

## A COMPARATIVE STUDY OF ANTIBACTERIAL AND ANTIFUNGAL EFFICACY OF SOFT CONTACT LENS DISINFECTING SOLUTIONS

Tomislav Kuzman<sup>1</sup>, Rajko Pokupec<sup>1</sup>, Miro Kalauz<sup>1</sup>, Jelena Juri<sup>1</sup>, Zdenko Bujger<sup>1</sup> and Aleksandra Presečki<sup>2</sup>

<sup>1</sup>University Department of Ophthalmology; <sup>2</sup>Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, Zagreb, Croatia

**SUMMARY** – The aim of this study was to compare antibacterial and antifungal efficacy of five soft contact lens disinfecting solutions. The following solutions were tested: Oxysept Comfort (Advanced Medical Optics), Nitolens One Step (Avizor), Opti-Free Express (Alcon Laboratories), Complete Moisture Plus (Advanced Medical Optics) and Nitolens All-in-one (Avizor). Their efficacy to disinfect saline solution experimentally contaminated with American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) and *Staphylococcus epidermidis* (isolated from our laboratory) was tested. All study solutions reduced concentrations of bacteria and fungi below 1000 CFU/mL (reduction by 3 log and 1 log, respectively) after 8-hour overnight disinfection. However, there were differences in their disinfecting efficacy. Generally, all contact lens care solutions showed good disinfecting activity against the bacteria and fungi tested. Yet, the differences recorded in their antimicrobial activity may become more evident among non-compliant patients, and therefore the results of the study might be valuable when selecting appropriate solutions for contact lens wearers.

**Key words:** *Contact lens; Disinfection; Multipurpose contact lens solution, antimicrobial*

### Introduction

Disinfecting solutions need to keep soft contact lenses in a hygienic condition in order to avoid infections. Contact lens wearers are in the risk group of acquiring bacterial and fungal keratitis<sup>1-3</sup>. These infections are often associated with contaminated contact lens solutions<sup>4</sup>. Compliance and proper hygienic care of contact lens wearers are also of great importance, as poor compliance is estimated to be responsible for 80% or more of all contact lens related problems<sup>5,6</sup>.

Contact lens care systems have changed in the last few decades, from multi component bottle systems to current single bottle multipurpose care solutions. Although easier to use by patients, in order to achieve all

of the intended tasks the manufacturers of multipurpose solutions have to make some compromises. It has been suggested that the disinfecting agents used are less efficient but have better wetting and comfort abilities<sup>7,8</sup>.

The International Organization for Standardization (ISO) has established microbiological requirements and test methods for products and regimens for hygienic management of contact lenses with methodology and acceptance criteria for stand alone disinfecting solutions (ISO/CD 14729). According to this standard for stand alone primary acceptance criteria, disinfecting solution must be able to reduce the starting concentration of bacteria (*Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) by 3 log and of fungi (*Fusarium solani* and *Candida albicans*) by 1 log at the minimum disinfection time recommended by the manufacturers<sup>9</sup>. However, two common bacteria, *Staphylococcus epidermidis* and *Escherichia coli*, which are not required by ISO, often cause

Correspondence to: Tomislav Kuzman, MD, University Department of Ophthalmology, Zagreb University Hospital Center, Kišpatičeva 12, HR-10000 Zagreb,  
E-mail: tkuzman@gmail.com

Table 1. Formulation of the soft contact lens disinfecting solutions compared

Soft lens solution	Code used in study	Manufacturer	Disinfecting agent	Surfactant, isotonic, buffering and other agents
Oxysept Comfort	A	Advanced Medical Optics	Hydrogen peroxide 3%	Neutralizing catalase tablet, vitamin B12
Nitilens One Step	B	Avizor	Hydrogen peroxide 3%	Neutralizing catalase tablet
Opti-Free Express	C	Alcon	0.001% Polyquad 0.0005% MAPD	Tetronic 1304, boric acid, AMP, sodium chloride, sodium citrate and sorbitol
Complete Moisture Plus	D	Advanced Medical Optics	0.0001% PHMB	Poloxamer 237, sodium phosphate, sodium chloride, potassium chloride, propylene glycol and taurine
Nitilens All-in-one	E	Avizor	0.0002% Polyhexanide	0.25% Poloxamer, 0.1% EDTA, 0.05% PVP

AMP – 2-amino-2-methyl-1-propanol; EDTA – ethylenediaminetetraacetic acid; PVP – polyvinylpyrrolidone

ocular pathology<sup>10-12</sup>. Therefore, the aim of our study was to compare relative antibacterial and antifungal efficacy of five soft contact lens disinfection solutions commercially available in Croatia. To the best of our knowledge, the Nitilens One Step and Nitilens All-in-one (Avizor) solutions were now for the first time included in this type of study.

## Material and Methods

The antimicrobial activity of five commercially available disinfecting solutions was assessed and compared. We included the following solutions: Oxysept Comfort (Advanced Medical Optics), Nitilens One Step (Avizor), Opti-Free Express (Alcon Laboratories), Complete Moisture Plus (Advanced Medical Optics) and Nitilens All-in-one (Avizor) (Table 1). Their efficacy to disinfect saline solution experimentally contaminated with American Type Culture Collection (ATCC): *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) and *Staphylococcus epidermidis* (isolated from our laboratory) was tested.

The bacteria were grown on the blood agar plate, and *Candida albicans* on the Emmons agar plate. Using physiological saline, the microbial suspensions were adjusted to contain  $1.0 \times 10^8$  colony-forming units per milliliter (CFU/mL) bacteria and  $1.0 \times 10^6$  CFU/mL fungi. The appropriate volume of the disinfection solution (10 mL A-B; 2 mL C-E) and 2 mL of physiological saline were inoculated with the appropriate volume of the microbial suspension (100  $\mu$ L in A-B; 20  $\mu$ L in C-E and in physiological saline) to achieve a final concentration of

$1.0 \times 10^6$  CFU/mL bacteria and  $1.0 \times 10^4$  CFU/mL fungi. This concentration was the first one in the series of dilutions ( $1.0 \times 10^6$  or  $1.0 \times 10^4$  to  $1.0 \times 10^1$ ) with which the plates were inoculated separately four times. The mixtures of the disinfection solution and the microbial suspension were stored at ambient temperature, which was  $25 \pm 1$  °C. After 8-hour incubation (overnight disinfection period), appropriate disinfectant neutralizer was applied and the plates were inoculated. The blood and the Mueller-Hinton agar plates were used for identification of bacterial growth and the Emmons agar plate for identification of fungal growth. The agar plates were cultured at  $35 \pm 2$  °C for 24-72 hours. In addition, sterility control of disinfection solutions (100  $\mu$ L of each solution were seeded in the blood and Mueller-Hinton agar plate) and microbial growth control (100  $\mu$ L of each microbial suspension in a series of dilutions  $1.0 \times 10^8$  or  $1.0 \times 10^6$  to  $1.0 \times 10^1$  CFU/mL were seeded in the blood or Emmons agar plate) were performed.

## Results

All study solutions reduced microorganism concentrations below 1000 CFU/mL (concentrations of bacteria and fungi reduced by 3 log and 1 log, respectively). However, there were differences in their disinfecting efficacy.

Solution A containing hydrogen peroxide as a disinfecting agent showed good efficacy against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* as the concentration of bacteria was below 100 CFU/mL, whereas it was the least effective against *Staphylococcus epidermidis* with a concentration of 900 CFU/mL (Fig. 1).

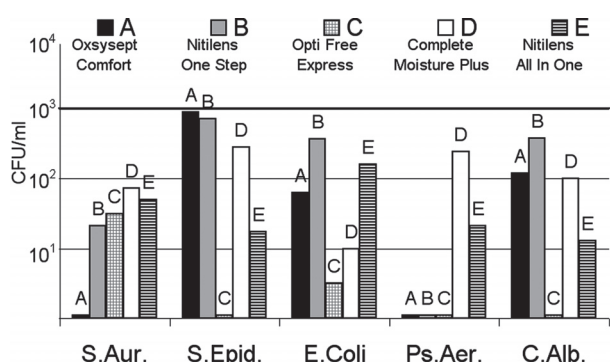


Fig. 1. Number of colony-forming units per milliliter (CFU/mL) of bacteria and fungi remained after 8-hour disinfection period for study solutions. Thick horizontal line represents 3 log and 1 log reduction criteria for bacteria and fungi, respectively.

Solution B, also containing hydrogen peroxide as a disinfecting agent showed good efficacy against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (<100 CFU/mL) but was not equally effective against *Staphylococcus epidermidis* and *Candida albicans*, showing a similar trend like solution A. However, solution A was more effective compared to solution B against *Escherichia coli* (<100 CFU/mL vs. <400 CFU/mL), although they contained the same disinfecting agent (Fig. 1).

Solution C showed very good disinfection activity against all microorganisms tested (all below 100 CFU/mL) (Fig. 1).

Solution D showed good efficacy in microorganism reduction against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (<100 CFU/mL) but was not as efficient against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Fig. 1).

Solution E showed good antimicrobial activity against all microorganisms tested (<100 CFU/mL), with the exception of *Escherichia coli* (Fig. 1).

The mean log reduction of microorganism concentrations for each of the soft contact lens solutions after 8-hour disinfection time is shown in Table 2.

Table 2. Mean log reduction after 8-hour overnight disinfection

Test solution	Staphylococcus aureus	Staphylococcus epidermidis	Escherichia coli	Pseudomonas aeruginosa	Candida albicans
A	>5.0	3.1	4.4	>5.0	1.9
B	4.8	3.3	3.6	>5.0	1.6
C	4.7	>5.0	>5.0	>5.0	>3.0
D	4.3	3.7	>5.0	3.8	2.0
E	4.5	4.8	3.8	4.8	2.9

## Discussion

The optimal contact lens care system should provide disinfection against a wide spectrum of different microorganisms avoiding toxicity to the eye. Antimicrobial agents can induce numerous adverse clinical effects like limbal and conjunctival hyperemia, punctate keratitis, superior limbal keratoconjunctivitis, papillary conjunctivitis, corneal staining and edema<sup>13-15</sup>. Therefore, the manufacturers should always balance the ability of solutions to retain a broad spectrum of antimicrobial activity while allowing for only minimal toxicity. These properties depend upon agents contained in the solution.

Although not required by ISO Guidelines, *Staphylococcus epidermidis* is one of the most common bacteria in the eyes of lens wearers<sup>11</sup>. Likewise, disinfecting activity against *Escherichia coli* is not required in the ISO Guidelines but it commonly contaminates contact lens accessories stored in bathrooms<sup>10</sup>. We believe that contact lens solution disinfecting activity should be extended to as much as possible resistant and common microbial species. If the goal is to achieve sterile contact lens, it might be important for the contact lens solution to exhibit antimicrobial activity also against the most common bacteria. It is frequently unnecessary to identify the bacteria that are contained in the solution<sup>16</sup>. Therefore, both *Escherichia coli* and *Staphylococcus epidermidis* were also tested in our study.

Disinfection time in our study was 8 hours, which is defined as overnight disinfection. We believe that 8-hour disinfection period is appropriate as it resembles everyday situations considering that lens wearers usually do not wear lenses overnight.

We tested several different contact lens disinfecting solutions containing different disinfecting agents: two containing hydrogen peroxides, one polyquad and myristamidopropyl dimethylamine (MAPD), one polyhexamethylene biguanide (PHMB), and one containing polyhexanide (Table 1).

The observed efficiency of Oxysept Comfort against all bacteria and fungi, with the exception of *Staphylococcus epidermidis*, was consistent with other studies, whereas there are no reports on studies investigating Nitilens One Step antimicrobial activity<sup>17-19</sup>. Both solutions were least effective against *Staphylococcus epidermidis*. The fact that *Staphylococcus epidermidis* was not standardized, but was isolated from our laboratory might provide an explanation for these results. However, considering that *Staphylococcus epidermidis* was isolated from our laboratory, our results add “real life” experience to this experiment. As we live surrounded by unstandardized bacteria, the efficacy of a disinfecting solution should be measured by its efficacy to kill most microorganisms that may be present<sup>16</sup>. Therefore, we believe our results are rather interesting. It should be noted that these systems are also preservative free, so they can be used in patients allergic to preservatives.

According to our results, Opti-Free Express showed highest antimicrobial activity against all bacteria and fungi tested. This might be attributed to the fact that it contains two antimicrobial agents, polyquad and MAPD. Polyquad is a quaternary ammonium-based antimicrobial agent providing predominantly antibacterial properties, whereas MAPD has a wider spectrum of antimicrobial activity, particularly for fungi<sup>20</sup>. Our results are in relatively consistent with other studies<sup>21,22</sup>. Opti-Free Express showed good efficacy against *Staphylococcus aureus*, unlike the results from a similar study which, however, was performed during a 6-hour disinfection period<sup>23</sup>. A different ATCC strain of *Staphylococcus aureus* might also provide an explanation for the observed difference. Although having strong antimicrobial activity, Opti-Free Express was shown to be more cytotoxic as compared with other solutions<sup>24</sup>. It should be noted that multipurpose solutions are intended to be applied into the eye, and therefore should not be rated solely based on their disinfecting capacity.

The latter two solutions tested, Complete Moisture Plus and Nitilens All-in-one, both contain biguanide-based antimicrobial agents, polyhexamethylene biguanide (PHMB) and polyhexanide, respectively. These antimicrobial agents contain cationic active sites that have the ability to lyse microbial cellular membranes by electrostatic interaction. PHMB is a polymer with 6 to 14 active sites, showing antimicrobial activity for gram-positive and gram-negative bacteria<sup>25</sup>. Both solutions showed similar antibacterial activity against all microorganisms tested, although Complete Moisture Plus

showed somewhat lower efficacy than Nitilens All-in-one. Likewise, previous studies have shown that multipurpose disinfecting contact lens solutions with identical concentrations of PHMB might behave differently depending on the solution formulation<sup>26</sup>. Furthermore, it is possible that besides solution components, other solution qualities, e.g., viscosity and ionic balance of the solution, contribute significantly to the overall antimicrobial activity<sup>27,28</sup>. Although having somewhat lower antimicrobial activity, Complete Moisture Plus was not found to be cytotoxic in previous studies<sup>24</sup>.

There is one issue that should be acknowledged. Antimicrobial activity was tested against microorganisms in suspension. As shown in a previous study, microorganisms adhering to the surface of the lens case may be more difficult to eliminate<sup>29</sup>.

Overall, all contact lens care solutions showed good antimicrobial activity against all bacteria and fungi. However, we noted differences in their antimicrobial activity that might become more evident among non-compliant patients. A solution that showed better antimicrobial efficacy could provide higher safety for non-compliant patients, as good disinfection capacities might be the most important solution quality that would help minimize the risk of eye infections.

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## Sažetak

## USPOREDBA ANTIBAKTERIJSKE I ANTIGLJIVIČNE UČINKOVITOSTI OTOPINA ZA DEZINFEKCIJU MEKIH KONTAKTNIH LEĆA

T. Kuzman, R. Pokupec, M. Kalauz, J. Juri, Z. Bujger i A. Presečki

Cilj studije bio je usporediti antibakterijska i antigljivična svojstva pet tekućina za dezinfekciju mekih kontaktnih leća. Ispitane su slijedeće tekućine: Oxysept Comfort (Advanced Medical Optics), Nitolens One Step (Avizor), Opti-Free Express (Alcon Laboratories), Complete Moisture Plus (Advanced Medical Optics) i Nitolens All-in-one (Avizor). Usporedili smo njihovu učinkovitost u dezinfekciji fiziološke otopine eksperimentalno kontaminirane slijedećim mikroorganizmima (American Type Culture Collection (ATCC)): *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028) i *Staphylococcus epidermidis* (soj izoliran u našem laboratoriju). Sve ispitivane tekućine su smanjile početnu koncentraciju bakterija i gljivica ispod 1000 CFU/mL (broj kolonija/mL; smanjenje 3 log za bakterije i 1 log za gljivice) nakon dezinfekcijskog razdoblja od 8 sati. Međutim, zabilježene su razlike u njihovoj dezinfekcijskoj učinkovitosti. Općenito su sve ispitane tekućine pokazale dobru dezinfekcijsku učinkovitost protiv testiranih bakterija i gljivica, ali uočene razlike u njihovoj učinkovitosti mogu postati važne kod nesuradljivih nositelja mekih kontaktnih leća. Stoga su rezultati ove studije korisni pri odabiru tekućina kod navedene skupine nositelja.

Ključne riječi: *Kontaktne leće; Dezinfekcija; Višenamjenska otopina za dezinfekciju kontaktnih leća, antibakterijska*