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LABORATORY STUDY

One- and two-step hydrogen peroxide contact lens disinfection solutions against *Acanthamoeba*: How effective are they?

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Abstract

Purpose **Effective contact lens disinfection solutions are important to keep the storage case free of *acanthamoebae* and thus prevent an infection of the eye. The aim of our study was to evaluate the effectivity of two new one-step hydrogen peroxide disinfecting solutions against *Acanthamoeba* spp. and compare it to the effectivity of other commercially available systems.**

Methods **Nine one-step 3% hydrogen peroxide systems including the new systems Silver Sept (platinum and silver disk for intensifying disinfection) and Blue Vision (newly composed catalytic tablet) and 2 two-step systems (0.6 and 3.0% H_2O_2) were tested for their effectivity against cysts of two *Acanthamoeba* keratitis isolates at different concentrations.**

Results **After a soaking time of 8 h (overnight soaking of contact lenses) the 2 two-step systems completely destroyed the cysts of both *Acanthamoeba* strains, even at the highest concentration of cysts tested. The nine tested one-step systems showed weaker effects. The new Blue Vision system was able to eradicate the cysts of one strain at the low concentration of cysts.**

Conclusions **One-step hydrogen peroxide systems do not have sufficient effects on *Acanthamoeba* cysts and therefore may not protect the contact lens user from a possible infection of the eye. Further development of tablets like the ones used in the Blue Vision system may result in better cysticidal effects for one-step systems.**

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Introduction

Contact lens wearing is the main risk factor for keratitis caused by *Acanthamoeba*.[1] Several studies have shown that the contact lens storage case is a potential source and reservoir of these parasites. Acanthamoebae have been isolated from the lens storage cases of many patients in whom *Acanthamoeba* keratitis has been diagnosed and also from contact lens cases of asymptomatic lens wearers.[1,2–6] *Acanthamoeba* trophozoites and cysts attach to the surface of the contact lens and can therefore be transmitted from the contaminated storage case onto the eye in high concentrations.[7] The adherence depends on the lens type and material and is significantly higher with high water content soft contact lenses than with rigid gas permeable lenses, which probably explains the greater risk for soft contact lens wearers.[7] Corneal microtraumata in connection with the use of contact lenses represent an open door for *Acanthamoeba* invasion and the devastating infection of the eye.[8,9]

In the early stage, the infection is often misdiagnosed as a Herpes simplex or fungal keratitis.[10,11] The treatment of *Acanthamoeba* keratitis is most successful in those cases in which the diagnosis is made very early.[11,12–14] However, the most important aspect in the management of *Acanthamoeba* keratitis is prevention. Therefore the use of contact lens storage solutions that reliably kill *Acanthamoeba* trophozoites and cysts is of great importance.

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Table 1 Contact lens solutions tested

Trade name	*Manufacturer*	*System*	*Hydrogen peroxide*	*Lens-Type*
Oxysept® 1	Pharm Allergan GmbH D-76260 Ettlingen	Two-step catalase solution 520 U/ml	3% H_2O_2	Soft
Titmus H_2O_2	CIBA Vision Corp. Duluth GA 30097, USA	Two-step catalase solution 170 U/ml	0.6% H_2O_2	Soft and rigid gas permeabel
Oxysept® Comfort	Pharm Allergan GmbH D-76260 Ettlingen	One-step catalase 0.1 mg/tablet	3% H_2O_2	Soft
ONS MERK 1	Oté Pharma Sol BV-NL 5406-XP Uden	One-step catalase tablet no details	3% H_2O_2	Soft
BLUE VISION	CIBA Vision Corp. Duluth GA 30097, USA	One-step catalase 0.3 mg/ tablet	3% H_2O_2	Soft
Silver Sept	MDLE Gmbh Germ.-87700 Memmingen	One-step platin disk and silver disk	3% H_2O_2	Soft
Concerto® platinum	Lab. Contapharm France-77390 Creteil	One-step platin disk	3% H_2O_2	Soft
easy® SEPT	Bausch&Lomb IOM S.p.A.-2005 Ital., Milano	One-step platin disk	3% H_2O_2	Soft
CONTACT	Wöhlk C.L. Gm-bh, Germ.-24232 Schönkirchen	One-step platin disk	3% H_2O_2	Soft
CARE SOFT contopharma	Contopharma AG CH –3800, Interlaken	One-step catalase solution 111 U/ml	3% H_2O_2	Soft
AOSEPT	CIBA Vision Corp. Duluth GA 30097, USA	One-step platin disk	3% H_2O_2	Soft

The two most popular types of contact lens storage solutions are multipurpose disinfection systems on one hand and one-step hydrogen peroxide systems on the other. In this study, the effect of nine one-step hydrogen peroxide contact lens storage and disinfection solutions and 2 two-step systems on different *Acanthamoeba* strains was tested.

Materials and methods

Contact lens solutions

All contact lens disinfection solutions tested (Table 1) were purchased from local retail stores. The solutions were taken from the original wrappings and used before their stated expiry date. In case of the two-step systems Titmus H_2O_2 [0.6% H_2O_2] and Oxysept® 1 [3% H_2O_2] neutralisation of the hydrogen peroxide is achieved by soaking the contact lenses in a second catalytic solution for 10 min before wearing.

In five of the tested one-step systems (Concerto® platinum, AOSEPT, Contact care soft, easy® SEPT and Silver Sept) neutralisation of the 3% hydrogen peroxide is achieved within 6 h by a catalytic platinum disk which is located in the contact lens storage case. In the Silver Sept system additionally to the platinum disk on the bottom of the storage case, a small silver disk is attached to the contact lens storage basket. This silver disk releases silver ions during the disinfecting process, intensifying disinfection and suppressing microbial multiplication.

In three of the four other one-step systems tested (Blue VISION, ONS MERK 1 and Oxysept® Comfort) a catalytic tablet is put into the storage case together with the 3% hydrogen peroxide solution requiring a minimum lens soaking time for disinfection and neutralisation of 6 h.

The one-step system contopharma® works with two solutions. After pouring 4 ml of a catalytic solution into a marked contact lens storage case, 4 ml of a 3% hydrogen peroxide solution are added and the contact lenses are soaked whereby disinfection and neutralisation should be achieved within 20 min of soaking time.

Acanthamoeba

Two different strains of *Acanthamoeba* spp. belonging to morphological group II were used in this study. Both strains, the 11DS strain of *Acanthamoeba hatchetti* and the 1BU strain of *Acanthamoeba castellanii*, had been isolated from patients suffering from a severe *Acanthamoeba* keratitis.[15,16] Isolation had been achieved by inoculating corneal epithelium onto non-nutrient agar plates covered with a 48-h-old culture of *Escherichia coli*.

The isolates were cloned by transferring a single cyst onto a fresh plate with a micromanipulator. Axenisation was achieved by harvesting cysts from parallel cultures and incubating them in 3% HCl overnight in order to eliminate coexisting bacteria. The cysts were then transferred to sterile filtrated proteose peptone-yeast extract-glucose (PYG),[17] that was used as axenic medium further on. Synchronised encystment of the amoebae was achieved by long-term storage without the addition of fresh medium. The process of encystment including morphological changes of the cyst wall was observed daily by phase-contrast microscopy. After 14 days the

cysts of both strains were in their mature stage. The cysts were harvested by centrifugation (500 *g*/7 min), resuspended in sterile 0.9% NaCl and counted in a Bürker-Türk haemocytometer. Of each strain two suspensions were prepared, one with 10^4 and one with 10^5 cysts/ml, respectively.

Performance of the tests

Tests were performed in 15 ml centrifugation tubes. A measure of 100 μl of the respective cyst suspension (10^3 and 10^4 cells) and 8 ml of the respective contact lens solution were added per tube. All solutions were used according to the manufacturers' instructions.

The two-step solutions were directly added to the tubes. For the one-step systems working with a catalytic disk, the platin ring was installed prior to the addition of the 3% hydrogen peroxide solution. For the systems working with a catalytic tablet, the tablet was added immediately after addition of the 3% hydrogen peroxide solution. Silver Sept tests were performed in the original contact lens case because of the silver disk attached to the contact lens basket.

After a soaking time of 8 h the pellets (after 500 g/7 min centrifugation) of the respective tubes were inoculated onto non-nutrient agar plates covered with a layer of *E. coli*. The plate cultures were sealed and incubated at 30°C for 14 days. Amoebic growth was observed daily by phase-contrast microscopy. All experiments were carried out in triplicate. The control groups were performed with sterile 0.9% NaCl.

Results

The effect of the 11 hydrogen peroxide contact lens disinfecting solutions on the two different *Acanthamoeba* strains is shown in Table 2. Generally, the two-step systems Titmus H_2O_2 and Oxysept® 1 showed the best amoebicidal effects. After 8 h of soaking, the cysts of both strains, *A. hatchetti* and *A. castellanii*, were completely destroyed independently from cyst concentration. The one-step systems Blue Vision (tablet) and AOSEPT (platin disk) were effective against 10^3 cysts of *A. hatchetti*, while cysts at higher concentration (10^4) and those of *A. castellanii* were still viable after exposure to these solutions for 8 h. All other solutions working with a platin disk (Concerto® platinum, easy Sept and Contact care soft) or with a catalytic tablet for neutralisation of the 3% hydrogen peroxide (Oxysept® Comfort and ONS MERK 1), were not able to eradicate the *Acanthamoeba* cysts at any concentration within 8 h of soaking time. Cysts of both tested strains and concentrations were still viable. Also Silver Sept, working with a platin and a silver disk and contopharma® (the one-step system with a hydrogen peroxide solution and a neutralising solution) gave positive cultures after 8 h of soaking time with either *Acanthamoeba* strain and at either concentration.

Table 2 Viability of *Acanthamoeba* cysts after 8 h of exposure time

	A. hatchetti (11 DS)		*A. castellanii (1 BU)*	
	10^3	10^4	10^3	10^4
Oxysept® 1	−	−	−	−
Titmus H_2O_2	−	−	−	−
BLUE VISION	−	+	+	+
AOSEPT	−	+	+	+
Oxysept® Comfort	+	+	+	+
Silver Sept	+	+	+	+
Concerto® platinum	+	+	+	+
Easy® Sept	+	+	+	+
Contact care soft	+	+	+	+
ONS MERK 1	+	+	+	+
Contopharma	+	+	+	+
Control 0.9% NaCl	+	+	+	+

Both *Acanthamoeba* strains belong to morphological group II, however, *A. castellanii* was found to be slightly more resistant than *A. hatchetti*.

Discussion

Our study has shown that not all of the different hydrogen peroxide contact lens storage and disinfecting systems tested are effective against *Acanthamoeba* cysts. After an exposure time of 8 h, the two tested two-step systems, Titmus H_2O_2 and Oxysept® 1, were able to completely eradicate the *Acanthamoeba* cysts at either concentration tested. The nine tested one-step systems working with 3% hydrogen peroxide were less effective.

Three per cent hydrogen peroxide is a very capable disinfectant against a wide variety of microorganisms and ocular pathogens.[18] An incubation of 15 min is sufficient to kill most bacteria and to inactivate HIV, for *Acanthamoeba* an incubation of 1–3 h with 3% hydrogen peroxide is necessary.[18–20]

However, hydrogen peroxide is toxic to the ocular epithelium.[21] In one-step systems neutralisation of the hydrogen peroxide is achieved by a catalytic platinum disk or a tablet containing catalase which is located into the contact lens storage case. These systems are very comfortable for the contact lens wearer. According to the manufacturers' instructions neutralisation and disinfection are achieved by one-step within a period of 6 h and the contact lens can be brought directly onto the eye.

Among the one-step systems working with a catalytic tablet Blue Vision (0.3 mg catalase/tablet) completely

destroyed *A. hatchetti* at a concentration of 10^3 cells/ml, whereas Oxysept® Comfort and Ons Merk 1 did not. In Oxysept® Comfort (0.1 mg catalase/tablet) and Ons Merk 1 the neutralisation of the hydrogen peroxide to oxygen and water by the catalase tablet starts immediately. In the Blue Vision system it takes 55–65 min of tablets' soaking time to decompose the blue capsule of the tablet and release catalase from the nucleus. After that time the neutralisation of the hydrogen peroxide starts promptly. These findings may explain the better results of the Blue Vision system compared to the two other one-step systems based on a catalase tablet.

Too early neutralisation of the 3% hydrogen peroxide could be avoided by the development of more durable tablet–capsules and therefore make one-step systems safer for contact lens users.

Among the systems working with a platin disk AOSEPT (3% hydrogen peroxide with 0.85% sodium chloride), completely destroyed *A. hatchetti* at a concentration of 10^3 cells/ml, whereas the other platin disk systems did not show enough effectivity to completely eradicate the *Acanthamoeba* cysts at either concentration. The reasons for the better activity of the AOSEPT system in comparison to the other platin disk systems are unclear. Silvany *et al*[19] demonstrated the viability of two *Acanthamoeba* species after 8 h exposure to AOSEPT and Hughes and Kilvington[20] described a 1.28 log reduction of *A. polyphaga* after 4 h contact time to AOSEPT. Differences in stabilising ingredients, pH, and neutralisation time of the hydrogen peroxide may result in the varying amoebicidal effect for different products containing the same concentration of hydrogen peroxide and a catalytic platinum disk for neutralisation.

Interesting results for platin disk systems have been shown by Hughes *et al*.[22] A combination of horseradish peroxidase, KI, and H_2O_2 showed an enhanced cysticidal activity compared to hydrogen peroxide alone, and was also effective when tested with a platinum disk system.

The normal period of time for contact lenses to be disinfected would be overnight while the wearer is not using them. Thus a soaking time of 8 h was of main interest in our study. Our results showing the good cysticidal activity of the two-step system Oxysept 1 correspond to those of other studies and can be explained by the long exposure time to 3% hydrogen peroxide which is not deactivated by a catalytic system (tablet or disk).[20,23] Although the Titmus H_2O_2 system for disinfection of soft and rigid contact lenses works with hydrogen peroxide at a concentration of only 0.6%, it is more effective than the one-step systems, that contain 3% H_2O_2, but in that the neutralisation process starts immediately. This shows clearly, that also with 0.6% H_2O_2 a complete destruction of *Acanthamoeba* cysts can be achieved, if this concentration is held up during 8 h and not deactivated by a catalytic system. However, the H_2O_2 concentration must not be lower than 0.6%. Zanetti *et al* tested the hydrogen peroxide of two contact lens solutions against a corneal isolate of *A. castellanii*. A 1 : 2 dilution of this solution (1.5%) killed the cysts after a 9 h exposure, but after exposure to a 1 : 10 dilution (0.3%), 25% of the cysts were still viable.[24]

In our study, *A. hatchetti* showed a higher degree of sensitivity against the tested contact lens solutions, than *A. castellanii*. The susceptibility of *Acanthamoeba* cysts to disinfection is dependent on the cyst age,[25,26] but also on the cyst structure of the particular strain. Several studies have shown that the sensitivity of *Acanthamoeba* cysts against contact lens disinfectants varies between the different strains and morphological groups.[19,23,27–28] *Acanthamoeba* cysts are slightly smaller than the trophozoites with a size of 12–28 μm in diameter. They are double-walled consisting of an interior endocyst, which can be stellate, polygonal or even round, and an exterior wrinkled ectocyst. The cyst walls are laminated and united at several sites. Where the endocyst and the ectocyst meet, characteristic pores are found.[12,29] The thickness of the cyst wall and the number of pores may influence the biocide susceptibility of the particular strain.

A contact lens disinfecting solution that does not kill both *Acanthamoeba* trophozoites as well as cysts is not suitable for protecting the contact lens user from a posssible infection with these pathogens. The results of our study demonstrate that commercially available one-step hydrogen peroxide disinfecting systems are not effective enough against *Acanthamoeba* cysts because of rapid hydrogen peroxide neutralisation.

Further development of catalytic tablets like tested in the Blue Vision system should make one-step systems safer for the contact lens wearer. Adequate exposure times to 3% H_2O_2 may be achieved by the development of more durable tablet capsules, protecting the catalytic nucleus for 2–3 h and therefore avoiding too early neutralisation of the hydrogen peroxide.

We recommend the tested two-step sytems (Titmus H_2O_2, Oxysept® 1) for the storage and disinfection of contact lenses.

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