Average log reduction across all microbes for Biotrue Hydration Plus (4.6 ± 0.1) was comparable to that for CLEAR CARE PLUS HPS (4.3 ± 0.1) and greater than those for OPTI-FREE puremoist (3.6 ± 0.1), OPTI-FREE Replenish (4.0 ± 0.2), ACUVUE RevitaLens (3.9 ± 0.03), and COMPLETE MPS (3.6 ± 0.1). Biotrue Hydration Plus was especially effective at reducing the population of *C. albicans* (4.2 ± 0.7 -log reduction).

Conclusion: Products marketed for planned replacement soft CL disinfection generally meet the ISO 14729 standard's primary criteria for reducing populations of compendial organisms, with larger differences between solutions noted with *C. albicans*.

Keywords: multi-purpose solution, MPS, hydrogen peroxide solution, HPS, disinfection, myristamidopropyl dimethylamine, MAPD, polyhexamethylene biguanide, PHMB, ISO 14729

Introduction

Contact lenses (CLs) for vision correction remain popular, with an estimated 45 million wearers in the United States (US)¹ and 150 million wearers world-wide.² Based upon a recent global survey, daily disposable contact lenses accounted for 45% of all lens fits worldwide in 2023, while planned replacement soft CLs accounted for 40%.³ A recent US survey found that a large majority (about 35 million wearers in the US) continued to wear planned replacement soft CLs in 2021.⁴ For these wearers, the choice of multi-purpose solution (MPS) may play a role in helping maintain successful CL wear.

CL disinfection is critical for the prevention of microbial keratitis (MK) associated with lens wear, which although infrequent is reported to occur in 2–40 per 10,000 CL wearers annually, depending upon lens type, and has remained relatively constant despite the introduction of silicone hydrogel lenses.⁵ While separate solutions for disinfecting and cleaning/conditioning lenses were once common,⁶ and still exist today, most current planned replacement soft CL wearers worldwide instead opt to use multi-purpose solutions (MPSs),³ owing to the convenience of a single solution

that disinfects, cleans, conditions, rinses, and stores lenses.^{7,8} In the US and most of the world, a CL disinfecting solution must meet stringent performance requirements before commercial sale,⁹ yet products differ in subtle ways that can affect their respective clinical performances with different CLs.⁷

MPSs have become more sophisticated since the introduction of the first commercial MPS in 1987 (ReNu MPS, Bausch & Lomb Incorporated, Rochester, NY). The original ReNu formulation was composed of sterile, borate-buffered, isotonic saline solution containing polyaminopropyl biguanide (PAPB) disinfectant, edetate disodium (EDTA) chelating agent, and poloxamine 1107 surfactant. More recently, manufacturers have added additional ingredients, including multiple disinfectants, demulcent/wetting agent(s) for comfort, and different surfactant(s) for lens cleaning, conditioning, and comfort.¹⁰ A single all-in-one solution is more convenient for the planned replacement soft CL wearer and aids in improving wearer compliance.⁴ Contrary to sterilization as achieved by heat during CL manufacturing, the goal of disinfection via rub, rinse, and soak in MPS between lens wearing periods is to reduce the population of pathogenic microorganisms well below the threshold that results in MK.¹¹ Disinfectants in the MPS must provide adequate biocidal activity at the concentrations used, yet be sufficiently biocompatible to minimize adverse interaction with the corneal epithelium after placement of the lens upon the ocular surface.¹⁰

Manufacturers must demonstrate contact lens care product safety and effectiveness to satisfy regulatory requirements before commercialization. Most rely upon International Organization for Standardization (ISO, Geneva, Switzerland) standardized test methods to demonstrate safety, efficacy, and compatibility of care solutions with CLs to do so.⁷ The ISO 14729 standard specifies two test protocols for evaluating the antimicrobial activity of CL-disinfecting products, the stand-alone test (to establish adequate antimicrobial activity of the solution only for use as CL-disinfecting product) and the regimen test (to establish adequate antimicrobial activity of the individual solution in combination with a CL as part of a multifunctional disinfecting regimen, eg, rub, rinse, and soak).¹² The stand-alone test specifies five challenges to the solutions, each containing a different microorganism representative of those associated with MK, these being three bacteria, *Staphylococcus aureus* (American Type Culture Collection, Manassas, VA; ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), and *Serratia marcescens* (ATCC 13880), and two fungi, *Candida albicans* (ATCC 10231) and *Fusarium solani* (ATCC 36031). Optionally, the standard allows for organic soil to be added to the challenge solutions prior to testing, as the presence of organic material can affect the biocidal activity of some disinfectants.^{13,14} The standard specifies organic soil as a certain concentration of heat-killed *Saccharomyces cerevisiae* (ATCC 4098) resuspended in heat-inactivated bovine serum, based upon ISO 14729, Annex E.¹² In the stand-alone test, a disinfecting solution that achieves a 3-log reduction in each of the bacteria and a 1-log reduction in each of the fungi after incubation for the manufacturers' respective minimum disinfection times (typically 4 or 6 hr) is deemed to meet the primary criteria and can thus be labeled as a contact lens disinfecting product. Solutions that do not satisfy the requirements to meet the primary criteria but do meet the secondary (lower antimicrobial activity) criteria may be evaluated in the regimen test with different lens types to demonstrate disinfection efficacy of both the CL and the soaking solution as part of a cleaning/disinfecting regimen. Regulatory bodies and experts advocate that eye care practitioners (ECPs) recommend a "rub and rinse" regimen with all MPS and soft CLs, particularly silicone hydrogel lenses, to maximize lens cleanliness, regardless of product labeling.¹⁵

This study was undertaken to evaluate the stand-alone disinfection efficacy against the ISO 14729 compendial organisms of five commercially available MPSs, including two recently introduced MPSs, Biotrue Hydration Plus Multi-Purpose Solution (launched in 2022) and Acuvue Revitalens Multi-Purpose Disinfecting Solution (launched in 2019), with a hydrogen peroxide solution (HPS) serving as control.

Materials and Methods

Multi-Purpose Solutions

Disinfection efficacies were evaluated for the five MPSs and one HPS control summarized in Table 1. Listed solution components, including surfactants/cleaners, disinfectants, and additional ingredients such as comfort agents, are based upon product package inserts.^{16–21}

Challenge Organisms

ISO 14729 standard compendial 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). Organism suspensions were filtered, as appropriate.

Organic soil was prepared according to Annex E of ISO 14729¹² and incorporated into each challenge organism suspension, achieving a final concentration of 1×10^7 to 1×10^8 colony-forming units (cfu)/mL.

Experimental Procedure

Disinfection efficacy was evaluated with three individual replicates of each organism in each solution according to ISO 14729 stand-alone test protocols.¹² For each replicate, the five MPSs (10mL) were dispensed into conical tubes (5 tubes per solution for the 5 organisms tested), and the HPS (10mL) was dispensed into the accompanying neutralizing lens cases provided by the manufacturer. Conical tubes and neutralizing lens 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 soak time (4 or 6 hours, Table 1). Each MPS was neutralized with Dey-Engley Broth (DEB; Remel™ D/E (Dey-Engley) Neutralizing Broth, Catalog Number R453042, ThermoFisher Scientific), 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 in triplicate and poured with Trypticase Soy Agar (bacterial species; BD Trypticase™ soy agar, SKU 299099, BD, Franklin Lakes, NJ) or Sabouraud Dextrose Agar (fungal species; SKU 299510 BD). Plates were incubated for recovery at 30°C to 35°C and 20°C to 25°C, respectively, for the appropriate incubation time. An inoculum control (IC) 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 (LRV) were calculated, based upon the mean log recovery of the IC, and mean LRV and standard deviation determined for each solution/organism combination.

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

Disinfecting Solution	Manufacturer	Buffer	Surfactants, Cleaners	Additional Ingredients	Disinfectant(s)	Manufacturer-Recommended Disinfection Time (Hours)
OPTI-FREE puremoist (MPS-1) ¹⁷	Alcon, Fort Worth, TX	Borate/citrate	Tetronic 1304 EOBO-41 EDTA	Sorbitol	PQ-I (0.001%) MAPD (0.0006%)	6
OPTI-FREE Replenish (MPS-2) ¹⁸		Borate/citrate	Tetronic 1304 C9-ED3A	Propylene Glycol	PQ-I (0.001%) MAPD (0.0005%)	6
CLEAR CARE PLUS (HPS) ¹⁶		Phosphate	Pluronic 17R4 EOBO-21		Hydrogen Peroxide (3%)	6
Biotrue Hydration Plus (MPS-3) ¹⁹	Bausch & Lomb Incorporated, Rochester, NY	Borate	Poloxamine 1107 Poloxamer 181 EDTA	Hyaluronan Erythritol Potassium	PAPB (0.00005%) Alexidine (0.00025%) PQ-I (0.00015%)	4
ACUVUE RevitaLens (MPS-4) ²⁰	Johnson & Johnson Vision, Jacksonville, FL	Borate/citrate	Tetronic 904 EDTA		PQ-I (0.0003%) Alexidine (0.00016%)	6
COMPLETE (MPS-5) ²¹		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-I, Polyquaternium-1.

Statistical Analysis

SAS software (Cary, NC) version 9.4 was used for data analysis.

Stand-alone disinfection efficacy was calculated as a 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 five challenge organisms (mean \pm standard deviation).

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

Results

Stand-alone disinfection efficacy (average log reduction of 3 replicates of each organism/disinfecting solution combination) is shown in Table 2. All disinfecting solutions evaluated achieved the primary criteria of the standard (3-log reduction in each of the bacteria and a 1-log reduction in each of the fungi after recommended 4 or 6 hr. disinfection times), except for MPS-5, which only achieved a 0.4 ± 0.1 log reduction for *C. albicans*.

Overall stand-alone disinfection efficacy (average reduction over all five challenge organisms) is shown for each disinfecting solution in Figure 1; disinfection efficacy against *C. albicans* is shown in Figure 2.

Discussion

One of the critical functions that CL care solutions serve is lens disinfection, without which wear of reusable lenses would not be possible. Both MPSs and HPS are formulated with antimicrobial agents to disinfect CLs between lens wearing periods and lessen the likelihood of MK associated with CL wear. Most contemporary solutions include additional ingredients to improve lens cleaning and comfort during lens wear. As the effect of these agents on the antimicrobial activity of solution disinfectants is not always known, every new solution must be evaluated to ensure that the disinfection properties of the solutions are not compromised. In this study, the disinfection efficacies of five MPSs as well as one HPS control were evaluated using the ISO 14729 stand-alone test procedure in the presence of organic soil.¹²

The best performing solutions overall based upon average log reduction across the compendial organisms were MPS-3 (4.6 ± 0.1) and HPS (4.3 ± 0.1), which were comparable ($p \geq 0.05$) and performed better than did MPS-2 (4.0 ± 0.2) and MPS-4 (3.9 ± 0.03 ; all $p < 0.05$), which were comparable and performed better than did MPS-1 and MPS-5 (both 3.6 ± 0.1 ; all $p < 0.05$), which were comparable ($p \geq 0.05$; Figure 1). While most planned replacement soft CL wearers choose MPS over HPS,³ both solution types performed well overall in this assay.

Table 2 Multi-Purpose (MPS) and Hydrogen Peroxide (HPS) Disinfection Efficacy by Microorganism

Microbe Solution	Stand-Alone Disinfection Efficacy (Mean Log Reduction of 3 Replicates \pm Standard Deviation)				
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>	<i>C. albicans</i>	<i>F. solani</i>
OPTI-FREE puremoist (MPS-1)	3.8 ± 0.6	4.6 ± 0.05	3.2 ± 0.2	1.6 ± 0.4	4.6 ± 0.1
OPTI-FREE Replenish (MPS-2)	4.0 ± 0.7	4.6 ± 0.05	4.1 ± 0.2	2.7 ± 0.6	4.6 ± 0.1
CLEAR CARE PLUS HPS	4.8 ± 0.04	4.6 ± 0.05	4.7 ± 0.1	3.0 ± 0.1	4.5 ± 0.2
Biotrue Hydration Plus (MPS-3)	4.8 ± 0.04	4.6 ± 0.05	4.7 ± 0.1	4.2 ± 0.6	4.5 ± 0.3
ACUVUE RevitaLens (MPS-4)	4.8 ± 0.1	4.6 ± 0.05	4.7 ± 0.1	1.1 ± 0.3	4.6 ± 0.1
COMPLETE (MPS-5)	4.8 ± 0.04	4.6 ± 0.05	4.6 ± 0.2	0.4 ± 0.1	3.5 ± 0.2

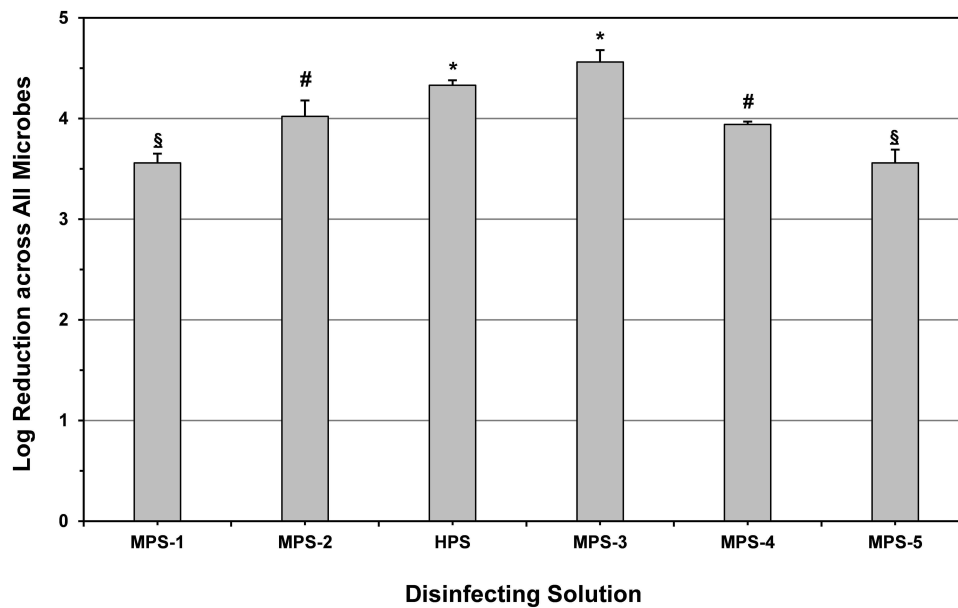


Figure 1 Overall disinfection efficacy of multi-purpose (MPS) and hydrogen peroxide (HPS) solutions (mean log reduction ± standard deviation).

Notes: Key to statistical comparisons, all at $\alpha = 0.05$: *Not significantly different from each other, but greater than all other solutions; #Not significantly different from each other, but less than all other solutions.

Abbreviations: 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.

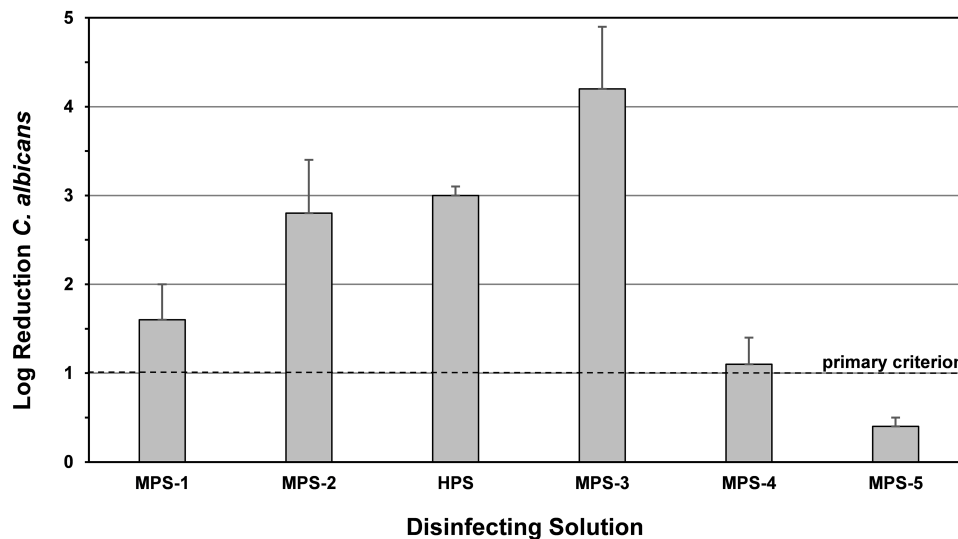


Figure 2 Disinfection efficacy of multi-purpose (MPS) and hydrogen peroxide (HPS) solutions against *C. albicans* (mean log reduction ± standard deviation). The dashed line represents the ISO primary criterion (1-log reduction in fungi species).

Notes: Statistical comparisons at $\alpha = 0.05$. MPS-3 is significantly greater than all other solutions; HPS is comparable to MPS-2 and significantly greater than all remaining solutions; MPS-2 is comparable to MPS-1 and significantly greater than all remaining solutions; MPS-1 is comparable to MPS-4 and significantly greater than MPS-5; MPS-4 is comparable to MPS-5.

Abbreviations: 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.

Literature generally confirms the in vitro efficacy of MPS and HPS against compendial organisms.^{22–27} Literature also suggests that HPS is effective at disinfecting CL-bound bacteria and fungi, and perhaps more effective than MPS with respect to the latter.²⁷ However, in this study MPS-3 performed better than did HPS at reducing the population of *C. albicans* (4.2 ± 0.7 , versus 3.0 ± 0.1 -log reduction, respectively; $p < 0.05$), as well as better than the other MPSs

evaluated (range 0.4 ± 0.1 to 2.8 ± 0.6 -log reduction, all $p < 0.05$; Figure 2). Further, MPS-2 and HPS were comparable (2.7 ± 0.6 , versus 3.0 ± 0.1 -log reduction, respectively; $p \geq 0.05$) and performed better than did both MPS-1 (1.6 ± 0.4) and MPS-4 (1.1 ± 0.03 ; all $p < 0.05$), which were comparable. All solutions performed better than did MPS-5 (all $p < 0.05$), which failed to meet the primary criteria (ie, achieved only 0.4 ± 0.1 log reduction of *C. albicans*) but did meet the secondary criteria (stasis for yeast and mold, which was the performance requirement at the time the solution was first cleared for sale in the US).¹⁴ There was less difference between all solutions with respect to *F. solani* compared to *C. albicans*. It should be noted that meeting the ISO 14729 disinfection criteria is a typical regulatory requirement for selling a CL disinfecting product in the US and most other countries. Failing to meet the primary criteria is not necessarily predictive of a solution's clinical performance in a real-world setting. In fact, widespread, problematic *C. albicans* infection among MPS-5 users has not been reported over the two decades since its commercial introduction.

Effective lens disinfection is a function of both the specific disinfectants used and their respective concentrations, the specific cleaning aids that facilitate separation of deposits from the lens, and the effects of other lens care solution components.⁷ Disinfecting solutions studied in this assay each contained either one disinfectant [MPS-5, PAPB (0.0001%); HPS, Hydrogen Peroxide (3%)], two disinfectants [MPS-1, PQ-1 (0.001%)/myristamidopropyl dimethylamine (MAPD; 0.0006%); MPS-2, PQ-1 (0.001%)/MAPD (0.0005%); MPS-4, PQ-1 (0.0003%)/Alexidine (0.00016%)] or three disinfectants [MPS-3, PAPB (0.00005%)/Alexidine (0.00025%)]/PQ-1 (0.00015%) (Table 1). The latter solution is relatively low in the concentration of each disinfectant compared with the other MPSs, yet as effective as HPS and more effective than the other MPSs in reducing the populations of the compendial organisms (Figure 1). Determining how individual MPS components or combinations of components contribute to disinfection efficacy is complicated, due to adverse and beneficial synergistic interactions between components. Further, efficacy against compendial and non-compendial organisms may differ.²⁷ For example, two MPSs containing PAPB, and one containing PQ-10 were reported more effective than two solutions containing PQ-1 and MAPD in killing four different clinical isolates of *S. marcescens* but equally effective at killing the compendial ATCC 13880 strain.²⁸

In addition to reducing microbial populations on lenses, contemporary solutions include ingredients that provide other benefits in addition to disinfection. Most include surfactant to aid in lens cleaning. MPS-5 includes poloxamer 237; MPS-1 and MPS-2 include Tetronic 1304; MPS-4 includes Tetronic 904; HPS includes Pluronic 17R4; MPS-1 and HPS include polyoxyethylene-polyoxybutylene (EOBO-41 and EOBO-21, respectively); MPS-3 includes poloxamer 181 and Tetronic 1107, the latter reported to improve subjective comfort and remain present in/on etafilcon A lenses for at least 8-hours of wear.²⁹ Some solutions include demulcent/wetting agents to improve comfort during lens wear.³⁰ MPS-1 includes sorbitol; MPS-2 includes propylene glycol; MPS-3 includes hyaluronan and erythritol, the latter acting both as a comfort agent and an osmoprotectant.³¹ Determination of how well these individual additives affect disinfection efficacy or provide other benefits during lens wear is beyond the scope of this study.

While CL-disinfecting solutions enable reusable CL-wear, they are most effective when wearers adhere to ECP and manufacturer's recommendations regarding lens wear and lens hygiene. CL wear itself is a risk factor for MK,³² which may be related to non-compliance or poor hygiene.³³ Poor handwashing (estimated to occur in up to half of all CL wearers) is a known risk factor for MK, independent of which CL care system is used.³⁴ With few CL wearers fully compliant with CL care and wear practice,^{35,36} and nearly four in five young adult wearers in a health care setting admitting to inadequate CL and lens case maintenance,³⁷ ECPs should consider CL disinfection efficacy when recommending lens disinfecting solutions to their patients, as well as encourage lens wear compliance (ie, washing hands, not reusing or topping off solution, not using expired solution, replacing lenses and cases at manufacturer-recommended intervals, refraining from overnight wear) to ensure maximal CL-disinfecting solution efficacy.³⁸ Regardless of the disinfecting solution used, compliance with ECP recommendations and manufacturer's instructions for use is paramount for effective lens disinfection and prevention of MK in the real-world setting.

Conclusion

All MPSs evaluated exceeded the ISO 14729 primary criteria for each of the five challenge organisms, except for MPS-5, which achieved only 0.4 log reduction for *C. albicans*. MPS-3 showed greater efficacy against *C. albicans* than did all

other solutions, and MPS-3 and HPS showed greater overall antimicrobial efficacy across the five ISO compendial organisms compared with the other MPS 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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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All the authors are employees of Bausch & Lomb Incorporated.

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