

# In Vitro Antimicrobial Efficacy of Silver Lens Cases Used With a Multipurpose Disinfecting Solution

Ananya Datta<sup>1</sup>, Mark Willcox<sup>1</sup>, and Fiona Stapleton<sup>1</sup>

<sup>1</sup> School of Optometry and Vision Science, University of New South Wales Sydney, Australia

**Correspondence:** Fiona Stapleton, School of Optometry and Vision Science, University of New South Wales, Sydney, NSW 2052, Australia. e-mail: f.stapleton@unsw.edu.au

**Received:** 10 January 2019

**Accepted:** 19 April 2019

**Published:** 27 June 2019

**Keywords:** antimicrobial efficacy; silver lens case; contact lenses; corneal infections

**Citation:** Datta A, Willcox M, Stapleton F. In vitro antimicrobial efficacy of silver lens cases used with a multipurpose disinfecting solution. *Trans Vis Sci Tech.* 2019;8(3):52. <https://doi.org/10.1167/tvst.8.3.52> Copyright 2019 The Authors

**Purpose:** We evaluate the in vitro antibacterial activity of silver-copolymerized barrel cases compared to nonsilver barrel cases in combination with a multipurpose disinfecting solution (MPDS).

**Methods:** The ability of silver and nonsilver cases to reduce the number of adherent Gram-positive and Gram-negative bacteria was assessed. Additionally, the efficacy of silver cases along with MPDS was investigated in the presence of organic soil and contact lenses. Contact lens cases were challenged with 10<sup>6</sup> colony-forming units (CFU)/mL of five bacterial species. Adherent bacteria were dislodged from lens cases and surviving organisms enumerated.

**Results:** Significantly lower numbers of microbes were recovered from silver cases compared to controls, for all bacterial strains ( $P < 0.005$ ). The combination of silver case along with MPDS showed added efficacy against Gram-positive and -negative bacteria with a maximum reduction of  $3.00 \pm 0.5 \text{ Log}_{10} \text{ CFU/mL}$ , compared to the efficacy of silver cases alone ( $1.97 \pm 0.4 \text{ Log}_{10} \text{ CFU/mL}$ ). The addition of organic soil and a contact lens resulted in a significant ( $P < 0.005$ ) decrease (a maximum of  $1.68 \pm 0.2 \text{ log}_{10} \text{ CFU/mL}$ ) in disinfection efficacy when MPDS and either silver or control cases were used.

**Conclusions:** Silver-copolymerized barrel cases work on conjunction with a hypochlorite producing MPDS in the presence of contact lenses and organic soil to reduce microbial contamination of lens cases.

**Transitional Relevance:** Silver-copolymerized barrel contact lens cases show promising in vitro antibacterial activity against bacterial types commonly implicated in contact lens-related corneal infections. This intervention may limit storage case contamination during use and reduce the frequency of contact lens-related microbial disease.

## Introduction

Microbial contamination of contact lens cases is a major concern for daily lens wearers because colonization by pathogenic microorganisms is a risk factor for microbial keratitis and sterile infiltrates.<sup>1–4</sup> The causative organisms for microbial keratitis have been recovered from the contact lens cases of affected individuals.<sup>5,6</sup> Approximately 30% to 85% of contact lens cases are contaminated even during asymptomatic daily wear.<sup>7–11</sup>

Multiple genera and species, predominantly bacteria, can be isolated from contact lens cases,<sup>7,8,10–14</sup> including the pathogenic micro-organisms, such as

*Pseudomonas aeruginosa* and *Serratia marcescens*, which are isolated frequently from contact lens-induced microbial keratitis.<sup>9,11,15–19</sup>

Bacterial biofilm formation in lens cases may be a risk factor for contact lens-associated corneal infection and may explain the persistence of organisms in lens storage cases. Biofilms are communities of sessile bacteria adherent to a surface and encased in polymeric material.<sup>20,21</sup> Clinical isolates have been shown to be more resistant to disinfection than standard strains,<sup>7</sup> and biofilms also are more resistant to disinfection than planktonic isolates.<sup>2,22</sup> Multipurpose disinfecting solutions (MPDSs) are required to demonstrate antimicrobial efficacy against selected

**Table 1.** Bacterial Strains Used in This Study

Type of Bacteria	Microorganisms	Sources
Gram-positive bacteria	<i>S. aureus</i> 31	Contact lens induced peripheral ulcer
	<i>S. aureus</i> ATCC 6538	Human lesion
	<i>S. epidermidis</i> ATCC 35984	Catheter sepsis
	<i>S. epidermidis</i> 22-1	Contact lens case of an asymptomatic wearer
	<i>M. luteus</i> 22-1	Contact lens case of an asymptomatic wearer
	<i>M. luteus</i> 14-1	Contact lens case of an asymptomatic wearer
Gram-negative bacteria	<i>S. marcescens</i> ATCC 13880	Pond water
	<i>S. marcescens</i> 27	Microbial keratitis
	<i>P. aeruginosa</i> 6294	Microbial keratitis
	<i>P. aeruginosa</i> ATCC 9027	Otic infection
	<i>A. radioresistens</i> 22-1	Contact lens case of an asymptomatic wearer
	<i>A. radioresistens</i> 14-1	Contact lens case of an asymptomatic wearer

reference strains of planktonic bacteria and fungi, but not against clinical isolates or biofilms of microorganisms.<sup>23–25</sup>

Various strategies have been adopted to limit the numbers of bacteria and biofilm formation on lens cases. Such strategies include incorporation of silver ions, selenium compounds, polyquaternary ammonium compounds, polymeric pyredium compounds, nitric oxide, furanones, and cationic peptides into cases.<sup>26–28</sup>

Silver ions, often in the form of nanoparticles, provide broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa, and certain viruses,<sup>29</sup> including antibiotic-resistant bacteria.<sup>30–32</sup> Slow release of silver ions inhibits bacterial growth by multiple methods, including enhancing structural deformities in nucleic acids,<sup>33</sup> membranes,<sup>34</sup> and the cell walls of bacteria.<sup>34–38</sup> The multiple mechanism of actions of silver ions does not allow bacteria to easily develop resistance.

Silver-copolymerized contact lens cases are commercially available. Silver-impregnated flat lens cases have robust activity against Gram-positive and -negative bacteria.<sup>22,28,39</sup> A silver-impregnated barrel lens case also is available, but its efficacy has not been reported. This study evaluated the in vitro antimicrobial activity of silver-copolymerized barrel cases compared to nonsilver cases in combination with a multipurpose disinfecting solution and in presence of a contact lens and organic soil.

## Material and Methods

Strains of *P. aeruginosa*, *S. marcescens*, *Acinetobacter radioresistens*, *Staphylococcus aureus*, *S. epi-*

*dermidis*, and *Micrococcus luteus* were used in this study to assess the antimicrobial efficacy of silver barrel lens cases in combination with a contact lens cleaning and disinfecting solution. The details of the strains and their sources are listed in Table 1.

## Bacterial Culture Preparation

Bacteria were revived from the School of Optometry and Vision Science, University of New South Wales culture collection by being inoculated into a tryptic soy broth (TSB; Oxoid, Scoresby, Australia) and incubated for 24 hours at 37°C. Bacterial cells were washed three times by centrifugation at 1175g for 15 minutes at 25°C in sterile phosphate buffered saline (PBS; NaCl 8 g/L, KCl 0.2 g/L, Na<sub>2</sub>HPO<sub>4</sub> 1.15 g/L, KH<sub>2</sub>PO<sub>4</sub> 0.2 g/L; pH 7.2). The optical density (OD) of the bacterial cell suspensions was adjusted to 0.1 at 660 nm (1 × 10<sup>8</sup> colony forming unit/mL [CFU/mL]) in PBS using a spectrophotometer (FLUOstar Omega; BMG Labtech, Ortenberg, Germany) and the cell suspensions were serially diluted to 1 × 10<sup>6</sup> CFU/mL in PBS for all bacteria and in 1:100 TSB for Gram-positive or 1:100 LB for Gram-negative bacteria (Luria broth; Sigma-Aldrich Corp., Castle Hill, NSW, Australia) to assess the effect of nutrition on adhesion and MPDS efficacy.

## The Effect of Lens Case and Solution on Antimicrobial Activity

Silver barrel lens cases (Sauflon Pharmaceuticals Ltd., Twickenham, UK) and nonsilver barrel lens cases (control; Sauflon Pharmaceuticals Ltd.) were used. An MPDS (Sauflon, Pharmaceuticals Ltd.) containing an oxychlorite complex (Oxipol; sodium

chloride and hydrogen peroxide), as the disinfecting component was used.

Lens cases were opened aseptically and inoculated with 10 mL freshly prepared bacterial suspensions ( $1 \times 10^6$  CFU/mL). The lid of the lens cases was loosely recapped and cases were incubated at 37°C with agitation (250 rpm) for 24 hours to allow bacterial adhesion.

To test for the effects of the MPDS, cases were incubated with the bacterial cell suspension ( $1 \times 10^6$  CFU/mL) for 6, 18, or 48 hours. Residual bacterial cells were discarded, and cases were rinsed once with PBS to dislodge any planktonic or weakly adherent cells. Following this, lens cases were filled with the MPDS (approximately 80% full), recapped, and stored at ambient temperature for 6 hours based on the manufacturer's recommended disinfection time. As a control, lens cases were incubated with 10 mL PBS after bacterial adhesion and left at ambient temperature for 4 to 6 hours.

After incubation to allow bacterial adhesion, residual bacterial suspensions were discarded, and the cases were rinsed twice with PBS to dislodge any planktonic or weakly adherent cells. Following this, 10 mL PBS was added to each lens case along with a sterile magnetic stirring bar and the case was vortexed for 1 minute to dislodge the bacterial cells. Ten-fold serial dilutions were conducted using Dey Engley Neutralizing Broth (DE Broth) and dilutions were plated onto the trypticase soy agar (TSA; Thermo Fisher Scientific, Scoresby, Australia) containing 0.05% (wt/vol) Tween 80 (Sigma-Aldrich Corp.) and 0.07% (wt/vol) lecithin (Sigma-Aldrich Corp.) and incubated at 37°C for 18 to 24 hours for the recovery of bacteria. The numbers of CFUs were counted and converted to CFU/mL. This assay was repeated in duplicate on three different occasions for each bacterial strain.

## Impact of Contact Lens and Organic Soil on Disinfection

For testing in the presence of added organic soil and contact lenses, a modified ISO 14729 Stand Alone Test<sup>25</sup> procedure was followed. After bacterial growth in TSB, bacteria were prepared in 0.4% (vol/vol) organic soil, consisting of fetal bovine serum (0.4% vol/vol) and heat-killed *Saccharomyces cerevisiae* OD of 1.5 at 660 nm, followed by adjusting the final concentration of the bacteria to  $1 \times 10^6$  CFU/mL. Bacteria were incubated in lens cases for 24 hours at 37°C followed by washing with PBS as described above. Contact lenses were removed aseptically from

their packaging, washed three times with PBS and placed in the basket of lens cases. The lens cases were filled with the MPDS (80% full) and incubated at ambient temperature for 6 hours (the manufacturer's recommended minimum disinfection time), 10 or 24 hours. Control lens cases were filled with MPDS after bacterial adhesion and no contact lenses were added during the disinfection.

After the designated disinfection time, the numbers of adherent bacteria in lens cases were evaluated as described above. The number of bacteria adherent to contact lenses was assessed by washing the removed lenses once in PBS, placing the lens into 2mL PBS and dislodging the adherent organisms by vortexing in the presence of a small magnetic stirring bar. The resulting lens slurry was diluted in PBS and dilutions plated onto tryptic soy agar containing Tween 80 and lecithin and the CFUs were enumerated after incubation for 24 hours at 37°C. This assay was repeated in duplicate on three different occasions for each bacterial strain.

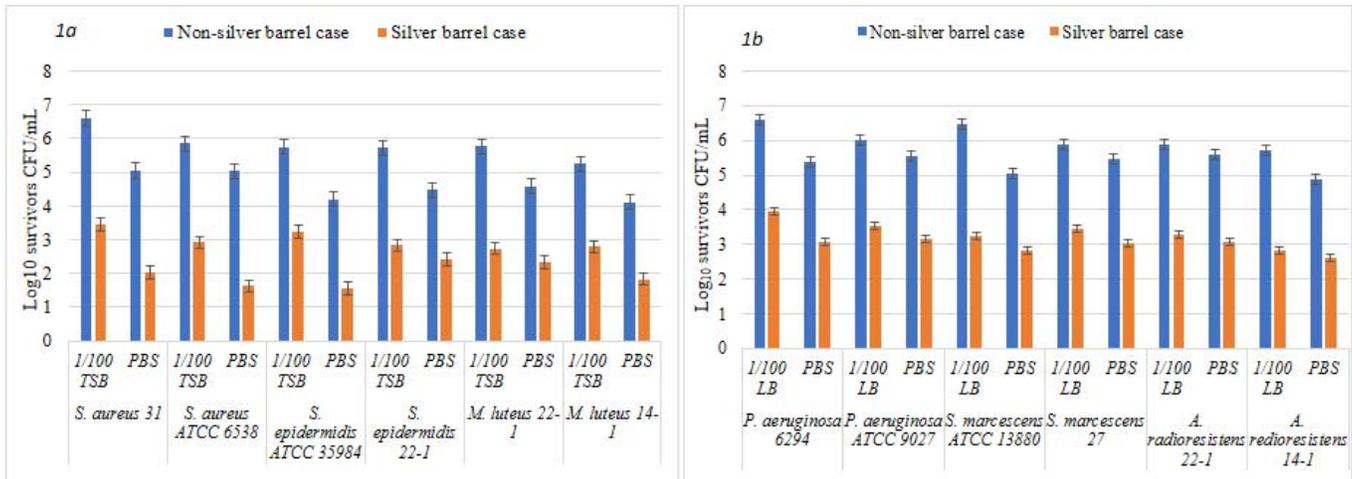
## Statistical Analysis

Data analyses were performed using Microsoft Excel 2010 and Statistical Package for Social Science for Windows version 20.0 (SPSS, Inc., Chicago, IL). The total number of viable organisms for each lens case was recorded as  $\text{Log}_{10}$  CFU/m and the standard deviation derived from the three independent biological replicates. A two-sample Wilcoxon rank-sum (Mann-Whitney) test was used to compare the rate of bacterial recovery from silver and nonsilver lens cases. Log differences were summarized as mean  $\pm$  SD and were compared using a repeated measures ANOVA for the test organisms at each incubation time. Bonferroni correction was used for post hoc multiple comparisons.

## Results

### Antibacterial Efficacy of Silver Cases

Silver cases significantly ( $P < 0.005$ ) reduced the number of adherent Gram-positive bacteria by an average of  $2.61 \pm 0.52 \text{ Log}_{10}$  CFU/mL when suspended in PBS and  $2.82 \pm 0.28 \text{ Log}_{10}$  CFU/mL when suspended in 1/100 TSB compared to nonsilver cases. There was no significant effect of suspending fluid on the activity of silver cases, but more Gram-positive bacteria (an average of  $1.2 \text{ log}_{10}$  CFU/mL) adhered when diluted in 1/100 TSB compared to PBS ( $P < 0.005$ ; Fig. 1). The maximum inhibition of  $3.41 \pm 0.06 \text{ Log}_{10}$  CFU/mL was observed for *S. aureus*



**Figure 1.** Numbers of (a) Gram-positive and (b) Gram-negative bacteria adherent to silver and nonsilver lens cases in different growth media.

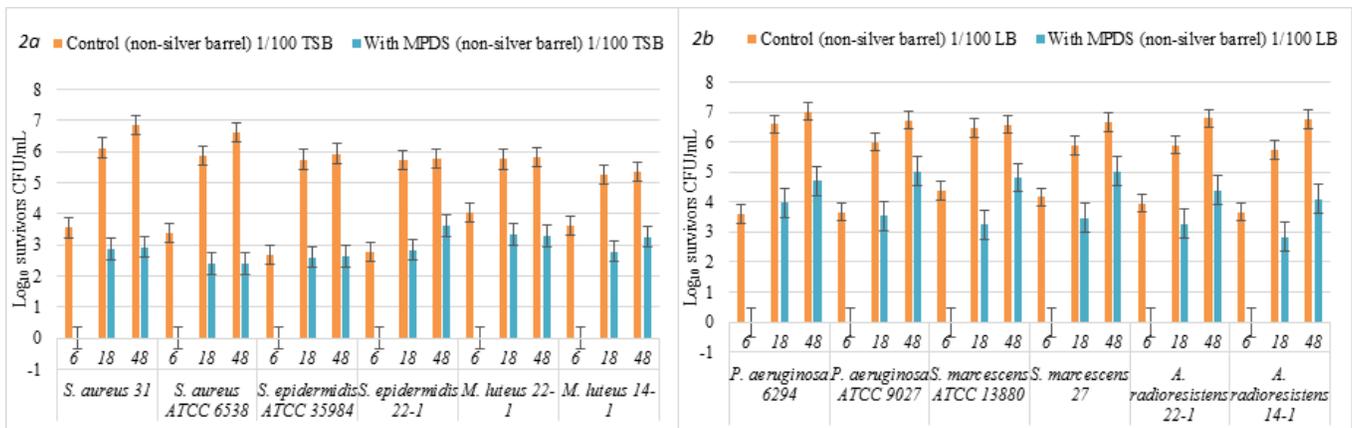
ATCC 6538 ( $P = 0.008$ ) when allowed to adhere in PBS, compared to nonsilver barrel cases as a control (Fig. 1). Within a species, there was a significant ( $P < 0.005$ ) difference in adhesion between the strains of *S. aureus* (ATCC 6538 and 31) and *S. epidermidis* (ATCC 35984 and 22-1) in lens cases, but not for strains of *M. luteus* (Fig. 1).

The effect of silver was greater for Gram-positive bacterial strains ( $P = 0.04$ ) compared to Gram-negative bacteria. Silver cases significantly reduced the number of adherent Gram-negative bacteria by  $2.36 \pm 0.11$  Log<sub>10</sub> CFU/mL when diluted in PBS and  $2.71 \pm 0.3$  Log<sub>10</sub> CFU/mL when diluted in 1/100 LB, compared to the nonsilver cases ( $P < 0.005$ ). There was no significant effect of diluent on the activity of silver cases, but more Gram-negative bacteria (an average of 0.8 Log<sub>10</sub> CFU/mL) adhered when diluted in 1/100 LB compared to

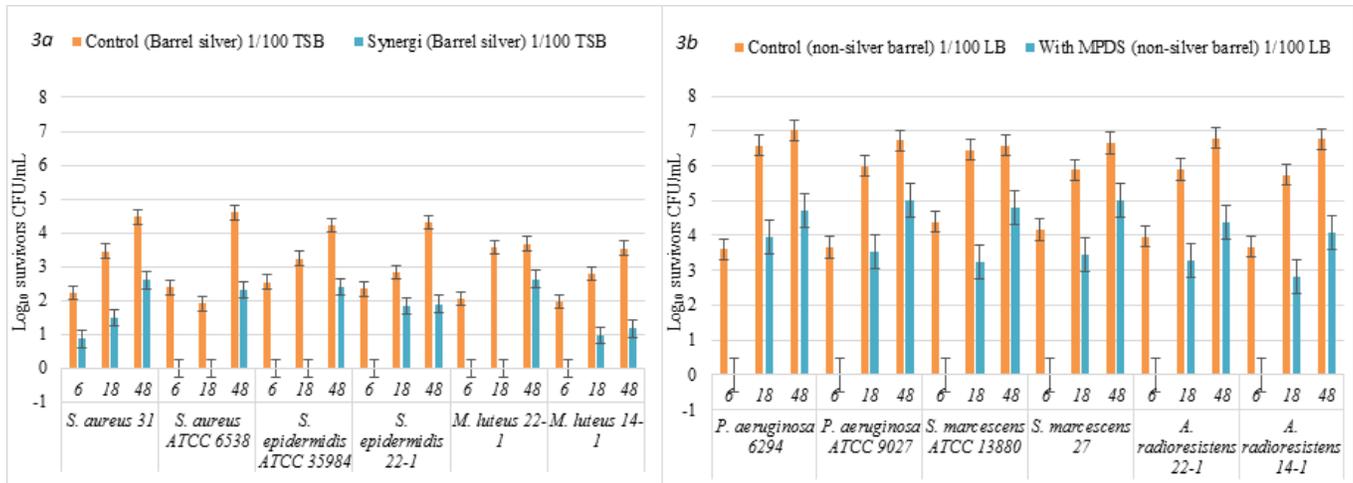
PBS ( $P = 0.009$ ; Fig. 1). For Gram-negative bacteria, a maximum of  $3.21 \pm 0.02$  Log<sub>10</sub> CFU/mL reduction in adhesion was seen with *A. radioresistens* 22-1 when prepared in 1/100 LB (Fig. 1). Within species, there were significant ( $P = 0.006$ ) differences in adhesion between the strains of *P. aeruginosa* (ATCC 9027 & 6294) and *S. marcescens* (ATCC 13880 & 27), but not for strains of *A. radioresistens* (Fig. 1), irrespective of the presence of silver.

### Efficacy of MPDS with or without Silver Lens Cases

In the absence of a silver lens case, the MPDS significantly reduced the numbers of adherent Gram-positive or Gram-negative bacteria ( $P = 0.003$ ; Fig. 2). The numbers of all bacteria that could be cultured



**Figure 2.** Biocidal efficacy of MPDS treatment for 6 hours against (a) Gram-positive bacteria and (b) Gram-negative bacteria, after allowing bacteria to adhere for 6, 18, and 48 hours in nonsilver barrel lens cases.



**Figure 3.** Biocidal efficacy of silver barrel lens cases in conjunction with 6 hours of MPDS exposure against (a) Gram-positive and (b) Gram-negative bacteria, after allowing the bacteria to adhere for 6, 18, and 48 hours.

from lens cases increased as the incubation time increased, particularly from 6 to 18 hours ( $P = 0.002$ ). For all bacteria, no colonies could be grown after addition of the MPDS to cells that had been allowed to adhere to cases for 6 hours. On average, the reduction in bacteria numbers with the use of the MPDS on Gram-positive bacteria grown for 6 hours in cases in PBS was  $2.92 \pm 0.3 \text{ Log}_{10} \text{ CFU/mL}$  and in 1/100 TSB it was  $3.34 \pm 0.52 \text{ Log}_{10} \text{ CFU/mL}$ . For Gram-negative bacteria grown for 6 hours in cases in PBS it was  $3.49 \pm 0.33 \text{ Log}_{10} \text{ CFU/mL}$  and in 1/100 LB it was  $3.91 \pm 0.32 \text{ Log}_{10} \text{ CFU/mL}$ . For bacteria adherent to lens cases for 18 or 48 hours, there were reductions in Gram-positive numbers after the addition of the MPDS of  $2.25 \pm 0.57$  or  $2.25 \pm 0.84 \text{ Log}_{10} \text{ CFU/mL}$ , respectively, for cells in PBS, and  $2.94 \pm 0.43$  or  $3.04 \pm 0.91 \text{ Log}_{10} \text{ CFU/mL}$ , respectively, for cells in 1/100 TSB. For Gram-negative bacteria there were reductions in bacteria adherent to lens cases for 18 or 48 hours after addition of the MPDS of  $2.3 \pm 0.38 \text{ Log}_{10} \text{ CFU/mL}$  or  $2.19 \pm 0.35 \text{ Log}_{10} \text{ CFU/mL}$ , respectively, in PBS, and  $2.71 \pm 0.3 \text{ Log}_{10} \text{ CFU/mL}$  or  $2.18 \pm 0.42 \text{ Log}_{10} \text{ CFU/mL}$ , respectively, in 1/100 LB. In the absence of a silver lens case, the MPDS was most effective against *S. aureus* ATCC 6538 within the Gram-positive bacteria, giving a reduction of  $4.22 \pm 0.2 \text{ Log}_{10} \text{ CFU/mL}$  (Fig. 2a) after 48 hours in 1/100 TSB, and against *S. marcescens* ATCC 13880 within the Gram-negative bacteria, giving a reduction of  $4.39 \pm 0.2 \text{ Log}_{10} \text{ CFU/mL}$  (Fig. 2b) after 6 hours exposure in 1/100 LB.

The combination of MPDS and silver in lens cases reduced bacterial adhesion over and above the effect of silver lens cases or MPDS alone. Compared to silver cases alone, the addition of MPDS significantly ( $P < 0.005$ ) reduced the numbers of all Gram-positive and -negative bacteria adhered to lens cases irrespective of the media in which they had been incubated or the time they had adhered. For all Gram-positive bacteria, addition of the MPDS with silver lens cases reduced the numbers of bacteria that could be cultured to  $<1 \text{ Log}_{10} \text{ CFU/mL}$  after 6 and 18 hours of adhesion to cases, compared to the silver lens cases alone. This also was the case for most of the strains of Gram-negative bacteria, the exception being for both strains of *P. aeruginosa* where there were 0.43 to 1.5  $\text{Log}_{10} \text{ CFU/mL}$  remaining after 6 and 18 hours of adhesion (Fig. 3). The greatest reduction in the numbers of bacteria that could be grown after addition of the MPDS to silver lens cases occurred at the 18 hour time point ( $P = 0.01$ ).

Compared to the use of MPDS without silver cases, the addition of the silver case significantly ( $P = 0.004$ ) reduced the numbers of all bacteria that could be cultured from cases after they had been allowed to adhere for 18 hours (Table 2, Fig. 3).

The use of MPDS with the silver case, significantly reduced bacterial adhesion compared to the silver case alone ( $P < 0.005$ , Table 2), although there were differences between strains (Table 2). Also, the magnitude of the reduction with MPDS was similar in the media and PBS conditions.

**Table 2.** Difference in Efficacy between Silver Cases Plus MPDS and MPDS Alone after Bacteria had Adhered to Lens Cases in PBS for 6,18, and 48 Hours

	Bacterial Strains	Change in Bacterial Adhesion Log <sub>10</sub> CFU/mL		
		6 Hrs.	18 Hrs.	48 Hrs.
Gram-positive bacteria	<i>S. aureus</i> 31	0	-2.57*	-0.2
	<i>S. aureus</i> ATCC 6538	0	-1.66*	-0.76*
	<i>S. epidermidis</i> ATCC 35984	0	-1.6*	0
	<i>S. epidermidis</i> 22-1	0	-1.03*	-1.9*
	<i>M. luteus</i> 22-1	0	-2.77*	-0.5*
	<i>M. luteus</i> 14-1	0	-2.23*	-1.77*
Gram-negative bacteria	<i>P. aeruginosa</i> 6294	1.3*	-1.73*	-0.38
	<i>P. aeruginosa</i> ATCC 9027	0.43	-2.19*	-0.46
	<i>S. marcescens</i> ATCC 13880	0	-3.26*	-0.43
	<i>S. marcescens</i> 27	0	-3.23*	-0.43
	<i>A. radioresistens</i> 22-1	0	-1.79*	0.1
	<i>A. radioresistens</i> 14-1	0	-2.64*	0.34

Negative values indicate numbers of adhered bacteria were reduced with the combination of MPDS plus silver lens cases compared to MPDS alone.

\* Significant difference ( $P < 0.05$ ).

### Antibacterial Efficacy of the MPDS with or without Silver Lens Cases in the Presence of Organic Soil and Contact Lenses

The addition of organic soil to the disinfection process resulted in a significant ( $P = 0.005$ ) decrease in disinfection efficacy for all bacteria with or without silver lens cases with 6 hours of disinfection (Table 3). The addition of a contact lens during the disinfection process resulted in reduction in the number of viable bacteria that could be cultured from lens cases regardless of the incubation media or use of silver in cases ( $P = 0.005$ ; Fig. 4). No bacteria were recovered from contact lenses for most of the strains of Gram-positive bacteria, when lenses were added for 6, 10, or 24 hours during disinfection, with the exception of *S. aureus* 31 ( $1.30 \text{ Log}_{10} \pm 0.2 \text{ CFU/mL}$ ) and *S. epidermidis* 22-1 ( $1.40 \text{ Log}_{10} \pm 0.1 \text{ CFU/mL}$ ,  $P < 0.005$ ), after 24 hours of incubation during disinfection in nonsilver cases. For Gram-negative bacteria, an average of  $1.50 \pm 0.1$  and  $1.40 \pm 0.1 \text{ Log CFU/mL}$  bacterial adhesion was seen in contact lenses after 24 hours of MPDS disinfection with or without silver cases, respectively, but no bacterial recovery was seen when lenses were added for 6 or 10 hours during disinfection.

Overall, the combination of silver lens cases along with MPDS was the most effective in reducing bacterial adhesion (Fig. 5). The combination of silver

lens cases with MPDS showed complete inhibition of bacterial adhesion against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 35984, *M. luteus* 22-1, and *S. marcescens* ( $P < 0.005$ ; Fig. 5).

## Discussion

This study reports for the first time the efficacy of a novel silver-impregnated barrel contact lens storage case with and without MPDS, organic soil, and contact lenses against both typed and clinical bacterial isolates. Silver barrel cases demonstrated robust activity against adherent Gram-positive and -negative bacteria. The greatest antimicrobial efficacy was achieved with the combined effect of silver lens cases and the MPDS. The presence of contact lenses in the lens case during the disinfection resulted in reduced numbers of bacteria adhered to lens cases compared to the absence of lenses. However, the addition of organic soil increased the bacterial adhesion to lens cases and significantly decreased the disinfection efficacy of the MPDS with or without silver lens cases.

For certain strains of *S. aureus*, *S. epidermidis*, *M. luteus*, *S. marcescens*, and *A. radioresistens*, there was complete bacterial inhibition after 6 hours of bacterial adhesion with the recommended 6 hours of disinfection. Mowrey-Mckee et al.,<sup>40</sup> using the ISO stand-

**Table 3.** The Biocidal Efficacy of MPDS in Presence or Absence of Organic Soil after 18 to 24 Hours of Bacterial Adhesion in Silver or Nonsilver Lens Cases

	Change in Bacterial Adhesion Log <sub>10</sub> CFU/mL With MPDS in Presence of Organic Soil	
	Nonsilver Lens Cases	Silver Lens Cases
Gram-positive bacteria		
<i>S. aureus</i> 31	1.49*	3.17*
<i>S. aureus</i> ATCC 6538	2.37*	3.17*
<i>S. epidermidis</i> ATCC 35984	2.40*	2.73*
<i>S. epidermidis</i> 22-1	1.68*	3.60*
<i>M. luteus</i> 22-1	1.23*	3.13*
<i>M. luteus</i> 14-1	1.07	2.89*
Gram-negative bacteria		
<i>P. aeruginosa</i> 6294	1.89*	-2.83*
<i>P. aeruginosa</i> ATCC 9027	1.63*	-3.01*
<i>S. marcescens</i> ATCC 13880	1.85*	4.30*
<i>S. marcescens</i> 27	1.45*	4.18*
<i>A. radioresistens</i> 22-1	1.62*	3.33*
<i>A. radioresistens</i> 14-1	1.96*	3.33*

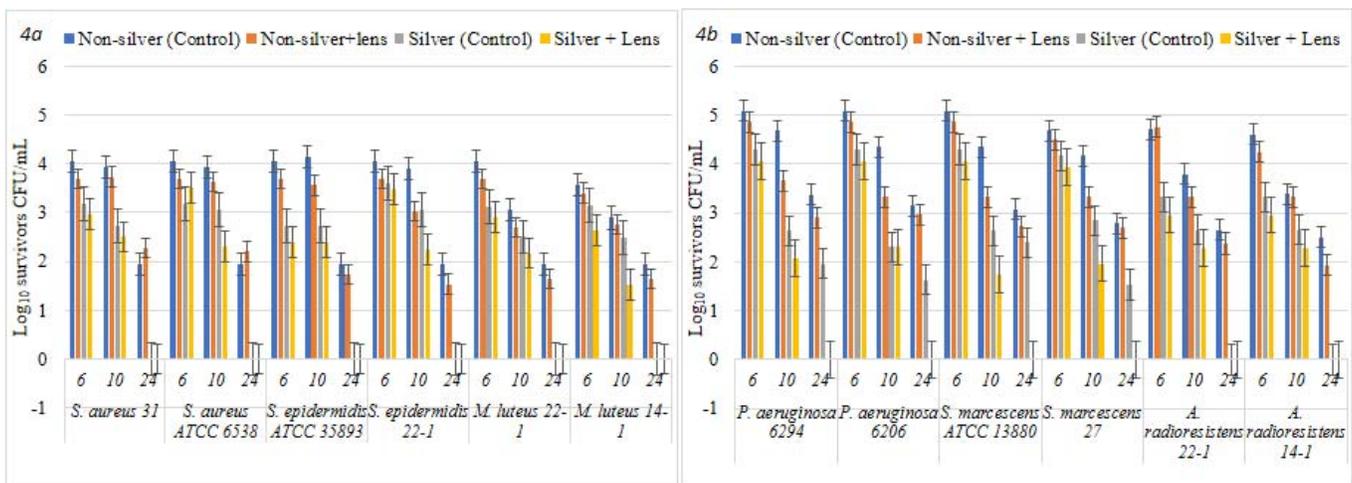
Negative values indicate the reduction in biocidal efficacy of the MPDS and silver case combination in presence of organic soil.

\* Significant difference ( $P < 0.05$ ).

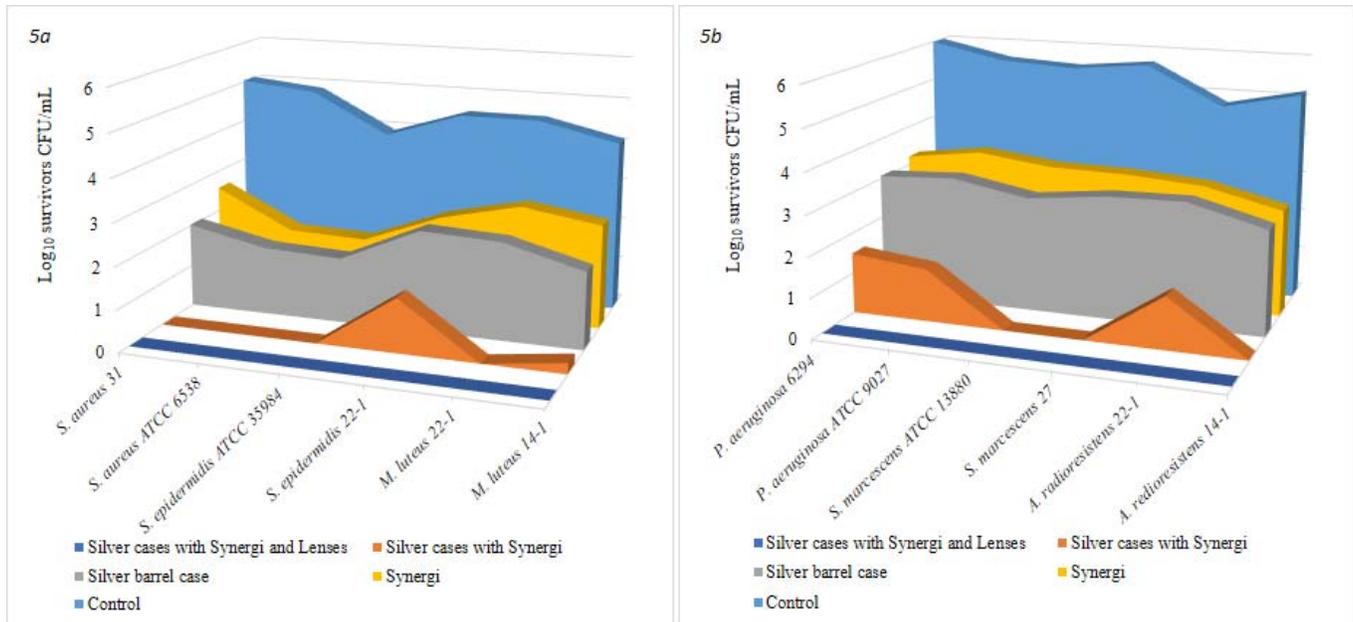
alone procedure, reported that the MPDS used in the current study reduced the numbers of planktonic *P. aeruginosa* by at least 3 Log<sub>10</sub> CFU/mL at the manufacturer’s recommended disinfection time (6 hours), but was not as effective against *S. aureus* ATCC 6538. However, in our study, the MPDS was active against adherent *P. aeruginosa* ATCC 9027 and *S. aureus* ATCC 6538, but did not show complete inhibition if bacteria had been allowed to adhere for 18 hours.

The most effective antimicrobial activity was achieved by the combined effect of silver cases and the MPDS compared to the individual efficacy of either silver cases or MPDS alone. Also, in our study, the efficacy of silver cases along with the MPDS was effective against a higher bacterial inoculum than used in a previous study with other silver lens cases and its polyhexanide-containing MPDS.<sup>28</sup> Furthermore, the MPDS used in our study has been shown to have greater antibacterial activity against Gram-negative bacteria in vitro than the polyhexanide-containing MPDS.<sup>41</sup> This may explain the greater effect of the silver/MPDS combination found in our study.

The presence of contact lenses has previously been shown to reduce the antimicrobial efficacy of MPDS,<sup>42</sup> possibly by absorbing the disinfecting solution during soaking.<sup>43</sup> However, our study demonstrated a greater bactericidal activity in the presence of lenses, especially for *S. marcescens* ATCC 13880. The reason for this is not known, but could be due to bacteria moving from the lens cases onto the lens surface and so depleting the



**Figure 4.** Efficacy of silver or nonsilver lens cases in conjunction with MPDS against (a) Gram-positive and (b) Gram-negative in organic soil. The three-time points (6, 10, and 24 hours) represent the disinfection time.



**Figure 5.** Summary of numbers of recovered bacteria of (a) Gram-positive and (b) Gram-negative bacteria from silver and control lens cases after different treatments after 18 to 24 hours of bacterial adhesion.

numbers adherent to lens cases, as bacteria were recovered from the lenses after disinfection. This transmission of the bacteria from lens cases to lenses has been shown previously.<sup>22</sup>

Organic soil reduced the antimicrobial activity of the current MPDS and silver cases against adherent bacteria. This is consistent with a previous report that used the ISO Stand Alone Test in the presence of organic soil, which found reduced antimicrobial efficacy of four commercially available contact lens MPDS with bacteria in suspension.<sup>44</sup> The use of organic soil is optional under ISO 14729, but mandatory under the Food and Drug Administration guidelines for their Regimen test.<sup>25,45</sup> The latter requires <10 CFU of a set of microbes to be present on contact lenses at the end of the testing procedure, which involves rubbing, rinsing, and disinfecting of contact lenses.

The use of MPDS with silver cases for 24 hours significantly reduced bacterial numbers in lens cases, which is consistent with previous study findings where extended exposure in silver cases with another MPDS showed better antimicrobial efficacy.<sup>46</sup> The volume of the barrel lens cases was higher (10 vs. 4 mL) than the more commonly used flat lens cases. The addition of the higher volume of MPDS in barrel lens cases during disinfection could enhance the efficacy of MPDS solution.<sup>39</sup> The surface area of the lens cases also can be a contributing factor in the

difference between the level of microbial adhesion to flat and barrel lens cases. One limitation of this work is that we examined the sole silver-impregnated barrel case available and the results may not be generalizable.

The oxychlorite complex in the MPDS disinfecting solution produces hypochlorite (bleach), which is antimicrobial.<sup>47</sup> A recent report demonstrated that the antimicrobial efficacy of the oxychlorite disinfecting solution was significantly better than other commercially available contact lens MPDSs and was equivalent to a one-step hydrogen peroxide disinfection system.<sup>41</sup>

In conclusion, silver-copolymerized barrel lens cases exhibited broad-spectrum antimicrobial efficacy with the greatest activity against Gram-positive bacteria. The addition of their MPDS to the silver cases enhanced the overall efficacy of the disinfection system. The use of silver cases can be an alternative to reduce bacterial adhesion in lens cases. Future studies will investigate whether silver barrel lens cases have a similar ability to control microbial colonization in vivo.

## Acknowledgments

Disclosure: **A. Datta**, None; **M. Willcox**, None; **F. Stapleton**, None

## References

- Bates AK, Morris RJ, Stapleton F, Minassian DC, Dart JK. "Sterile" corneal infiltrates in contact lens wearers. *Eye (Lond)*. 1989;3:803–810.
- McLaughlin-Borlace L, Stapleton F, Matheson M, Dart JK. Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. *J Appl Microbiol*. 1998;84:827–838.
- Bharathi MJ, Ramakrishnan R, Meenakshi R, Kumar S, Padmavathy S, Mittal S. This ulcerative keratitis associated with contact lens wear. *Indian J Ophthalmology*. 2007;55:64–67.
- Stapleton F, Dart JK, Seal DV, Matheson M. Epidemiology of *Pseudomonas aeruginosa* keratitis in contact lens wearers. *Epidemiol Infect*. 1995; 114:395–402.
- Mayo MS, Schlitzer RL, Ward MA, Wilson LA, Ahearn DG. Association of *Pseudomonas* and *Serratia* corneal ulcers with use of contaminated solutions. *J Clin Microbiol*. 1987;25:1398–1400.
- Ogushi Y, Eguchi H, Kuwahara T, Hayabuchi N, Kawabata M. Molecular genetic investigations of contaminated contact lens storage cases as reservoirs of *Pseudomonas aeruginosa* keratitis. *Jpn J Ophthalmol*. 2010;54:550–554.
- Wu Y, Willcox M, Zhu H, Stapleton F. Contact lens hygiene compliance and lens case contamination: A review. *Contact Lens Anterior Eye*. 2015;38:307–316.
- Gray TB, Cursons RT, Sherwan JF, Rose PR. Acanthamoeba, bacterial, and fungal contamination of contact lens storage cases. *Br J Ophthalmol*. 1995;79:601–605.
- Devonshire P, Munro FA, Abernethy C, Clark BJ. Microbial contamination of contact lens cases in the west of Scotland. *Br J Ophthalmol*. 1993;77: 41–45.
- Wu Y, Zhu H, Harmis N, Iskandar S, Willcox M, Stapleton F. Profile and frequency of microbial contamination of contact lens cases. *Optom Vis Sci*. 2010;87:152–158.
- Willcox MDP, Carnt N, Diec J, et al. Contact lens case contamination during daily wear of silicone hydrogels. *Optom Vis Sci*. 2010;87:456–464.
- Szczotka-Flynn LB, Pearlman E, Ghannoum M. Microbial contamination of contact lenses, lens care solutions, and their accessories: a literature review. *Eye Contact Lens*. 2010;36:116–129.
- Capriotti JA, Pelletier JS, Shah M, Caivano DM, Ritterband DC. Normal ocular flora in healthy eyes from a rural population in Sierra Leone. *Int Ophthalmol*. 2009;29:81–84.
- Dong Q, Brulc JM, Iovieno A, et al. Diversity of bacteria at healthy human conjunctiva. *Invest Ophthalmol Vis Sci*. 2011;52:5408–5413.
- Cheng KH, Leung SL, Hoekman HW, et al. Incidence of contact-lens-associated microbial keratitis and its related morbidity. *Lancet*. 1999; 354:181–185.
- Mayo MS, Cook WL, Schlitzer RL, Ward MA, Wilson LA, Ahearn DG. Antibiograms, serotypes and plasmid profiles of *Pseudomonas aeruginosa* associated with corneal ulcers and contact lens wear. *J Clin Microbiol*. 1986;24:372–376.
- Yung MS, Boost M, Cho P, Yap M. Microbial contamination of contact lenses and lens care accessories of soft contact lens wearers (university students) in Hong Kong. *Ophthalmic Physiol Opt*. 2007;27:11–21.
- Kuzman T, Kutija MB, Juri J, et al. Lens wearers non-compliance-is there an association with lens case contamination? *Contact Lens Anterior Eye*. 2014;37:99–105.
- Sankaridurg PR, Sharma S, Willcox M, et al. Bacterial colonization of disposable soft contact lenses is greater during corneal infiltrative events than during asymptomatic extended lens wear. *J Clin Microbiol*. 2000;38:4420–4424.
- Heydorn A, Ersbll BK, Hentzer M, Parsek MR, Givskov M, Molin S. Experimental reproducibility in flow-chamber biofilms. *Microbiology*. 2000; 146:2409–2415.
- Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. *Prog Nat Sci*. 2008;18:1049–1056.
- Vermeltfoort PBJ, Hooymans JMM, Busscher HJ, van der Mei HC. Bacterial transmission from lens storage cases to contact lenses-Effects of lens care solutions and silver impregnation of cases. *J Biomed Mater Res B Appl Biomater*. 2008;87:237–243.
- Larkin DF, Kilvington S, Easty DL. Contamination of contact lens storage cases by *Acanthamoeba* and bacteria. *Br J Ophthalmol*. 1990;74: 133–135.
- Szczotka-Flynn LB, Imamura Y, Chandra J, et al. Increased resistance of contact lens related bacterial biofilms to antimicrobial activity of soft contact lens care solutions. *Cornea*. 2014;28:918–926.
- ISO. International Organization for Standardization. ISO 18259:2014. Ophthalmic optics—contact lens care products—method to assess contact lens care products with contact lenses in a lens case,

- challenged with bacterial and fungal organisms. Geneva, Switzerland; 2014.
26. Weisbarth RE, Gabriel MM, George M, et al. Creating antimicrobial surfaces and materials for contact lenses and lens cases. *Eye Contact Lens*. 2007;33:426–429.
  27. Reid TW, Tran PL, Mosley T, et al. Selenium covalently incorporated into the polymer of contact lens case material inhibits bacterial biofilm formation. In: *Asia ARVO Program Number: 497 Poster Board Number: B0134*;2013:B0134.
  28. Dantam J, Zhu H, Stapleton F. Biocidal efficacy of silver-impregnated contact lens storage cases in vitro. *Invest Ophthalmol Vis Sci*. 2011;52:51–57.
  29. Balazs DJ, Triandafillu K, Wood P, Chevolut Y, Delden C Van. Inhibition of bacterial adhesion on PVC endotracheal tubes by RF-oxygen glow discharge, sodium hydroxide and silver nitrate treatments. *Biomaterials*. 2004;25:2139–2151.
  30. Gurunathan S, Han JW, Kwon D-N, Kim J-H. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Res Lett*. 2014;9:373.
  31. Taheri S, Cavallaro A, Christo SN, et al. Substrate independent silver nanoparticle based antibacterial coatings. *Biomaterials*. 2014;35:4601–4609.
  32. Stobie N, Duffy B, McCormack DE, et al. Prevention of Staphylococcus epidermidis biofilm formation using a low-temperature processed silver-doped phenyltriethoxysilane sol – gel coating. *Biomaterials*. 2008;29:963–969.
  33. Dallas P, Sharma VK, Zboril R. Silver polymeric nanocomposites as advanced antimicrobial agents: classification, synthetic paths, applications, and perspectives. *Adv Colloid Interface Sci*. 2011;166:119–135.
  34. Abu-youssef MAM, Soliman SM, Langer V, et al. Synthesis, crystal structure, quantum chemical calculations, DNA interactions and antimicrobial activity of [Ag (2-amino-3-methylpyridine) 2] NO 3 and [Ag (pyridine-2-carboxaldoxime) NO 3]. *Inorg Chem*. 2010;49:9788–9797.
  35. Dias HVR, Batdorf KH, Fianchini M, et al. Antimicrobial properties of highly fluorinated silver(I) tris(pyrazolyl)borates. *J Inorg Biochem*. 2006;100:158–160.
  36. Cavicchioli M, Massabni AC, Heinrich TA, et al. Pt(II) and Ag(I) complexes with acesulfame: crystal structure and a study of their antitumoral, antimicrobial and antiviral activities. *J Inorg Biochem*. 2010;104:533–540.
  37. Ramstedt M, Cheng N, Azzaroni O, Mossialos D, Huck WTS. Synthesis and characterization of poly (3-Sulfopropylmethacrylate) brushes for potential antibacterial applications. *Langmuir*. 2007;23:3314–3321.
  38. Sambhy V, Macbride MM, Peterson BR, Sen A, Park UV, Pennsylv V. Silver bromide nanoparticle / polymer composites: dual action tunable antimicrobial materials. *J Am Chem Soc*. 2006;128:9798–9808.
  39. Dantam J, Zhu H, Willcox M, et al. In vivo assessment of antimicrobial efficacy of silver-impregnated contact lens storage cases. *Invest Ophthalmol Vis Sci*. 2012;53:1641–1648.
  40. Mowrey-McKee M, George M, Ajello M, Tolliver C. Comparative efficacy of currently marketed one-bottle peroxide solutions and silver containing lens cases. *Contact Lens Ant Eye*. 2008;31:245.
  41. Tilia D, Zhu H, Thomas V, Willcox M, Holden B, Lazon P de la J. Associations between solution induced corneal staining, corneal infiltrative events, and Gram-negative bacteria case contamination. *Br Contact Lens Assoc Clin Conf Exhibit*. 2012:e25–e26.
  42. Gabriel MM, Mcanally C, Bartell J. Antimicrobial efficacy of multipurpose disinfecting solutions in the presence of contact lenses and lens cases. *Eye Contact Lens*. 2018;44:125–131.
  43. Clavet CR, Chaput MP, Silverman MD, et al. Impact of contact lens materials on multipurpose contact lens solution disinfection activity against *Fusarium solani*. *Eye Contact Lens*. 2012;38:379–384.
  44. McGrath D, Costanzo SP, Manchester RJ, Kaiser JJ, Norton SE, McCormick PJ. Antimicrobial activity of no-rub multipurpose lens care solutions in the presence of organic soil. *Eye Contact Lens*. 2003;29:245–249.
  45. FDA. Contact lens and care product guidance documents. FDA Executive Summary. Prepared for the meeting of the ophthalmic devices panel of the medical devices advisory committee; May 13, 2014. Silver Spring, MD, U.S. Food and Drug Administration, 2014. Ava. 2008:1–3.
  46. Hiti K, Walochnik J, Faschinger C, Haller-Schober E-M, Aspöck H. One- and two-step hydrogen peroxide contact lens disinfection solutions against *Acanthamoeba*: how effective are they? *Eye (Lond)*. 2005;19:1301–1305.
  47. Falah-Tafti A, Jafari A, Lotfi-Kamran M. Comparison of the effectiveness of sodium hypochlorite and dentamize tablet for denture disinfection. *World J Med Sci*. 2008;3:10–14.