

Reduction of disinfection efficacy of contact lens care products on the global market in the presence of contact lenses and cases

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ABSTRACT

Objective Sight-threatening infections can be caused by pathogenic micro-organisms colonising the cornea, leading to microbial keratitis (MK). These micro-organisms can be introduced to the eye via improper contact lens use and care. MK can also result from ineffective contact lens care solutions (CLCs), even if the patient is following best practice guidelines. Therefore, it is critical to understand the differences between the effectiveness of popular CLCs on the global market.

Methods and analysis Following the International Standards Organisation standards 14 729 and 18259, bacteria (*Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*), fungi (*Candida albicans*, *Fusarium* strains) and *Acanthamoeba* strains were inoculated into each CLC with and without contact lenses, and held for the manufacturer's stated disinfection time. Plate counts were conducted to determine the number of surviving micro-organisms.

Results All CLCs examined met the primary log reduction criteria during stand-alone testing for *Pseudomonas*, *Staphylococcus*, *Candida* and *Fusarium*. *renu Multiplus*, All Clean Soft, and Kombiösung Super did not meet the primary criteria when challenged with *Serratia*. Only OPTI-FREE Express exceeded 4 log reduction for both strains of *Acanthamoeba* tested. We noted a substantial reduction in disinfection efficacy when CLCs were challenged with *Fusarium* in the presence of lenses and cases versus stand-alone testing. OPTI-FREE Express demonstrated significantly less net log reduction loss than the other four CLCs tested.

Conclusion Of the popular CLCs on the global market, the product which relies on dual biocides polyquaternium-1 and myristamidopropyl dimethylamine demonstrated the highest disinfection efficacy in microbial disinfection challenges in the absence and presence of contact lenses.

INTRODUCTION

Corneal ulceration leading to loss of sight is a serious side effect of the ocular infections caused by opportunistic pathogens, a condition known as microbial keratitis (MK).¹ MK is a serious affliction known to affect over 30 000 people in the USA every year.¹ The

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ While contact lens care efficacy is routinely demonstrated in a stand-alone testing setting, and most products on the market meet or exceed the required disinfection efficacy set forth by the International Standards Organisation, it is important to examine how effective the contact lens care products are in the presence of contact lenses and cases.

WHAT THIS STUDY ADDS

⇒ We show here that, when challenged with one of the most common causative agents of microbial keratitis, *Fusarium*, most market contact lens care products demonstrate a substantial loss of disinfection efficacy in the presence of lenses and cases.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND/OR POLICY

⇒ The real-world disinfection efficacy of any contact lens care product is critical information for clinicians and patients to know when selecting their contact lens care products.

largest risk factors for the development of MK are the introduction of these pathogens to the eye via improper use of contact lenses or inefficient lens disinfection solutions.^{1 2} Failure to adhere to suggested practices include skipping the rub and rinse step, lack of fresh disinfection solution daily (ie, topping off of solutions), infrequent case replacement, wearing contact lenses during showering or swimming, or failure to replace contact lenses at the recommended time. Further, even if patients adhere to best practices, inefficient contact lens care solutions (CLCs) have been shown to possess ineffective biocides, leading to outbreaks of *Fusarium* keratitis^{3 4} and *Acanthamoeba* keratitis.^{5 6} Unfortunately, both *Fusarium* and *Acanthamoeba* pose unique challenges as difficult organisms to disinfect against. Bacterial pathogens which are the most common sources of MK are routinely



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highly susceptible to CLCs.^{7–9} However, *Fusarium* and *Acanthamoeba* stand out as highly differentiating, challenging micro-organisms against which not all products are highly effective. Thus, it is imperative that we understand which CLCs on the global market are effective against these two pathogens.

In particular, not only are these two species difficult to disinfect against *in vitro*, but they are also extremely challenging to treat once the infection has flourished *in vivo*. Compared with viruses and bacteria, these two micro-organisms are highly similar to mammalian cells, severely limiting treatment options which are otherwise available for other MK cases as effective treatments would be equally damaging to cornea cells. Fortunately, while these micro-organisms should be taken seriously, the incidence rate of infection is relatively low, and these are organisms which are more commonly found in the environment as opposed to more ubiquitous human colonisers such as *Staphylococcus*. Nonetheless, demonstrating CLC disinfection efficacy against both *Fusarium* and *Acanthamoeba* is critical. Previous investigations have indicated that the biocides included in each CLC govern the differences in disinfection efficacy against any particular organism. CLCs are evaluated for antimicrobial activity by the International Standards Organisation (ISO). The ISO protocols dictate testing requirements for the disinfection efficacy of CLCs when they are challenged with contact lenses and lens cases (18259)¹⁰ and without lenses (primary criteria for 14729).¹¹ It has been recently demonstrated that both contact lenses and the lens cases themselves can have an impact on disinfection efficacy due to the different materials of the lenses and cases taking the biocides out of solution,^{7,12} thereby reducing the antimicrobial activity of the CLC during actual patient use. While we have recently demonstrated that most of the common CLCs on the market are effective against pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*,⁷ previous investigations have yet to examine the efficacy of CLCs on the global market against *Fusarium* and *Acanthamoeba*, particularly in the presence of more recently released lenses.

Thus, the present investigation aims to investigate the disinfection efficacy of five different preserved CLCs containing a range of biocides, both in the absence and the presence of contact lenses and lens cases, when challenged with eight different common ocular micro-organisms. We also demonstrate the substantial difference in efficacies of the ISO 14729 and ISO 18259 protocols to assess the antimicrobial ability of these CLCs when used in a stand-alone test compared with when they are used with contact lenses and cases, as in a real-world setting.

MATERIALS AND METHODS

Acanthamoeba trophozoite culturing

As previously described,¹³ trophozoites were axenically cultured in AC6 media (axenic culture medium; containing 20 g biosate peptone, 5 g glucose, 0.3 g

KH_2PO_4 , 10 µg vitamin B₁₂, and 1 glass 5 mg L-methionine per litre of distilled deionised water). AC6 was adjusted to a pH 6.6–6.95 with 1M NaOH and autoclaved at 121°C for 20 min before being stored at room temperature for use within 2 months. Organisms were harvested using ¼ Ringer's solution. *Acanthamoeba* strains were obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA). *Acanthamoeba polyphaga* (ATCC 30461), Group T4, isolated from human eye infection (Namibia or South Africa, 1973) and *Acanthamoeba castellanii* (ATCC 50370), also Group T4, isolated from human eye infection (New York, New York, 1978) were the two strains used in this study. Importantly, these two commonly used clinical strains belong to the T4 genotype, which is the most commonly associated genotype with *Acanthamoeba* keratitis. To create a homogenous population of *Acanthamoeba* trophozoites, amoeba were scaled up in fresh AC6 media 24 hours prior to testing. Cells were then collected and centrifuged at 500g for 5 min, followed by a wash and resuspension using ¼ Ringer's solution. Count seeding was confirmed via haemocytometer.

Preparation of bacterial and fungal suspensions

Fusarium strains were acquired from ATCC and the Alcon Laboratories Microbial Collection (AMC, Fort Worth, Texas, USA). Mould cultures (*Fusarium keratoplasticum* (formerly identified as *Fusarium solani*), ATCC 36031; *Fusarium chlamydosporum*, AMC 5663; and a clinical isolate of *Fusarium*, AMC 1620) were transferred to potato dextrose agar and incubated for 10–14 days at 20°C–25°C. Spores were harvested using Dulbecco's phosphate buffered saline with 0.05% polysorbate 80 and filtered through glass wool.

Bacterial cultures (*Pseudomonas aeruginosa*, ATCC 9027; *Serratia marcescens*, ATCC 13880; *Staphylococcus aureus*, ATCC 6538) were transferred to soybean casein digest agar, while yeast cultures (*Candida albicans*, ATCC 10231) were transferred to sabourad dextrose agar, and were incubated for 18–24 hours at 30°C–35°C. Cells were harvested using 0.9% saline with 0.1% peptone.

Following this, micro-organism suspensions were adjusted to a final concentration of approximately 10⁵–10⁶ colony forming units (CFU) per mL, and resuspended in a 10% organic soil suspension containing heat-killed *Saccharomyces cerevisiae* cells (ATCC 9763; 10⁷ to 10⁸ CFU/mL) and heat-inactivated fetal bovine serum (VWR, Radnor, PA, USA).

ISO protocols used to examine disinfection efficacy

The disinfection efficacy of the examined CLCs was determined by using ISO 14729 test methods and criteria. In addition, antimicrobial efficacy endpoint methodology compatibility was performed in accordance with the ISO 18259 protocol methodology. Both ISO 14729 (stand-alone tests) and ISO 18259 (with-lens tests) antimicrobial efficacy testing were performed. While ISO 14729 also includes testing for *Candida albicans*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*,

commercially available products typically demonstrate a high degree of disinfection efficacy against most of these organisms with and without lenses present, although some products struggle to show with-lens disinfection efficacy against *Candida*.^{7,9} Therefore, of the ISO 14729 organisms, we chose the *Fusarium* spp as a highly challenging (and also common pathogenic organism causing MK)⁴ organism to differentiate between products. Further, while no standard yet exists for determining disinfection efficacy against *Acanthamoeba*, a protocol for examining this is currently being developed by the American National Standards Institute (ANSI).¹⁴ *Acanthamoeba* can also be considered a difficult and differentiating organism to outline the overall disinfection efficacy of any CLC.^{13,15,16}

Contact lenses and CLC solution used

The CLC solutions and their manufacturers, biocides, and stated disinfection times are as follows: OPTI-FREE Express (Alcon, Fort Worth, Texas, USA; polyquaternium-1 (0.001%), myristamidopropyl dimethylamine (0.0005%); 6 hours), Kombi-Clean & Moist, (Acumed, Hillsboro, Oregon, USA; polyhexamethylene biguanide (0.0002%), polyquaternium (0.004%); 6 hours), All Clean Soft (Avizor, Madrid, Spain; polyhexanide (0.0002%); 4 hours), Kombilösung Super (VISIOMAX, Neuberger, Germany; polyhexamethylene biguanide (0.0002%); 4 hours), and renu Multiplus (Bausch+Lomb, Rochester, NY, USA; polyaminopropyl biguanide (0.0001%); 4 hours). The contact lenses were all Group five contact lenses. Their manufacturers and compositions are: AIR OPTIX AQUA plus HydraGlyde (Alcon, Fort Worth, TX, USA; lotrafilcon B), ACUVUE VITA (Johnson & Johnson, New Brunswick, New Jersey, USA; senofilcon C), ACUVUE OASYS (Johnson & Johnson; senofilcon A), ULTRA (Bausch+Lomb; samfilcon A), and Avaira Vitality (CooperVision, Lake Forest, California, USA; fanfilcon A).

Stand-alone inoculation with micro-organisms

As previously described,⁹ ISO 14729 testing was performed by inoculating 1.0×10^5 to 1.0×10^6 CFU/mL of the tested micro-organisms into test tubes containing 10 mL of the required CLC. Test tubes were made of compatible materials based on the biocides within the CLC (Grenier round bottom polystyrene tubes, Millipore Sigma #Z617776, Burlington, Massachusetts, USA). Test samples and controls were evaluated to determine the number of surviving micro-organisms at the recommended disinfection time.

Contact lens inoculation with *Fusarium*

As previously described,⁹ ISO 18259 was performed by aseptically removing contact lenses from the blister package and soaked in phosphate buffered saline for 18 hours. The lenses were briefly blotted on sterile gauze to remove excess solution, and placed in the matching manufacturer's contact lens case concave side up.

Lenses were then inoculated to contain a final count of 1×10^5 to 1×10^6 CFU/mL of the specified micro-organism. Following a contact time of 3 min, the required CLC was added to the lens case to the fill line and the cases were closed, giving special attention to not contaminate the cap. Closed cases were stored at 20°C–25°C. Separate lenses and cases were prepared for each specific sampling time to avoid opening and closing, or re-entering, cases before their final endpoint. Test samples and controls were evaluated to determine the number of surviving micro-organisms at the recommended disinfection time. The lens cases were vortexed vigorously for 30 s prior to sampling.^{10,17} Lenses were then removed from the lens cases and discarded.

Micro-organism recovery

To recover surviving micro-organisms for both ISO standards, aliquots of 1 mL of the solution or lens/solution combination and their controls were transferred to test tubes containing 9 mL of Dey-Engley neutralising broth (DE broth, Difco, Detroit, MI). Serial 1:10 dilutions were conducted using additional test tubes containing DE broth. Appropriate neutralisation times were validated prior to testing such that products had sufficient contact time with the neutraliser to ensure any surviving micro-organisms were recoverable. DE broth was shown to be effective at neutralising antimicrobial agents contained in the test solutions. The recovery of micro-organisms from the neutralising broth with products was within 50% of the recovery of micro-organisms from the control tube (containing no CLC product) for all test micro-organisms.

Micro-organism quantification

Dilutions were then plated to quantify the CFU/mL. Bacterial and fungal pour plates were prepared with Soyabean Casein Digest Agar containing 0.07% lecithin and 0.5% polysorbate 80. Bacterial and yeast plates were incubated for 2–5 days at 30°C–35°C, and mould plates were incubated for 5–7 days at 20°C–25°C. Following the incubation period, plate counts were conducted and the CFU/mL was calculated based on the average from duplicate plates. Colonies resulting from *Fusarium* spores (ie, hyphae) were quantified.

Acanthamoeba was prepared on non-nutrient agar with 100 µL of *Escherichia coli* (10^8 CFU/mL) and incubated for 14 days at 26°C–30°C. Positive wells were identified and surviving trophozoites quantified using the 50% endpoint following the Reed and Muench computation.¹⁸ The 50% endpoint calculation is used to determine where exactly in a dilution series the 50% mortality of an organism lies. In this instance, for each experiment, the number of wells were counted which contained live organisms following the CLC challenge and 2-week incubation period. Each dilution is plated into four wells, and there are six dilutions per condition per replicate. By determining the two consecutive dilutions in which there were over 50% positive wells and under 50% positive wells, respectively, the

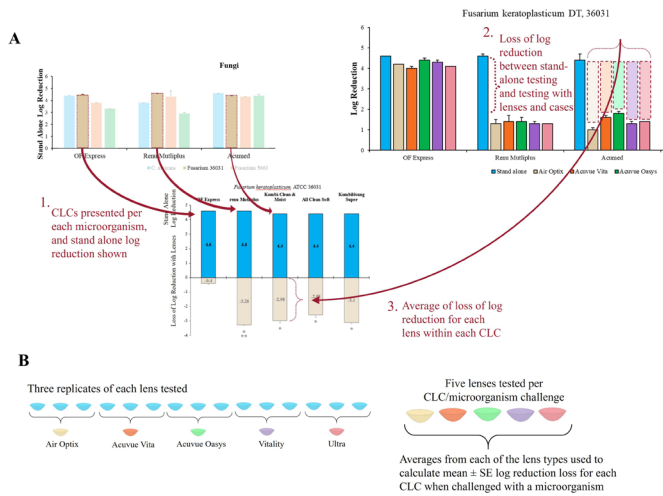


Figure 1 Representative depiction of the calculation and comparison of average of log reduction loss when products are challenged with lenses and cases. (A) (1) Stand-alone log reduction data (2) are represented on the positive log reduction y-axis, while loss of log reduction when CLCs are challenged with micro-organisms using lenses and cases (3) is represented in the negative y-axis. (B) The cumulative data from each lens experiment (composed of 3 replicates for each CLC-micro-organism challenge) are then counted as one grouped replicate within the loss of log reduction statistical comparison. Therefore, as five different lens types are used for each CLC-micro-organism challenge, the sample size for log reduction loss is calculated as five, and SE is calculated using the five grouped lenses. CLCs, contact lens care solutions.

proportionate distance between those dilutions and from there the surviving cells/mL of the original sample were calculated. Each *Acanthamoeba* strain was tested in triplicate and the results averaged.

Statistical analysis

Stand-alone log reduction and with-lens log reduction was calculated and depicted in mean±SE as described above. Loss of log reduction when comparing stand-alone results to with-lens results was calculated by averaging the three with-lens replicates for any lens-CLC-micro-organism combination. Those averages were then considered as one data point within any CLC-micro-organism disinfection challenge. Thus, as five lenses were tested within any CLC-micro-organism challenge, each CLC-micro-organism combination maintained a sample size of 5. Statistical analysis and SE were calculated based on this average loss of log reduction across the five lenses tested (figure 1). Quantifications were analysed via one-way analysis of variance with a post hoc Tukey's test, with $p < 0.005$ being used for significance.

RESULTS

Stand-alone testing (ie, CLCs in a test tube, challenged with micro-organisms directly) was conducted according to ISO 14729. CLCs on the global market were challenged with micro-organisms required in ISO 14729. The results

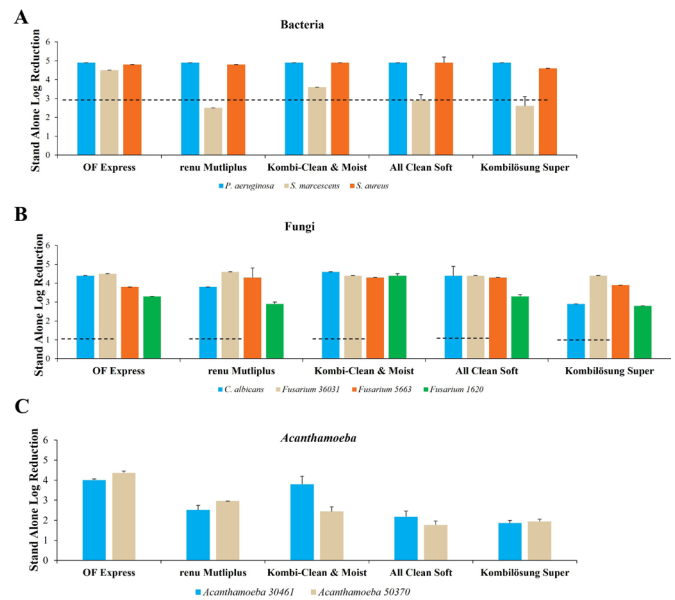


Figure 2 Stand-alone testing results, according to iso 14729, for (A) required bacterial species, (B) required fungal species, in addition to two other *Fusarium* strains, and (C) *Acanthamoeba polyphaga* (ATCC 30461) and *Acanthamoeba castellanii* (ATCC 50370). Dotted lines represent the primary criteria-required log reduction stated in iso 14729: 3 log reduction for CLCs challenged with bacterial species, 1 log reduction for CLCs challenged with fungal species. there are no criteria specified for *Acanthamoeba*. n=3/group. CLC, contact lens care.

of these challenges are presented in figure 2A, which are the bacterial pathogens, and in figure 2B, which are the yeast and mould pathogens. Of the yeast and mould, only *Candida albicans* (ATCC 10231) and *Fusarium keratoplaticum* (ATCC 36031) are required. We additionally tested two clinical isolates, *Fusarium chlamydosporum* (AMC 5663) and *Fusarium spp.* (AMC 1620). The primary criteria of ISO 14729 requires that CLCs demonstrate a minimum of a 3 log reduction when challenged with *Pseudomonas aeruginosa* (ATCC 9027), *Serratia marcescens* (ATCC 13880) or *Staphylococcus aureus* (ATCC 6538). All CLCs challenged met these requirements, except for renu Mutliplus, All Clean Soft and Kombilösung Super when challenged with *Serratia marcescens* (ATCC 13880). The primary criteria of ISO 14729 also requires that CLCs demonstrate a 1 log reduction when challenged with *Candida albicans* (ATCC 10231) or *Fusarium keratoplaticum* (ATCC 36031). All CLCs tested met this requirement. While the ISO is currently undertaking analysis and testing to add *Acanthamoeba* to the ISO requirements for CLC disinfection efficacy, this micro-organism is not currently mandated part of the compulsory testing. However, we here undertook stand-alone testing for two of the most commonly examined *Acanthamoeba* strains, *A. castellanii* and *A. polyphaga* (figure 2C). While all CLCs demonstrated at least a 1.5 log reduction, only OPTI-FREE Express demonstrated greater than 4 log reduction for both *Acanthamoeba* trophozoite strains.

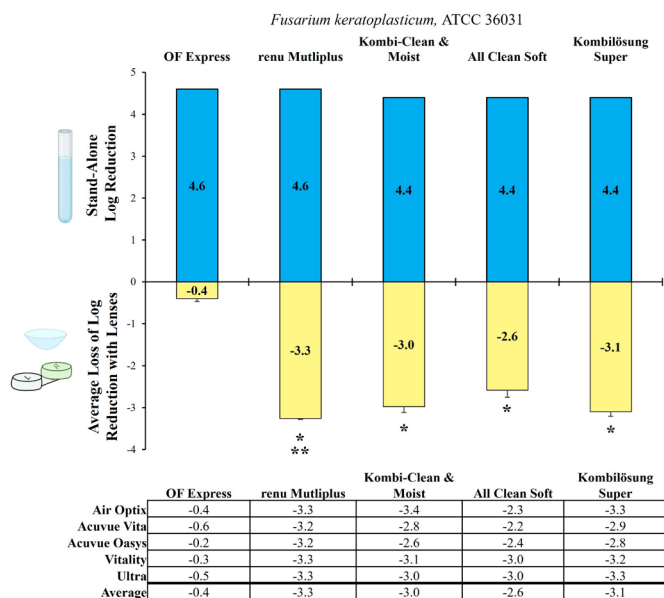


Figure 3 Comparison of the average loss of log reduction when CLCs are challenged with *Fusarium keratoplasticum* (ATCC 36031) in the presence of lenses and cases. Top panel: stand-alone testing (positive y-axis) was undertaken according to iso 14729, and testing with lenses and cases was performed according to iso 18259. Loss of log reduction when comparing disinfection efficacy between stand-alone and with-lenses is presented on the negative y-axis. Bottom panel: loss of log reduction for each lens type and CLC is shown, as a comparison to the stand-alone log reduction calculated for each CLC. * $P < 0.005$ vs OPTI-FREE express. ** $P < 0.005$ vs. All Clean Soft. $n = 5$ /group. CLC, contact lens care.

We next examined the differences in disinfection efficacy when a CLC was used in stand-alone testing vs when the CLC was used in a real-world scenario, with a contact lens and contact lens case. As all CLC products are meant to be used with contact lens cases and contact lenses, assessing them in their absence fails to truly describe their effectiveness. This with-lens testing is governed by ISO 18259, although no log reduction requirements are mandated. Three replicate lenses were tested for each lens-CLC-micro-organism combination. Each lens was then counted as one replicate within each CLC-micro-organism combination. Thus, within each CLC-micro-organism combination, there is a sample size of 5, accounting for the five lenses tested (figure 1). We used the three *Fusarium* strains as indicator organisms, as *Fusarium* is often one of the most challenging organisms to disinfect against. We examined the reduction of disinfection efficacy caused by lenses and cases for *Fusarium keratoplasticum* (figure 3), *Fusarium chlamydosporum* (figure 4) and the *Fusarium spp* clinical isolate (figure 5) as compared with the disinfection efficacy found in stand-alone testing.

Within *Fusarium keratoplasticum* (figure 3), we found that OPTI-FREE Express demonstrated an average loss of 0.4 log reduction when tested with lenses and cases vs stand-alone testing. Conversely, we found that renu

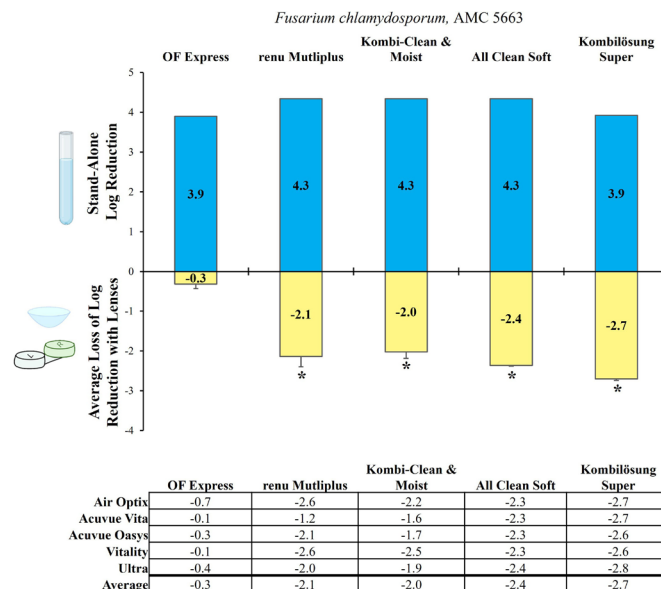


Figure 4 Comparison of the average loss of log reduction when CLCs are challenged with *Fusarium chlamydosporum* (AMC 5663) in the presence of lenses and cases. Top panel: stand-alone testing (positive y-axis) was undertaken according to iso 14729, and testing with lenses and cases was performed according to iso 18259. Loss of log reduction when comparing disinfection efficacy between stand-alone and with-lenses is presented on the negative y-axis. Bottom panel: loss of log reduction for each lens type and CLC is shown, as a comparison to the stand-alone log reduction calculated for each CLC. * $P < 0.005$ vs OPTI-FREE express. $n = 5$ /group. CLC, contact lens care.

Multiplus, Kombi-Clean & Moist, All Clean Soft, and Kombilösung Super demonstrated average losses of 3.3, 3.0, 2.6 and 3.1 log reduction, respectively, versus stand-alone testing. Thus, we found that OPTI-FREE Express demonstrated significantly less log reduction loss versus the other four CLCs ($p < 0.005$), and that All Clean Soft demonstrated significantly less log reduction loss vs renu Multiplus ($p < 0.005$).

When challenging these products with *Fusarium chlamydosporum* (figure 4), we found that OPTI-FREE Express demonstrated an average loss of 0.3 log reduction, while renu Multiplus, Kombi-Clean & Moist, All Clean Soft and Kombilösung Super demonstrated average losses of 2.1, 2.0, 2.4 and 2.7 log reduction, respectively, when tested with lenses and lens cases versus stand-alone testing. Accordingly, the loss of disinfection efficacy demonstrated by OPTI-FREE Express was significantly lower compared with the other four CLCs tested ($p < 0.005$).

Finally, we similarly examined these CLC products when challenged with a clinical isolate of *Fusarium* (figure 5). OPTI-FREE Express, renu Multiplus, Kombi-Clean & Moist, All Clean Soft and Kombilösung Super demonstrated average losses of 1.0, 2.5, 2.9, 3.0, and 2.3 log reduction, respectively, when tested with lenses and cases vs stand-alone testing. When comparing products, we found that OPTI-FREE Express demonstrated significantly less log reduction loss compared with the other

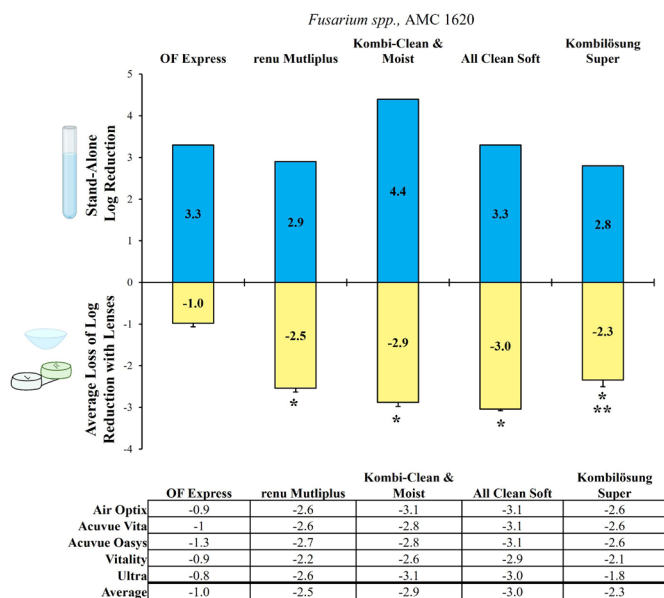


Figure 5 Comparison of the average loss of log reduction when CLCs are challenged with a clinical *Fusarium* isolate (AMC 1620) in the presence of lenses and cases. Top panel: stand-alone testing (positive y-axis) was undertaken according to iso 14729, and testing with lenses and cases was performed according to iso 18259. Loss of log reduction when comparing disinfection efficacy between stand-alone and with-lenses is presented on the negative y-axis. Bottom panel: loss of log reduction for each lens type and CLC is shown, as a comparison to the stand-alone log reduction calculated for each CLC. * $P < 0.005$ vs OPTI-FREE express. ** $P < 0.005$ vs All Clean Soft. $n = 5$ /group. CLC, contact lens care.

four CLCs ($p < 0.005$), and that All Clean Soft demonstrated significantly more log reduction loss compared with Kombilösung Super ($p < 0.005$).

DISCUSSION

MK is a sight-threatening ocular infection caused by ubiquitous micro-organisms which can opportunistically infect a host's cornea.¹ These infections are often caused or exacerbated by inappropriate contact lens use or care.¹ This includes infrequently changing lenses, failing to follow manufacturer directions for nightly disinfection, wearing contact lenses for longer than they are meant to be worn, rinsing contact lenses in tap water, and swimming or showering while wearing contact lenses.² However, while this range of potential contamination activities are ever-present, it's important that the CLC solutions commonly available to consumers are able to robustly disinfect lenses when used as directed, to otherwise protect patients against the micro-organisms that their lenses may come in contact with. Additionally, it is equally important that products are not only tested in the test tube stand-alone scenario, but also in the real-world common-use scenario involving contact lenses and cases, and that they maintain similar disinfection efficacy to the oft-reported stand-alone results.^{9 13} It has previously been shown that contact lenses themselves, as well

as the contact lens cases packaged with CLCs, can take up biocide out of solution, thereby making any CLC less effective in this real-world situation.^{7 19}

Therefore, we first conducted the stand-alone testing in accordance with ISO 14729 using five of the most common CLCs on the global market. The bacterial and fungal strains used to challenge these CLCs are those required by ISO 14729,¹¹ in addition to two other *Fusarium* isolates and *Acanthamoeba* strains. The bacterial strains used, ATCC 9027, ATCC 13880 and ATCC 6538, which are human clinical isolates, have shown reduced virulence in mice. However, these strains are a critical and commonly used piece of evidence when testing for sterility and microbial contamination, as well as biofilms.^{20–24} Numerous studies have shown that ATCC 9027, ATCC 6538 and ATCC 13880 are similarly susceptible to antibacterial activity as other strains and species,^{22 25–32} and that these strains have similar virulence or virulence factors when used in other species or with human cells.^{33–39} As we expected, and similar to results we have reported previously for products on the American market,⁹ most CLCs meet or exceed the primary criteria set forth by the ISO standard. The only exception found in this study was when renu Multiplus, All Clean Soft, and Kombilösung Super were challenged with *Serratia marcescens*. While these three did not meet the primary criteria of 3 log reduction, they did all exceed 2 log (99%) reduction. We also applied the stand-alone testing method, slightly modified to be appropriate to *Acanthamoeba*. While this species is not yet required by the ISO standards, it is currently under consideration by ANSI and the ISO, as it is the causative micro-organism in recent keratitis outbreaks, and is notoriously difficult to treat. While all CLCs examined in this study exceeded 1.5 log reduction when challenged with either *Acanthamoeba polyphaga* or *Acanthamoeba castellanii* trophozoites, only the OPTI-FREE Express product exceeded 4 log reduction of these two micro-organisms.

Following this, we challenged each CLC product with each micro-organism in the presence of contact lenses and cases, in accordance with ISO 18259. We noted that the majority of the CLCs demonstrated a substantial loss of disinfection efficacy when lenses and cases are added to the disinfection challenge as opposed to the stand-alone (test tube challenge) only. Therefore, we averaged the loss of disinfection efficacy across all lenses within any CLC-micro-organism challenge, and compared the loss of log reduction between CLCs within any micro-organism challenge. As most products are highly efficacious against the bacterial organisms required by the ISO standard, and as *Acanthamoeba* is not yet required by the ISO, we chose the *Fusarium* species as a challenging organism, which highlights the differences between products. We here also chose to not only use the strain required by ISO 14729, *Fusarium keratoplasticum* (previously named as *Fusarium solani*), but also another common *Fusarium* strain, *Fusarium chlamydosporum*, as well as an unknown strain which was clinically isolated. For all three of

these strains, OPTI-FREE Express maintained a significantly lower net loss of log reduction compared with renu Multiplus, Kombi-Clean & Moist, All Clean Soft or Kombilösung Super. All Clean Soft also demonstrated a significantly lower loss of disinfection efficacy than renu Multiplus when challenged with *Fusarium keratoplasticum*, and significantly greater loss than Kombilösung Super when challenged with the *Fusarium* clinical isolate. This data indicates that the relatively higher amount of polyquaternium-1 (0.001%) and the addition of myristamidopropyl dimethylamine (0.0005%) in OPTI-FREE Express are able to much more efficiently eradicate *Fusarium* compared with the lower amounts of polyhexanide (0.0001%–0.0002%), polyquaternium (0.004%) or polyaminopropyl biguanide (0.0001%) present in the other CLCs.

However, it is overall important to note that the majority of the CLC products tested—renu Multiplus, Kombi-Clean & Moist, All Clean Soft, and Kombilösung Super—lost at least 50% of their disinfection efficacy in almost all challenges when they were challenged in the presence of contact lenses and cases, as an everyday patient would use them, versus stand-alone testing. To note, these ISO standards do not require a rub and rinse step to meet the primary criteria. However, even if the manufacturer's stated directions indicate a rub and rinse step, the vast majority of patients do not do it.² Therefore, these CLC products must be able to adequately disinfect a contact lens without the rub and rinse step, yet our findings indicate a significant loss in efficacy when solutions are challenged in a real-world scenario compared with the test tube methodology. While all of the CLC products tested were able to achieve the ISO 14729 requirement of 1 log reduction even with lenses and cases added after challenge with *Fusarium keratoplasticum* and *Fusarium chlamydosporum*, renu Multiplus, All Clean Soft, and Kombilösung Super were not able to achieve this level of disinfection efficacy when challenged with the clinical isolate of *Fusarium*. Finally, it is important to note that all findings of disinfection efficacy or loss of disinfection efficacy were highly consistent regardless of which contact lens or which contact lens case were used.

In conclusion, we have here determined that, except for when challenged with *Serratia marcescens*, five of the most common CLC products on the global market meet and exceed the primary testing criteria when challenged with the five required ISO 14729 organisms, as well as two additional *Fusarium* strains, when tested according to the stand-alone ISO 14729 testing method. These products were also highly effective against two *Acanthamoeba* strains in stand-alone testing. However, when these same products were challenged in a real-world setting, in the presence of contact lenses and cases with those same three *Fusarium* strains, most of the CLCs demonstrated a substantial loss of disinfection efficacy compared with the previous stand-alone tests. OPTI-FREE Express maintained significantly less loss of log reduction compared with the other four products, followed by All Clean

Soft, which also demonstrated some instances of less log reduction loss compared with other products. It is critical to examine the disinfection efficacy of CLCs in the presence of contact lenses and lens cases, without the rub and rinse step, to appropriately mimic the most common scenario carried out by contact lens users.

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