

Removal of Biofilm from Contact Lens Storage Cases

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PURPOSE. Lens case hygiene practices are important in maintaining safe contact lens wear. However, the effectiveness of various lens case cleaning practices have not been evaluated and compared. This in vitro study aimed to evaluate and compare the efficacy of cleaning practices that are most commonly carried out by lens wearers and recommended by practitioners.

METHODS. *Pseudomonas aeruginosa* 122, *Serratia marcescens* ATCC 13880, and *Staphylococcus aureus* ATCC 6538 were the challenge bacteria for biofilm formation on unused lens cases from two different manufacturers. After establishment of the biofilm, each lens case was subjected to one of the six cleaning regimens: "rinsed," "rubbed and rinsed," "air-dried," "soaked in a multipurpose contact lens solution," "tissue-wiped," and "lids recapped." The level of residual biofilm was quantified at the end of each cleaning regimen. The efficacy of each cleaning regimen was then compared.

RESULTS. Mechanical rubbing and wiping of lens cases were the most effective cleaning regimen tested in reducing biofilm. Soaking lenses in disinfecting solution for 6 hours removed the majority of biofilm from lens cases. Rinsing lens cases alone provided only minimal efficacy in reducing biofilm. Air-drying or recapping the cases with the lid without any other additional cleaning methods were the least efficient at removing biofilm.

CONCLUSIONS. Based on this study, digital rubbing and rinsing and/or wiping the lens cases with tissue is recommended. Air-drying or recapping the lens case lids after use without any additional cleaning methods should be discouraged with non-antimicrobial lens cases. (*Invest Ophthalmol Vis Sci.* 2010;51:6329–6333) DOI:10.1167/iovs.10-5796

A recent epidemiologic study has confirmed that poor lens case hygiene is strongly associated with contact lens-related corneal infections¹ and other ocular complications.² It is important therefore that contact lens wearers perform effective lens case hygiene practice to minimize lens case contamination. Lens case contamination is frequent in the contact lens-wearing population,^{3–6} and the lens case is often the most contaminated lens accessory item.⁵ Recommended hygiene practices do not necessarily ensure a lens case free of contamination.^{7,8} Factors such as biofilm formation^{9,10} and inherent microbial resistance¹¹ may be associated with persistent microbial contamination of contact lens storage cases.

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Current multipurpose disinfecting solutions may not be effective against the biofilm forms of bacteria.¹² Thus, cleaning steps in addition to the use of these solutions may be relevant in lens case hygiene practices. However, only limited information relating to lens case hygiene practice is available to lens wearers, and such advice varies among manufacturers and eye care practitioners.¹³ The recommendations given to lens wearers are often lacking in detail, compromising lens wearer compliance. At the same time, it is difficult to identify the reasons for a wearer's noncompliant behavior: whether there is intentional noncompliance, whether lens wearers are following outdated instructions, or whether the lens wearers never received adequate lens case hygiene instructions from their eye care practitioner in the first place or were confused by different recommendations from each manufacturer. It is also a challenge for eye care practitioners when delivering detailed and uniform instructions given limited evidence-based findings on the effectiveness of lens case guidelines.

Much needs to be done to provide more comprehensive and detailed instructions to lens wearers. In view of this, the present study aimed to evaluate and compare the effectiveness of lens case cleaning practices that are most commonly carried out by lens wearers and recommended by practitioners.

MATERIAL AND METHODS

Bacterial Strains

Pseudomonas aeruginosa 122, *Serratia marcescens* ATCC 13880, and *Staphylococcus aureus* ATCC 6538 were selected as the challenge bacteria. These strains were chosen because they demonstrated the ability to form adequate biofilms on lens storage cases in a pilot study. The strains of *S. marcescens* and *S. aureus* were standard strains, whereas *P. aeruginosa* 122 was isolated from a case of microbial keratitis. Each strain was obtained from a bacterial stock stored at -80°C and streaked on a chocolate agar plate (Oxoid Australia, Sydney, NSW, Australia). Plates were incubated in O_2 at 37°C for 24 hours. After 24 hours, colonies were harvested and suspended in PBS with 1% Luria broth (PBS-LB; 10.0 g/L tryptone, 5.0 g/L yeast extract, and 5.0 g/L NaCl) and adjusted spectrophotometrically to achieve an optical density (OD) reading of 0.1 (approx. 10^8 CFU/mL) at 660 nm wavelength. Subsequently, the concentration of each inoculum was adjusted to approximately 10^6 per mL using serial dilution in PBS-LB.

Contact Lens Case

The first type of lens case (OPTI-FREE Replenish; Alcon, Fort Worth, TX) had 16 ridges in the inner surface well (Fig. 1A) and was molded from polypropylene.

The second type of lens case (Complete EasyRub; Advanced Medical Optics [AMO], Santa Ana, CA) had a smoother inner surface well (Fig. 1B). Lens case bases were made from acrylonitrile butadiene styrene, and the lids were made from polypropylene.

Disinfecting Solution

The ingredients of the disinfecting solution (OPTI-FREE Replenish; Alcon) are sodium citrate, sodium chloride, sodium borate, propylene glycol, TearGlyde (Tetric 1304 and nonanoyl ethylenediaminetriacetic

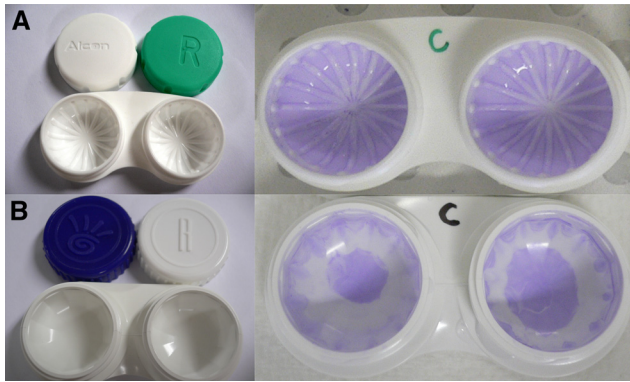


FIGURE 1. (A) Blank case (OPTI-FREE Replenish; Alcon) and CV-stained *S. marcescens* biofilm (control). (B) Blank case (Complete EasyRub; AMO) and CV-stained *S. marcescens* biofilm (control).

acid) as wetting agents with Polyquad (polyquaternium-1) 0.0001% and Aldox (myristamidopropyl dimethylamine) 0.0005% as active ingredients.

Tissue

Two-ply, non-lint-free facial tissues without moisturizer (Kleenex; Kimberly-Clark Australia, Milsons Point, NSW, Australia) were used and were handled with aseptic gloves.

Biofilm Formation on Lens Storage Case

Each lens case well was opened aseptically and inoculated with 2 mL freshly prepared bacterial suspension (10^6 CFU/mL). To allow for biofilm formation, lids of the lens cases were loosely recapped, and these lens cases were incubated in a 37°C digital agitator at 120 rpm for 24 hours. Bacterial suspensions in both wells were then discarded, and all wells were rinsed with distilled water twice to dislodge planktonic cells.

Cleaning Regimens

After biofilm formation, the lens cases were divided into seven groups. Each group underwent one of the following independent cleaning regimens:

- Control ($n = 8$): No treatment;
- Rinse ($n = 8$): Lens case rinsed once with OPTI-FREE Replenish solution;
- Rub and rinse ($n = 8$): Case filled 80% full with OPTI-FREE Replenish solution, then rubbed clockwise and anticlockwise for 5 seconds with a finger wearing disposable aseptic gloves (Gelteck Powder-Free latex gloves; Livingstone International, Rosebery, NSW, Australia);
- Air-dry ($n = 8$): Case air-dried face down on clean facial tissue paper for 6 hours;
- Recap ($n = 8$): Case recapped with the lids for 6 hours;
- Tissue-dry and recapped ($n = 8$): Case well wiped dry using a facial tissue (same as above) in circular motions against the side of the well twice and then recapped the lids for 6 hours;
- Soak in disinfecting solution ($n = 8$): Case well filled 80% full with OPTI-FREE Replenish solution and soaked for 6 hours.

The method described above was repeated for three challenged strains and two types of lens cases. On completion of each cleaning regimen, the remaining biofilm on the lens case was quantified by crystal violet and MTT staining.

Biofilm Quantification

Crystal Violet (CV) Assay for Total Bacteria in Biofilm.

Two milliliters of CV solution (0.5%, w/v) was added to each lens well and incubated statically for 15 minutes at ambient temperature. The

unbound CV was then removed by rinsing the well with distilled water five times. After air-drying for 2 hours, 2 mL of 100% ethanol was then added to the lens well to dissolve the CV staining. Fifteen minutes later, 1 mL of the solubilized CV was extracted from the lens well into a well of a flat-bottom, 24-well polyvinyl chloride microtitre plate (Greiner Bio-One, Frickenhausen, Germany) and absorbance measured at OD_{590} .¹⁴

MTT Assay for Viable Bacteria in Biofilm. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO) solution was aseptically prepared by dissolving the MTT powder at a concentration of 5 mg/mL in sterile PBS at room temperature and stored at 4°C in a dark, screw-cap container. After each cleaning treatment, 2 mL of LB and 0.2 mL of MTT solution were added to each case well. Lens cases were incubated at 37°C on a digital agitator at 120 rpm for 2 hours. The culture supernatant was then discarded and dimethyl sulfoxide (2 mL) was added to each well to solubilize the MTT, which had been cleaved into an insoluble purple formazan through the metabolism of the live cells. The levels of viable bacteria in the biofilm were determined by loading 1 mL of the solubilized MTT into a flat-bottom, 24-well polyvinyl chloride microtitre plate, and absorbance measured at OD_{570} nm with a reference wavelength of OD_{630} .¹⁵

Validation of MTT Assay. One additional set of Alcon lens cases (OPTI-FREE Replenish) incubated with *S. aureus* ATCC 6538 underwent the cleaning regimens and was sampled with a sterile calcium alginate swab premoistened with PBS. Calcium alginate swabs were vortexed in PBS containing 1% hexametaphosphate for 3 to 5 seconds, and the aliquots were plated onto heated blood (“chocolate”) agar (Oxoid Australia). The chocolate agar plates were incubated in O_2 for 24 hours at 37°C for bacterial recovery and enumeration in colony-forming units per milliliter. The level of viable bacteria in each cleaning regimen was plotted against the level of biofilm measured by MTT.

Statistical Analysis

The biofilm absorbance levels after each cleaning regimen were compared within each strain and lens case and for live and total biofilm using one-way ANOVA. Post hoc multiple comparisons were made using the Games-Howell correction, and $P < 0.05$ was considered statistically significant.

RESULTS

Figures 2.1–2.3 show the mean value of absorbance level for CV staining (total biofilm), and Figures 2.4–2.6 show the mean value of absorbance level for MTT staining (viable cells) and the effects of cleaning regimens for the Alcon lens cases (OPTI-FREE Replenish) in three challenged bacteria. The error bars represent ± 1 SD.

For the ridged cases (OPTI-FREE Replenish), “rubbing and rinsing,” “soaking in solution,” and “tissue-wiping” all showed a consistent significant reduction in CV and MTT staining ($P < 0.001$) for each bacterial type compared with that of the control group (Figs. 2.1–2.6). Rinsing alone showed significant reduction in CV ($P = 0.04$; Fig. 2.2) and MTT staining ($P = 0.03$; Fig. 2.5) for *S. marcescens* and for *S. aureus* in MTT staining only ($P < 0.001$; Fig. 2.6); rinsing alone had no significant effect on the level of biofilm produced by *P. aeruginosa* ($P > 0.05$). Air-drying lens cases and recapping the lids did not reduce the level of biofilm for any bacterial type when compared with the control group ($P > 0.9$). Overall, the most effective cleaning methods in reducing biofilm were rubbing and rinsing, soaking in solutions, and tissue-wiping for all three strains.

Figures 3.1–3.3 show the mean value of absorbance level for CV staining (total biofilm), and Figures 3.4–3.6 show the mean value of absorbance level for MTT staining (viable cells)

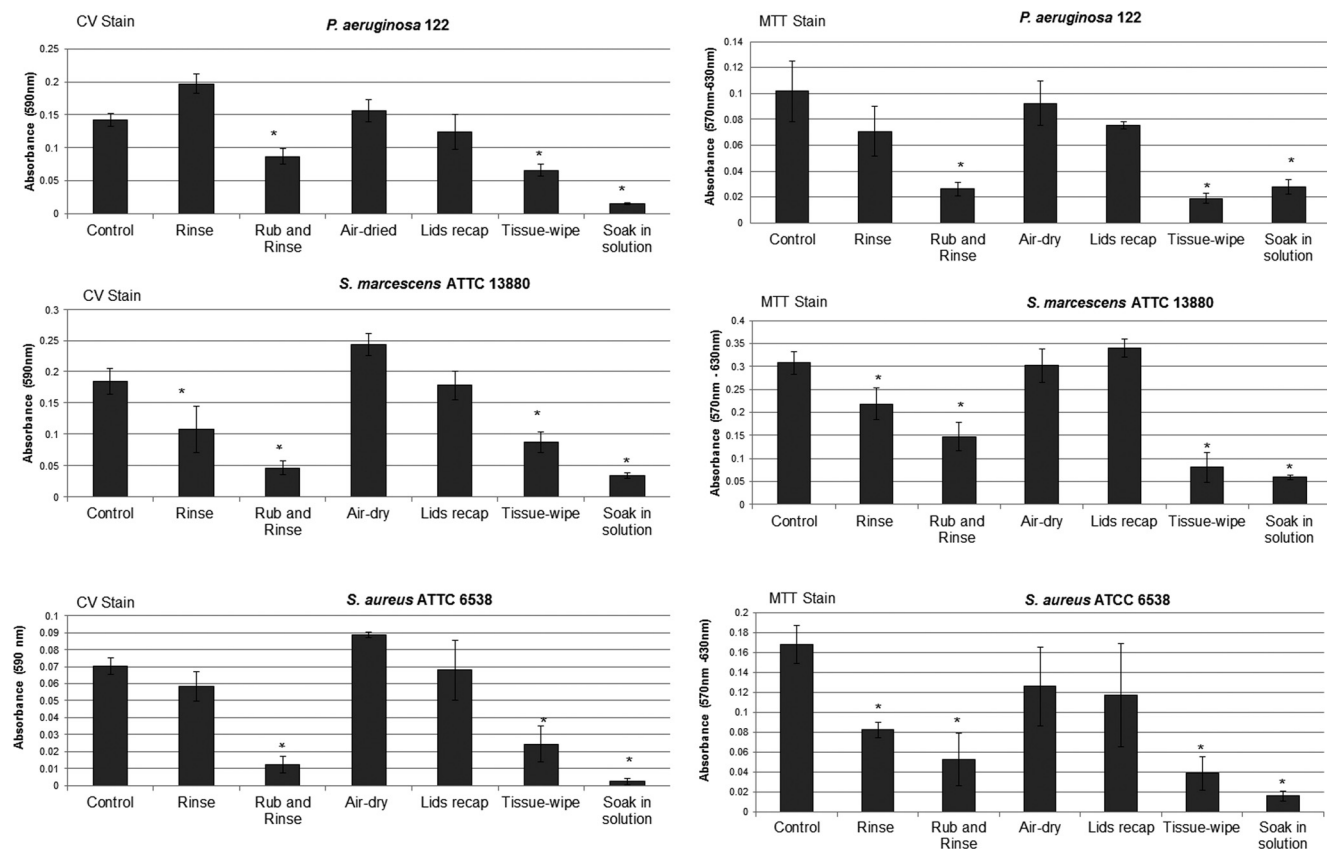


FIGURE 2. 1–2.6: Total biofilm (CV) and viable cells (MTT) in biofilm remaining on Alcon lens cases (OPTI-FREE Replenish) after different cleaning regimens. * $P < 0.05$, significant biofilm reduction when compared with the control group. Bars, mean of CV and MTT; error bars, ± 1 SD.

and the effects of cleaning regimens for AMO lens cases in three challenged bacteria. The error bars represent ± 1 SD.

For the smooth cases (Complete EasyRub), “rubbing and rinsing” or “tissue-wiping” cases demonstrated the most consistent and significant reduction in biofilm formation for various test strains when compared with the control group ($P < 0.001$; Figs. 3.1–3.6). Soaking in solutions was effective in reducing the bacterial loads for most bacterial types apart from the CV staining in *S. aureus* (Fig. 3.3) and MTT staining in *P. aeruginosa* (Fig. 3.4). Rinsing alone was effective only in reducing CV staining in *S. aureus* ($P = 0.023$; Fig. 3.3). Air-drying and recapping lids had no effect on biofilm levels or viable bacterial numbers for any strain.

In the validation experiment, there was a similar overall pattern for CFU recovery and MTT staining, indicating that the level of MTT absorbance is in concordance with the viable cells in biofilm (data not shown).

DISCUSSION

Contact lens storage cases are used along with disinfecting solution to store contact lenses after removal from eyes. However, the cleaning procedure required to maintain lens case hygiene between each use cycle is often not clear to contact lens wearers. Limited evidence-based information is available to allow comparison of the effectiveness of each lens case cleaning method on removal of biofilms. This makes it difficult to derive optimum cleaning methods to reduce biofilm formation in lens cases. To our knowledge, this is the first study to present evidence-based descriptions of the effectiveness of each of the lens case hygiene practices that are commonly carried out by lens wearers.

This study used an in vitro model of three strains of bacterial biofilm on lens cases that were measured using CV and MTT. CV binds to the negatively charged surface molecules and polysaccharides in the cell, staining both live and dead cells, and MTT provides a rapid and indirect measure of viable cells by detecting the amount of metabolic activity in the lens case. MTT dye is reduced by dehydrogenase in living cells to produce purple MTT formazan,¹⁶ which can be examined visually or quantified by a spectrophotometer. Our data (not shown) indicate that MTT staining is representative of viable counts in biofilm, and the CV and MTT assay approach has been widely applied in quantifying biofilm.^{15,17–19}

The most effective cleaning regimens to reduce bacteria load from the lens cases were “rub and rinse” and “tissue-wiping” of the lens cases. About one third of optometrists recommend that their lens wearers rub and rinse their contact lens case after use.¹³ “Rub and rinse” and “tissue-wiping” procedures both demonstrated significant reduction of biofilm. The removal of bacterial biofilm attributed to “rub and rinse” and “tissue-wiping” is likely to be due to the mechanical friction and shearing forces applied by the fingers and tissue. The mechanical interaction has been proven to be effective in cleaning contact lenses even without using a disinfectant.^{20,21} Rubbing of lens cases may also reduce the presence of potentially inflammatory microbial products such as endotoxin and result in reduced rates of inflammatory adverse events such as infiltrative keratitis. In this study, “tissue-wiping” was followed by recapping the case lids. This may better represent the use of lens storage cases in the community, for example, if wearers carry their lens cases during the day or insert lenses at work or at the gym and air-drying the case is therefore not possible.

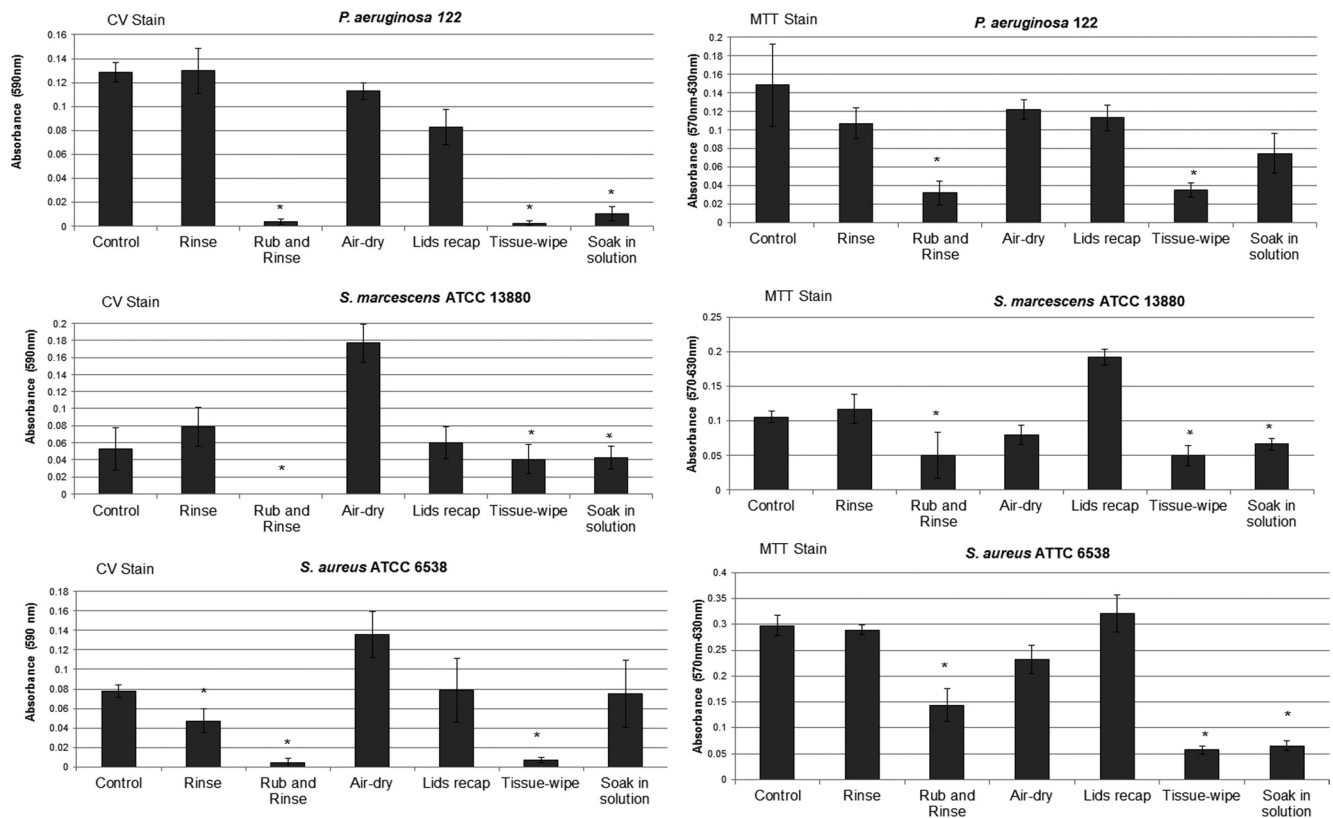


FIGURE 3. 1–3.6: Total biofilm (CV) and viable cells (MTT) in biofilm remaining on AMO lens cases (Complete EasyRub) after different cleaning regimens. * $P < 0.05$, significant biofilm reduction when compared with the control group. Bars, mean of CV and MTT; error bars, ± 1 SD.

Tissue-wiping was proven to remove a significant amount of biofilm, despite the absence of air-drying.

It is conceivable that facial tissues may shed fibers in the lens storage case and result in potential adverse responses in wearers. Although this article describes proof of concept in mechanical removal of biofilm, it has not established an optimum protocol for wearers.

We also noted that using gloved hands during the experiments may change the effectiveness of the rubbing regime compared with use of naked fingers. However, wearing gloves was necessary to maintain sterile technique and to protect the researchers from potentially harmful bacteria.

Rinsing lens cases alone has no significant effect in reducing biofilm. Transient exposure of bacteria to solutions while being rinsed elicited a marginal antimicrobial effect. A recommendation of a longer rinsing time may not be strictly adhered to by lens wearers who are cost conscious because it speeds up the consumption of disinfecting solutions. A better alternative would be rubbing and rinsing.

Air-drying the lens cases alone cannot be relied on to prevent bacterial growth. Microorganisms may decline in number initially; however, substantial regrowth may occur within 24 hours.²² Another study has shown that air-drying biofilm for 10 hours may decrease recovery of challenged microorganisms,²³ but drying was not enough to decrease results to zero recovery. Also, the ability of bacteria to survive and regrow on surfaces may depend on numerous factors, such as the drying speed, surrounding temperature, antimicrobial effect of a given multipurpose disinfecting solution, and level of nutrient available. Therefore, additional cleaning steps, such as rubbing and rinsing before air-drying, should be recommended not only to dislodge the bacteria, but also to reduce nutrients that may promote bacterial growth.

The effect of recapping the lids was similar to that of the control group (no treatment). Silver-impregnated cases, however, appeared to perform better when the lids were recapped, retaining some moisture in the lens case.²⁴ For non-silver-coated flat lens cases, recapping the lids should be discouraged. It is recommended that lens cases be cleaned after use and air-dried face down.²⁵

Soaking lens cases in disinfecting solution for the minimum disinfection time recommended by manufacturers (6 hours) was able to reduce the bacterial load in cases. Two of the test strains in the present study were identical with the strains that are used in International Organization for Standardization testing of contact lens care products. Thus, it was anticipated that the commercially available disinfecting solution would have potent antimicrobial effects on these strains. However, the antimicrobial effects of different multipurpose solutions may differ for different species and strains of organisms.^{12,26} Also, in practice, lens wearers may not ensure that the wells are fully immersed in disinfecting solution with every use. Therefore, soaking lens cases alone may not provide optimum biofilm removal in real life, and additional cleaning methods may be required to remove biofilm on lens cases. Indeed, lens cases commonly harbor microbes during normal use.^{3,4,6,8}

As the material and surface topology of the two storage cases differed, it was not possible to make comparisons between biofilm formation on the two different cases. There was visible residual biofilm between the grooves of one of the cases (Alcon), and the surface of the other cases (AMO) became soft with dimethyl sulfoxide when extracting the MTT from the case. Despite these difficulties, the comparisons between different cleaning regimens within each case type seem robust.

Manually cleaning lens cases after use remains important in maintaining good lens case and contact lens hygiene. The

present study has demonstrated that “rub and rinse” and “tissue-wiping” the lens cases are both effective in reducing biofilm on lens cases. “Rinse only” provides a limited cleaning effect in removing biofilm on lens cases. “Air-drying alone” and “recapping the lids” of either type of case after use should be discouraged. Further studies combining these lens case cleaning methods in a sequential manner and in combination with different disinfecting solutions may provide further useful information in the establishment of detailed lens case cleaning guidelines.

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