Resistance of *Acanthamoeba* Cysts to Disinfection in Multiple Contact Lens Solutions

Stephanie P. Johnston*, Rama Sriram¹, Yvonne Qvarnstrom¹, Sharon Roy¹, Jennifer Verani¹, Jonathan Yoder¹, Suchita Lorick²,³, Jacquelin Roberts¹, Michael J. Beach¹, and Govinda Visvesvara¹

Division of Parasitic Diseases¹ and Division of Immunization Services², and Epidemic Intelligence Service Program³, Centers for Disease Control and Prevention, Public Health Service, Department of Health and Human Services, Atlanta, Georgia

Corresponding Author and Address for Reprints:

Stephanie P. Johnston  
Division of Parasitic Diseases  
Centers for Disease Control and Prevention  
4770 Buford Highway, NE, MS F-36  
Atlanta, Georgia 30341-3724  
Phone: (770) 488-7044  
Fax: (770) 488-3115  
E-mail: sjohnston@cdc.gov
ABSTRACT

Acanthamoebae are free-living amoebae found in the environment including soil, fresh, brackish and sea water, and hot tubs and Jacuzzis. Acanthamoeba can cause keratitis, a painful vision-threatening infection of the cornea, and fatal granulomatous encephalitis in humans. More than 20 species of Acanthamoeba belonging to morphological groups I, II, and III distributed in 15 genotypes have been described. Among these, A. castellanii, A. polyphaga, and A. hatchetti are frequently identified as causing Acanthamoeba keratitis (AK). Improper contact lens care and contact with non-sterile water while wearing contact lenses are known risk factors for AK. During a recent multi-state outbreak, AK was found to be associated with the use of Advanced Medical Optics Complete® MoisturePlus™ multipurpose contact lens solution, which was hypothesized to have had insufficient anti-Acanthamoeba activity. As part of this outbreak investigation, we compared the efficacy of 11 different contact lens solutions against cysts of A. castellanii, A. polyphaga, and A. hatchetti, genotype T4, which were isolated in 2007 from specimens obtained during the outbreak investigation. The data, generated using A. castellanii, A. polyphaga, and A. hatchetti cysts, suggest that contact lens solutions containing hydrogen peroxide were the only solutions that showed any disinfection ability with 0% and 66% growth respectively for A. castellanii and 0% and 33% growth respectively for A. polyphaga. There was no statistically-significant difference in disinfection efficacy between the 11 solutions for A. hatchetti.
Acanthamoeba, a free-living amoeba, occurs worldwide in soil and water. It has been isolated from ponds, lakes, brackish and sea water, filters of heating, ventilating and air conditioning units, medical equipment such as gastric wash tubing, dental irrigation units, contact lenses and contact lens solutions, as well as vegetables, cell cultures, and even from human and animal tissues (7, 23, 38). It has also been isolated from toxic waste dumpsites with high levels of pesticides, herbicides, pharmaceuticals, heavy metals, and polychlorinated biphenyls (PCBs) (35). Acanthamoeba has two stages in its life cycle: a vegetative or trophozoite stage which reproduces by binary fission and feeds voraciously on bacteria and detritus present in the environment and a non-dividing, cyst stage that is resistant to environmental stress. Acanthamoeba causes different types of human disease including central nervous system infection — granulomatous amebic encephalitis (GAE), cutaneous infection — Acanthamoeba dermatitis, and ocular infection — Acanthamoeba keratitis (AK). GAE and cutaneous infections occur principally in immunocompromised individuals, including patients with HIV/AIDS (17, 23, 37, 43). In contrast, AK principally occurs in immunocompetent individuals.

AK is a painful vision-threatening infection, which if not treated promptly may lead to ulceration of the cornea, loss of visual acuity, and eventually blindness (7, 15, 16). AK is associated with trauma to the cornea and with contact lens wear as a result of poor lens care and hygiene. Introduced into the eye by a contaminated contact lens, Acanthamoeba may adhere to the corneal surface and subsequently infiltrate the stoma and cause tissue damage (10). Both Acanthamoeba cysts and trophozoites can be isolated...
Confocal microscopy has been used as an aid in the diagnosis of AK (29). Molecular techniques such as real-time PCR assays have been developed for the identification of *Acanthamoeba* (32, 33). Sequencing analysis of the 18SrRNA gene has been used to identify as many as 15 genotypes of *Acanthamoeba*, of which the T4 genotype appears to be the most common in the environment and in patients with AK (2, 23).

The first documented case of AK in the United States occurred in 1973 in a south Texas rancher following trauma to his right eye (15, 40, 42). Both trophozoite and cyst stages of *A. polyphaga* were demonstrated in corneal sections. Between October 1985 and August 1986, Stehr-Green, et al (41) conducted a case-control study to investigate an outbreak of AK in the United States. The majority of case-patients wore contact lenses and illness was most highly associated with the use of homemade saline solutions and lens care practices, such as disinfecting lenses less frequently than recommended, and swimming while wearing lenses (8, 41). Contact lens use is now considered to be an important risk factor for AK in the United States. AK cases have continued to be diagnosed since the 1986 outbreak but, because AK is not a reportable disease in the US, the actual number of cases occurring each year is not known.

A recent study indicated a dramatic increase in AK cases in the Chicago, IL area (16). An investigation conducted by the Centers for Disease Control and Prevention (CDC) revealed that this increase in the number of AK cases was occurring nationwide, starting in 2004 and continuing through 2007 (7). A subsequent investigation identified the use of Advanced Medical Optics Complete® MoisturePlus™ multipurpose contact lens solution as the primary risk factor, leading to an international recall of this product.
by the manufacturer (7, 16). We therefore decided to examine this and other frequently
used major contact lens solutions for their efficacy against *Acanthamoeba* species
isolated from clinical samples collected during the 2007 AK outbreak investigation.

MATERIALS AND METHODS

**Isolation of *Acanthamoeba***. During the 2007 AK outbreak investigation, 94
specimens from patients were collected and cultured on non-nutrient agar plates coated
with a layer of *Escherichia coli*. In the 24 plates that were positive, the amoebae
consumed bacteria, multiplied, and encysted after most of the bacteria were gone. Both
trophozoites and cysts were examined microscopically and assigned to the morphological
group II. In addition, cyst morphology was used for identification at the species level (28,
34). One isolate of *A. castellanii* (isolate CDC:V568), *A. polyphaga* (isolate CDC:V572),
and *A. hatchetti* (isolate CDC:V573) were selected for genotyping as these species of
*Acanthamoeba* are commonly found in the U.S. All three isolates were found to be
genotype T4 using sequencing analysis of the 18SrRNA gene as previously described (2,
22, 36).

**Contact lens solutions**. Eleven different contact lens solutions were tested: Alcon
OPTI-CLEAN® II, Alcon OPTI-FREE® Express®, Alcon OPTI-FREE® RepleniSH®,
AMO Complete® MoisturePlus™, AMO UltraCare®, Bausch & Lomb Boston
Simplus®, Bausch & Lomb ReNu MoistureLoc®, Bausch & Lomb ReNu MultiPlus®,
Ciba Vision Clear Care®, Ciba Vision AQuify®, and Kirkland Signature Multipurpose
Solution. These 11 solutions were selected for this study because they were brands used
by case-patients in the 2004–2007 AK outbreak. Ten of the 11 solutions were purchased from retail stores in the Atlanta area. The remaining solution, Bausch & Lomb ReNu MoistureLoc®, was removed from the market in 2006 because it was the brand of multipurpose contact lens solution associated with an outbreak of Fusarium keratitis (9). This solution was provided by colleagues at the Centers for Disease Control and Prevention who had kept a supply of this solution following the investigation of the Fusarium keratitis outbreak. The solutions used in this study along with their active ingredients and disinfectant properties are listed in Table 1.

To study the effects of various contact lens solutions against the cysts of the three species of Acanthamoeba, the amoebae were grown on agar plates for three weeks with E. coli. When most of the bacteria were consumed trophozoites began to differentiate into cysts and by the third week the agar plates were covered with cysts. Cysts were harvested from the agar plates and washed three times with 50 ml of amoeba saline, counted in a hemacytometer and adjusted to yield 100 cysts per 10 µl.

The lens cases used with the nine non-hydrogen peroxide containing solutions hold 1 ml of contact solution. Therefore, ten microliters of the cyst-containing solution was added to one ml of each contact solution (Alcon OPTI-CLEAN® II, Alcon OPTI-FREE® Express®, Alcon OPTI-FREE® RepleniSH®, AMO Complete® MoisturePlus™, Bausch & Lomb Boston Simplus®, Bausch & Lomb ReNu MoistureLoc®, Bausch & Lomb ReNu MultiPlus®, Ciba Vision AQuify®, and Kirkland Signature Multipurpose Solution) in 15 ml tubes, in triplicate, and incubated at 24°C for either 4 or 6 hours (according to manufacturer’s contact lens soaking-time recommendations) and for 24 hours.
The two hydrogen peroxide-containing solutions (AMO UltraCare® and Ciba Vision Clear Care®) require the use of lens cases, provided in the box, that need to be filled up to the fill line (approximately 5 ml) of the case with the contact lens solution. Therefore, ten microliters of the cyst-containing solution was added to the contact lens cases already filled with the contact lens solutions (along with the AMO UltraCare® - provided neutralizing tablet), in triplicate, and incubated at 24°C for either 6 or 24 hours. AMO UltraCare® includes a neutralizing tablet that must be added to the contact lens solution in the contact lens case while Ciba Vision Clear Care® has a built in neutralizing disc within the contact lens case.

After incubation, the cysts were washed by centrifugation for 10 minutes at 1500 x g, inoculated on agar plates coated with E. coli, and incubated at 24°C. Plates were examined daily for two weeks with an inverted microscope for the presence of trophozoites and the efficacy of the solutions was recorded as positive or negative.

**Statistical Analysis.** The Cochran-Mantel-Haenszel Test was used to test for overall association between the number of positive plates and contact lens solutions, controlling for the three Acanthamoeba species at 4–6 hours and 24 hours incubation. Fisher’s Exact test was used to compare the number of positive plates within each species. All analyses were performed using SAS, version 9.1, SAS Institute Inc., Cary, NC, USA. Statistical significance was set at alpha=0.05.
RESULTS

Of the 11 contact lens solutions that were examined for their efficacy in inactivating cysts of the three *Acanthamoeba* species, one of these solutions that contained hydrogen peroxide (Ciba Vision Clear Care®) demonstrated the greatest inactivation of cysts of all three species of *Acanthamoeba* (Table 3). Overall, there were no statistically-significant differences between the three *Acanthamoeba* species in their susceptibility to the contact lens solutions tested. All three were most responsive to the Ciba Vision Clear Care®, which was the only solution that prevented excystation under these experimental conditions.

Considering all *Acanthamoeba* species together, there were statistically-significant differences in the efficacy of the different brands of contact lens solutions at both 4–6 hours (p<0.0001) and 24 hours (p<0.0001) incubation. At 4–6 hours incubation, there were statistically-significant differences in disinfection efficacy between the 11 solutions for *A. castellanii* (p=0.008) and *A. polyphaga* (p=0.0014). Specifically, Ciba Vision Clear Care® and AMO Ultracare, both containing hydrogen peroxide, were the only solutions that showed any disinfection ability with 0% and 66% growth respectively for *A. castellanii* and 0% and 33% growth respectively for *A. polyphaga*. There was no statistically-significant difference in disinfection efficacy between the 11 solutions for *A. hatchetti*. Overall, differences in solution efficacy between species at 4–6 hours incubation were not significant with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* showing 87.9% (29/33), 84.9% (28/33), and 90.9% (30/33) positive plates respectively.
At 24 hours incubation, there were statistically-significant differences in disinfection efficacy between the 11 solutions for *A. castellanii* \((p=0.0081)\) and *A. hatchetti* \((p=0.0264)\), but not against *A. polyphaga*. In addition to Ciba Vision Clear Care® and AMO Ultrace, several non-hydrogen peroxide solutions also showed some disinfection ability at 24 hours incubation (Tables 2 and 3). Overall, differences in solution efficacy between species at 24 hours incubation were not significant with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* showing 81.8% (27/33), 69.7% (23/33), and 78.8% (26/33) positive plates respectively.

**DISCUSSION**

Contact lens wear is the most common risk factor for the development of *Acanthamoeba* keratitis in the United States; 85% of cases occur in contact lens wearers (30). Studies demonstrate that nearly all United States rigid and soft contact lens solutions have inadequate *Acanthamoeba* disinfection efficacy (1, 3, 4, 5, 12, 13, 14, 16, 19, 20, 25, 26, 37, 38).

The two most common types of solution used for contact lens disinfection are (1) the multipurpose solution, in which a single solution is used for cleaning, disinfecting, and storing the lenses, and (2) the hydrogen peroxide-based system, in which either a single solution or multiple products are used for disinfecting and storing the lenses (13, 38). Hydrogen peroxide is known to be very effective at contact lens disinfection due to its broad antimicrobial activity against bacteria, fungi, and *Acanthamoeba* and its ability to destroy these pathogens by oxidation (13). It is active against *Acanthamoeba* cysts when a concentration of 3% and an exposure time of at least 6 hours are used (13).
Currently, there are only 2 hydrogen peroxide-based contact lens disinfection systems available in the United States. Only one of these is based on a single-step hydrogen peroxide solution, Ciba Vision Clear Care®, which does not require a separate neutralization step. This solution disinfects and cleans the lenses if soaked for 6 hours or overnight. AMO UltraCare® is also a hydrogen peroxide-based contact lens system that is available in the U.S. but it includes a neutralization tablet that is added to the solution while lenses are being disinfected. Other two-step hydrogen peroxide solutions employing a separate neutralization step are no longer available in the United States. (38).

The results of this study indicated that Ciba Vision Clear Care® containing 3% hydrogen peroxide was 100% effective at killing cysts of *A. castellanii* and *A. polyphaga* at both 6 and 24 hours. For *A. hatchetti*, it was 66% effective at killing cysts at 6 hours but 100% effective at 24 hours, although this difference was not statistically significant. Surprisingly, AMO UltraCare®, which also contains 3% hydrogen peroxide, did not show the same disinfection efficacy. Of the 9 non-hydrogen peroxide solutions tested in the current study, only 4 solutions, Bausch & Lomb Boston Simplus® (used for gas permeable contact lenses and not soft lenses), Bausch and Lomb ReNu with MoistureLoc®, Ciba Vision AQuify®, and Kirkland Signature Multi-Purpose Solution had any effect on *Acanthamoeba* cysts (Table 2). We tested the efficacy of Bausch & Lomb ReNu MoistureLoc®, even though production on this product ceased after the *Fusarium* outbreak of 2006 (9), because this contact lens solution was very popular before it was pulled from the market. These solutions without hydrogen peroxide had varying degrees of activity against *Acanthamoeba*, but none had activity at 4–6 hours.
incubation. Although the above 4 contact lens solutions had some activity with particular species of *Acanthamoeba* after 24 hours incubation, these differences were not statistically-significant and most contact lens wearers do not soak lenses longer than 8–12 hours (overnight).

Current International Organization for Standardization (ISO) and Food and Drug Administration (FDA) regulations do not provide guidelines for testing the efficacy of contact lens solutions against *Acanthamoeba* species (3, 16, 30). Without an accepted standard for testing, the procedures used and reported in contact lens testing studies are highly variable. Strains differ and the methods of cultivation and cyst production vary, thus clouding interpretation of results (1, 3, 5, 11, 12–14, 19, 20, 25, 26, 31, 38, 39).

Shoff et al. (38) used five different *Acanthamoeba* strains, all belonging to genotype T4, but isolated from different sources (including AK patients and tap water), and found a differential response among the various isolates to the different contact lens solutions. They found an overall survival of 54.4% for Ciba Vision Clear Care® and 25.5% survival for AMO UltraCare® (38). One isolate recovered from Chicago tap water was the most resistant strain; it survived in all solutions tested at 24 hours incubation except AMO UltraCare®. The reason for the variance in results between studies is unclear but might be due to inherent differences that exist in strains isolated from different geographic areas, possibly because of development of resistance after exposure to different toxic chemicals in the environment.

In one study by Borazjani, et al., (3) existing ISO/FDA guidelines for efficacy testing against bacteria and fungi were modified to test for *Acanthamoeba* species. A 3-log reduction of *Acanthamoeba* was required to establish efficacy using these guidelines.
Of the 4 no-rub/rinse solutions tested, Bausch & Lomb ReNu with MoistureLoc® achieved a ≥ 3-log reduction in trophozoites and cysts of *Acanthamoeba* species; Alcon Opti-Free® Express® was also highly effective with a ≥ 3-log reduction within 6 hours against trophozoites.

In another study, it was determined that certain commercial products that contain propylene glycol induce *Acanthamoeba* encystment (20). However, reduction or absence of encystment has been observed with other commercial solutions containing propylene glycol, suggesting that additional factors, such as buffering systems, may be involved (20).

Testing standards need to be developed to evaluate the efficacy of contact lens solutions against *Acanthamoeba* cysts. To date different strains and species of *Acanthamoeba* have been used by various investigators and this presents several challenges. First, most investigators have used strains that were isolated many years ago and that are continuously grown axeni
cally for many years. Hence, these strains are highly selected and may not truly represent the isolates that are currently causing AK in patients. In a recent paper Köhsler et al. (21) demonstrated that *Acanthamoeba* strains, especially those that have been in axenic cultivation for a number of years, not only lose their ability to encyst synchronously but also decline in their encystment potential. This is in part because of the down regulation of certain genes that are essential for the survival of strains under inhospitable conditions. Amoebae grown continuously in axenic medium are provided with abundant nutrition and a constant temperature and hence, do not need to develop strategies for survival. In contrast, newly isolated strains from AK patients have been subjected to inhospitable conditions including desiccation and contamination.
with toxic substances in milieu. Further, it has been shown that continuous cultivation in an axenic medium makes the amoebae lose their virulence (24, 37).

A second challenge is in the way the amoebae are processed for testing. Most of the researchers have used axenically grown amoebae that have been induced to produce cysts by nutrient deprivation in the presence of Mg\(^{++}\) (11, 27). Encystment in such media may not always produce 100% mature cysts, which may in turn affect biocide resistance of the cysts. A mature cyst has two layers in the cyst wall: an outer wrinkled ectocyst that is made of protein, and an inner thick, stellate, polygonal, triangular or round endocyst consisting largely of cellulose that is very resistant to physical and chemical agents. Any interference in the maturation process will unduly affect the resistance of the endocyst because resistance to biocides develops during the cellulose-synthesis phase of encystment. Previous studies have shown that inadequate aeration and improper control of pH may also hamper encystment (e.g. 8% encystment vs. >80% encystment [6, 27]) leading to imperfect cyst wall synthesis. Variation in buffers and the inclusion of a chelating agent (EDTA) or the use of dimethyl sulfoxide (DMSO) in the test solutions may also adversely affect the efficacy of the biocides (18, 42).

Hughes et al. (14) showed that strain age, number of passages in axenic culture, and method of encystment has great influence on the efficacy of therapeutic agents used to kill cysts. Kilvington and Anger (19) also suggested these differences may be due to the different methods of cyst production, which may explain the discrepancies in the cysticidal efficacies of disinfectants reported by many investigators. Another important factor to consider is the time the cysts were stored prior to their use in testing.
Because of all these challenges, we elected to use amoebae that were directly isolated from patient specimens and then grown with *E. coli*. Since encystation in starvation medium does not always produce synchronized cyst formation, we used cysts that were generated by growing the amoebae on agar plates coated with bacteria, a process that occurs in nature (19, 21, 26).

Prevention of future cases of AK will require contact lens solutions that are effective against *Acanthamoeba* and continued emphasis on proper lens care hygiene. Educating contact lens wearers about the risk factors for AK, including improper use of contact lens solutions is important, but a systematic method for evaluating contact lens solutions will reduce the chance that inefficacious solutions are available. We strongly urge adoption of standardized procedures for determining disinfection efficacy for *Acanthamoeba* in order to reduce the incidence of AK associated with use of inefficacious contact lens solutions. An initial meeting was held by FDA in June 2008 to begin addressing the need for standardizing procedures for disinfection efficacy for *Acanthamoeba* ([www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfadvisory/details.cfm?mtg=699](http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfadvisory/details.cfm?mtg=699)).

Subsequently, FDA held a workshop in Silver Springs, Maryland in January 2009 titled “Microbiological Testing of Contact Lens Care Products” in which it was decided to include cysts and trophozoites of *Acanthamoeba* in manufacturer’s testing against contact lens solutions ([http://www.jcahpo.org/clmw/pdf/FDA_PostMeeting2.pdf](http://www.jcahpo.org/clmw/pdf/FDA_PostMeeting2.pdf)). These meetings are the first steps toward improving testing of contact lens solutions against *Acanthamoeba* and AK disease in an area that has not been well standardized.
ACKNOWLEDGMENTS

The findings and conclusions in this journal article are those of the author(s) and do not necessarily represent the official position of [the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry]. The use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the U.S. Department of Health and Human Services.

REFERENCES


429 43. Visvesvara, G. S., H. Moura, and F. L. Schuster. 2007. Pathogenic and
430 opportunistic free-living amoebae: Acanthamoeba spp., Balamuthia mandrillaris,
### Table 1: Contact lens solutions tested and their ingredients.

<table>
<thead>
<tr>
<th>Contact Lens Solution</th>
<th>Active Ingredients</th>
<th>Other Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcon OPTI-CLEAN® II</td>
<td>PolyQuad® 0.001%</td>
<td>Tween® 21, MicroClens™, edetate disodium 0.1%</td>
</tr>
<tr>
<td>Alcon OPTI-FREE® Express®</td>
<td>PolyQuad® (0.001%), ALDOX® (0.0005%)</td>
<td>Sodium citrate, sodium chloride, boric acid, sorbitol, AMP-95, Tetronic® 1304, edetate disodium 0.05%</td>
</tr>
<tr>
<td>Alcon OPTI-FREE® Replens®</td>
<td>Propylene glycol, PolyQuad® 0.001%, ALDOX® 0.0005%</td>
<td>Sodium citrate, sodium chloride, sodium borate, TearGlyde™, Tetronic® 1304, nonamoyl ethylenediaminetriacetic acid</td>
</tr>
<tr>
<td>AMO Complete® Moisture Plus</td>
<td>Polyhexamethylene biguanide 0.0001%, Poloxamer 237</td>
<td>Hydroxypropyl methylcellulose, Propylene Glycol, phosphate, taurine, edetate disodium, sodium chloride, potassium chloride, water</td>
</tr>
<tr>
<td>AMO UltraCare®*</td>
<td>3 % Hydrogen peroxide</td>
<td>Sodium stannate, sodium nitrate, buffered with phosphates and water</td>
</tr>
<tr>
<td>Bausch &amp; Lomb Boston Simplus®</td>
<td>Chlorhexidine gluconate 0.003%, polyaminopropyl biguanide 0.0005%</td>
<td>Poloxamine, hydroxyalkylphosphonate, boric acid, sodium Borate, sodium chloride, hydroxypropylmethylcellulose, Glucam</td>
</tr>
<tr>
<td>Bausch &amp; Lomb ReNu MoistureLoc®</td>
<td>Alexidine 0.00045%</td>
<td>Boric acid, sodium chloride, sodium phosphate, Hydranate®, poloxamine, MoistureLoc™</td>
</tr>
<tr>
<td>Bausch &amp; Lomb ReNu MultiPlus®</td>
<td>DYMED 0.0001% (polyaminopropyl biguanide)</td>
<td>HYDRANATE, boric acid, edetate disodium, poloxamine, sodium borate, sodium chloride</td>
</tr>
<tr>
<td>Ciba Vision Clear Care®*</td>
<td>3 % Hydrogen peroxide</td>
<td>Sodium chloride 0.79%, phosphonic acid, phosphate buffered system, Pluronic 17R4</td>
</tr>
<tr>
<td>Ciba Vision AQuify®</td>
<td>Polyhexanide 0.0001%</td>
<td>Sorbitol, tromethamine, pluronic F127, sodium phosphate, dihydrogen, dexamethenol, edetate disodium dehydrate</td>
</tr>
<tr>
<td>Kirkland Signature Multi-Purpose Solution</td>
<td>Polyaminopropyl biguanide 0.0001%</td>
<td>Poloxamer 237, edetate disodium, sodium chloride, potassium chloride, water</td>
</tr>
</tbody>
</table>

*Hydrogen peroxide-containing solution
Table 2: Nine non-hydrogen peroxide contact lens solutions tested with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* at 4–6 hours and 24 hours incubation.

<table>
<thead>
<tr>
<th>Contact Lens Solution (manufacturer-recommended contact time)</th>
<th>Plates Positive 4–6 hr</th>
<th>Plates Positive 4–6 hr</th>
<th>Plates Positive 24 hr</th>
<th>Plates Positive 24 hr</th>
<th>A. castellanii</th>
<th>A. polyphaga</th>
<th>A. hatchetti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcon OPTI-CLEAN® II (4 hour)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Alcon OPTI-FREE® Express® (6 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Alcon OPTI-FREE® ReplenSH® (6 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>AMO Complete® Moisture Plus (4 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Bausch &amp; Lomb Boston Simplus® (4 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>1/3 +</td>
<td>33%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Bausch &amp; Lomb ReNu MoistureLoc® (4 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Bausch &amp; Lomb ReNu MultiPlus® (4 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Ciba Vision AQuify® (4 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
<tr>
<td>Kirkland Signature Multi-Purpose Solution (6 hours)</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
<td>100%</td>
<td>3/3 +</td>
</tr>
</tbody>
</table>
Table 3: Two contact lens solutions containing hydrogen peroxide tested with *A. castellanii*, *A. polyphaga*, and *A. hatchetti* at 6 hours and 24 hours contact.

<table>
<thead>
<tr>
<th>Contact Lens Solution (manufacturer-recommended contact time)</th>
<th>6 hr</th>
<th>24 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. castellanii</strong> Plates Positive</td>
<td>% Positive</td>
<td>Plates Positive</td>
</tr>
<tr>
<td>AMO UltraCare® (6 hours)</td>
<td>2/3 +</td>
<td>66%</td>
</tr>
<tr>
<td>Ciba Vision Clear Care® (6 hours)</td>
<td>0/3 +</td>
<td>0%</td>
</tr>
<tr>
<td><strong>A. polyphaga</strong> Plates Positive</td>
<td>% Positive</td>
<td>Plates Positive</td>
</tr>
<tr>
<td>AMO UltraCare® (6 hours)</td>
<td>1/3 +</td>
<td>33%</td>
</tr>
<tr>
<td>Ciba Vision Clear Care® (6 hours)</td>
<td>0/3 +</td>
<td>0%</td>
</tr>
<tr>
<td><strong>A. hatchetti</strong> Plates Positive</td>
<td>% Positive</td>
<td>Plates Positive</td>
</tr>
<tr>
<td>AMO UltraCare® (6 hours)</td>
<td>2/3 +</td>
<td>66%</td>
</tr>
<tr>
<td>Ciba Vision Clear Care® (6 hours)</td>
<td>1/3 +</td>
<td>33%</td>
</tr>
</tbody>
</table>