Efficacy of multipurpose solutions for rigid gas permeable lenses

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Abstract

The use of multipurpose solutions for cleaning and disinfecting rigid gas permeable lenses has replaced single purpose solutions, but there are no reports of the efficacy of these multipurpose solutions, or of the effects of storage conditions on their disinfecting capacities. This study investigated activity against four bacterial and two fungal species, and the effects of storage in a refrigerator, at room temperature, at elevated temperature in both dry and humid conditions and with exposure to sunlight. The disinfecting solutions were challenged with the micro-organisms initially upon opening and then at 2-weekly intervals up to 12 weeks after being stored under the different conditions. Solutions were opened daily to simulate use. One solution failed to meet Food and Drug Administration (FDA) criteria to reduce numbers of bacteria by three log dilutions and of fungi by one log dilution. Storage reduced activity of all solutions over the 12-week period, but not below the requirements of the FDA. Storage in the refrigerator tended to reduce disinfecting capacity more quickly. Multipurpose solutions for rigid gas permeable (RGP) lenses lose activity over the 3 months recommended time of use but remain satisfactory for use over this time in the conditions tested. Practitioners need to remind patients to replace their solutions regularly and should advise against storage in the refrigerator. Multipurpose solutions for RGP lenses have simplified cleaning and disinfecting processes and the current formulations have improved disinfecting capacity compared to former disinfecting solutions, which is particularly important for wearers of orthokeratology lenses.

Keywords: contact lens, disinfection, rigid gas permeable

Introduction

Several studies have examined the solutions originally designed for use with hard and rigid gas permeable (RGP) lenses, but these were conducted several years ago when it was usual for several solutions to be used in the care of such lenses (Keeven et al., 1995; May et al., 1995; Modi et al., 1995; Landa et al., 1998). In recent years, the use of multipurpose solutions for contact lenses has dominated the market. In order to fulfill their intended range of functions, the manufacturers may have to make some compromises in their disinfecting abilities (Greco, 1985; McLaughlin, 2001). It has been suggested that the strength of the disinfecting agents may have been reduced as a trade-off for their ability to be used as wetting solutions, and more toxic ingredients such as mercury compounds are no longer used in disinfecting solutions.

The guidelines for disinfecting performance circulated by the US Food and Drug Administration (FDA) refer to the ability of contact lens disinfecting solutions, when freshly opened, to reduce the levels of typical strains of bacteria by three log dilutions and of fungi by one log dilution (Food and Drug Administration, 1997). The organisms recommended for testing are Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Serratia marcescens ATCC 13880, Candida albicans ATCC 10231 and Fusarium solani ATCC 36031. Several studies have evaluated multipurpose solutions for use with soft contact lenses (Cano-Parra et al., 1999;
Rosenthal et al., 1999; Miller et al., 2001; Leung et al., 2004), but there have been no recent studies performed on multipurpose solutions for RGP lenses.

There are no guidelines concerning the keeping quality of multipurpose solutions, though patients are generally recommended to discard solutions 3 months after breaking the seal of the bottle. In a recent study, it was reported that newly-opened multipurpose solutions for soft contact lenses were able to successfully reduce the numbers of bacterial pathogens, but only one solution met the requirements for fungi (Leung et al., 2004). However, it was found that several of the solutions lost their ability to fulfill the FDA criteria when testing was performed regularly over a 3-month period. The solutions were used in a way which simulated daily use by patients, being opened daily and a portion being poured out for use. In addition to time, disinfection capacities were also shown to be affected by storage conditions including temperature and humidity. As storage conditions of solutions can vary between contact lens users, it is important to be aware of these effects. These effects may be increased in tropical and sub-tropical regions where temperatures and humidity are higher.

In recent years, the problem of viable but non-culturable (VBNC) organisms has been raised with regard to effective disinfection, especially in the food industry (Cho and Kim, 1999; Wong et al., 2004). If organisms are rendered unable to grow but are not killed, they may be able to resume growth when the disinfecting agent is removed (Rompre et al., 2002). In the case of contact lens use, it is possible that such organisms could be transferred to the eye via the lens if a lens solution containing VNBC is used for rinsing of the lens. The presence of VBNC has not been previously investigated in multipurpose solutions.

This study aimed to investigate the effects of storage parameters on disinfecting capacity for six commercially-available RGP solutions, and to determine if VNBC organisms were present in the solutions after they had been in use. Storage conditions previously investigated were extended to include: refrigerator temperature (4°C), room temperature (22°C), room temperature with exposure to sunlight (window ledge of south facing window), warm dry condition (30°C incubator), and warm humid condition (30°C water bath).

### Materials and methods

#### Contact lens solutions

Six brands of solution for RGP lenses available locally were investigated: Boston Simplicity, Boston Advance (Polymer Technology Corporation, Rochester, NY, USA), Menicon O₂ (Menicon Company Ltd, Japan), Menicare Plus (Menicon Pharma, Illkirch-Graffenstaden, France), Unique pH (Alcon Laboratories, Fort Worth, TX, USA) and TotalCare (Advanced Medical Optics, Australia). Their components are shown in Table 1. Three bottles of each solution from the same batch of manufacture were stored at each of five conditions: 4°C in a refrigerator, 22°C out of direct sunlight, 22°C exposed to sunlight, 30°C in a dry oven and 30°C in a covered water bath. Each bottle was opened daily and approximately 2 mL poured out to simulate daily use. At 2-weekly intervals, a sample from each bottle was taken and challenged with six potential contact lens-related ocular pathogens. Their disinfection activities were monitored for a period of 12 weeks.

#### Microorganisms and culture conditions

Four bacterial and two fungal strains were used in this study, namely *P. aeruginosa* ATCC 9027, *S. aureus* ATCC 6538, *S. marcescens* ATCC13880, *Escherichia coli* ATCC 25922, *C. albicans* ATCC 10231, and *F. solani* ATCC 36031. Sabouraud’s dextrose and Luria-Bertani (LB) agar plates were used for cultivation of the fungal and bacterial strains, respectively. The plates were incubated at 37°C for 16–18 h for bacteria, 48 h for *C. albicans*, and 72 h for *F. solani*.

#### Preparation of bacterial and fungal inocula

A single bacterial or fungal colony from the agar plate was inoculated into 10 mL LB broth or yeast peptone

| Table 1. Active agents of rigid gas permeable contact lens solutions |
|----------------------|----------------------|
| **Solution**          | **Ingredients**       |
| Boston Advance        | Polyaminopropyl biguanide (0.0005% w/v) |
|                      | Chlorhexidine gluconate (0.003% w/v) |
|                      | Disodium edetate (0.05% w/v) |
| Boston Simplicity     | Polyaminopropyl biguanide (0.0005% w/v) |
|                      | Chlorhexidine gluconate (0.003% w/v) |
|                      | Disodium edetate (0.05% w/v) |
|                      | PEO sorbitan, monolaurate and betaine surfactant |
| Unique pH             | Antimicrobial (0.001%) |
|                      | Disodium edetate (0.01%) |
|                      | AL 12355 wetting/conditioning polymer system |
|                      | Polyethylene glycol |
|                      | Polquad preservative |
| AMO TotalCare         | Polixetonium chloride (0.006% w/v) |
|                      | Lauryl quaternised protein (0.085% w/v) |
|                      | Disodium edetate (0.127% w/v) |
| Menicon Plus          | Polyhexamethylene biguanide (0.005% w/v) |
|                      | Poloxamer (0.5% w/v) |
|                      | Hypromellose (0.275%) |
| Menicon O₂ Care       | Sodium olefin sulphonate (0.75%) |
|                      | PEG octylphenyl ether (0.45%) |
|                      | EDTA (0.02%) |
|                      | DMDM hydantoin (0.03%) |
extract broth (2% peptone, 1% yeast extract and 2% dextrose), which was incubated at 37°C for 16–18 h. The concentration of each inoculum was adjusted to 10^6 CFU mL^{-1} by dilution with physiological saline.

**Time kill curves**

To determine the effectiveness of killing of the organisms studied by the solutions chosen, time kill curves were constructed to determine the density of an initial inoculum which could be reduced by three log dilutions for bacteria or one log dilution for fungi. It was found that an initial cell density of 10^6 CFU mL^{-1} was suitable for all but one of the solutions for bacterial cultures, and for all solutions for fungal cultures.

**Determination of antimicrobial activities of the lens solutions**

An aliquot of 1.8 mL of each brand of lens solutions stored under different ambient conditions was placed into a polystyrene test tube. To each tube, 0.2 mL diluted culture was added, and the solutions mixed, giving a final concentration of 10^5 CFU mL^{-1}. The resulting mixtures were incubated at 25°C for 4 h, with the disinfection time chosen according to the manufacturers’ recommendations. Two samples were withdrawn from the mixture by means of an autopipette immediately after mixing, and after four or 6 h depending on the manufacturer’s recommended soaking time, for viable microbial counts (an additional sample was withdrawn from the Menicare Plus bottle after 5-min incubation, as the manufacturer suggested that rapid disinfection could be achieved with this product). For the first sample, 0.1 mL of the mixture was removed immediately and added to 10 mL saline solution (1/100-dilution), 0.1 mL of the 1/100-diluted mixture was then spread over the appropriate agar plate using a glass spreader. For the second sample, 0.1 mL of the mixture was removed after four (or six) hours’ incubation, mixed with an equal volume of Dey-Engley neutralizing broth (Difco, Detroit, MI, USA), and spread over a second agar plate. The additional Menicare Plus sample was treated as for the second sample. Each culture was performed in duplicate and repeated for each bottle of RGP solution stored at each condition. Agar plates were incubated at 37°C as described above, and colonies were counted after incubation using an automated counter (Acolyte Supercount, Synbiosis, Frederick, MD, USA). The average number of CFU from the duplicate plates was recorded. The procedures were repeated at fortnightly intervals for 12 weeks, with 1.8 mL aliquots of lens solutions being withdrawn from the bottles for the monitoring. Log reduction was calculated according to the following formula:

$$\log(\text{viable count at 0 hour}) - \log(\text{viable count at 4th hour})$$

**Viable but non-culturable organisms**

At the end of the 12 week assessment period, during which time the bottles of multipurpose solutions had been opened daily, allowing for contamination to have occurred, a 1 mL aliquot of each solution was transferred to a bottle of Robertson’s cooked meat (RCM) broth. The broth was incubated at 30°C for 14 days, with subcultures on to blood agar and chocolate agar being performed after 7 and 14 days. The agar plates were incubated in CO2 for 7 days, being examined for growth daily.

**Statistical analysis**

Trend analysis of the means of the log reductions for each month was performed and differences in the monthly mean log reduction between solutions were determined by means of post-hoc tests (Tukey). In addition, the post-hoc test was used to compare the effects of storage conditions over the entire 12-week period for each of the solutions.

**Results**

Initial kill curves revealed that all solutions except Menicon O2 were able to achieve reductions meeting the FDA standards of three log dilutions for bacteria and one log dilution for fungi after incubation for 4 h. Log reductions achieved after 5 min for Menicare Plus also met FDA Guidelines for all organisms except S. aureus. These results are shown in Table 2. As Menicon O2, which is not designated as a disinfecting solution, did not meet FDA guidelines, further testing was not carried out on this product.

Storage under any of the conditions used did not lead to reduction in activity below FDA Guidelines for stand alone procedures against Pseudomonas for any of the solutions tested (Figure 1). The disinfectant activity remained unchanged for this organism throughout the period of the testing under all conditions for three of the solutions (Boston Advanced, Unique pH and Menicare Plus) (trend analysis p > 0.05). Storage at room temperature had no effect on the remaining solutions, but refrigerator temperature and 30°C with increased humidity reduced the activity of TotalCare (p < 0.001), and 30°C with increased humidity reduced the activity of Boston Simplicity (p = 0.009) against...
P. aeruginosa, though in neither case to below FDA guidelines.

With the other organisms tested however, storage conditions and time since opening of the RGP solution did cause reductions in activity, but for all solutions this loss in activity was not sufficient for the solution’s disinfecting capacity to drop below the FDA’s guidelines.

Prolonged storage time had an effect on the disinfecting capacity against Serratia and the fungi (Figure 2), but there was little difference in disinfecting capacity against these organisms between the bottles of RGP solutions stored under different conditions (post-hoc test, \( p > 0.05 \) for all). All solutions displayed a significant reduction in activity under all conditions (trend analysis \( p \leq 0.005 \)). A similar but less pronounced effect of reduction in activity over time was observed for E. coli, and this loss of activity was greatest at elevated temperature and humidity for Boston Advanced (\( p < 0.001 \)). Once again, the activity did not fall below a three-log reduction for any condition although the reduction in activity was significant (trend analysis \( p \leq 0.003 \)) for all solutions and conditions tested.

For S. aureus, the storage conditions appeared to have a more important effect with reduced activity observed both at refrigerator and elevated storage temperatures at 2 months for both Unique pH (post-hoc test \( p = 0.047 \)) and Menicare Plus (post-hoc test \( p = 0.03 \)). Only refrigerator temperature affected Boston Simplicity (Figure 3), in which a faster fall-off in activity at 2 months was noted (post-hoc test, \( p = 0.009 \)). However, the loss of disinfection capacity did not reduce activity below the FDA requirements for any of the solutions.

<table>
<thead>
<tr>
<th>Brand</th>
<th>Pseudomonas</th>
<th>Serratia</th>
<th>S. aureus</th>
<th>E.coli</th>
<th>Candida</th>
<th>Fusarium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplicity</td>
<td>5.4 (0.07)</td>
<td>5.3 (0.02)</td>
<td>4.6 (0.10)</td>
<td>5.5 (0.05)</td>
<td>5.2 (0.05)</td>
<td>4.8 (0.32)</td>
</tr>
<tr>
<td>Advance</td>
<td>5.4 (0.06)</td>
<td>5.5 (0.03)</td>
<td>4.3 (0.10)</td>
<td>5.1 (0.09)</td>
<td>5.2 (0.05)</td>
<td>4.9 (0.45)</td>
</tr>
<tr>
<td>TotalCare</td>
<td>5.5 (0.04)</td>
<td>5.6 (0.02)</td>
<td>4.1 (0.09)</td>
<td>4.6 (0.18)</td>
<td>2 (0.09)</td>
<td>2.1 (0.54)</td>
</tr>
<tr>
<td>Unique pH</td>
<td>5.4 (0.06)</td>
<td>5.4 (0.07)</td>
<td>5.4 (0.02)</td>
<td>5.4 (0.41)</td>
<td>5.2 (0.05)</td>
<td>4.9 (0.56)</td>
</tr>
<tr>
<td>Menicare Plus</td>
<td>5.4 (0.08)</td>
<td>5.4 (0.04)</td>
<td>5.4 (0.02)</td>
<td>5.2 (0.39)</td>
<td>5.1 (0.17)</td>
<td>4.8 (0.57)</td>
</tr>
<tr>
<td>Menicare Plus (5 min)</td>
<td>5.3 (0.08)</td>
<td>3.6 (0.05)</td>
<td>2.5 (0.03)</td>
<td>4.4 (0.39)</td>
<td>3.3 (0.32)</td>
<td>3 (0.72)</td>
</tr>
<tr>
<td>Menicon O₂</td>
<td>2.8 (0.07)</td>
<td>2.2 (0.07)</td>
<td>1.9 (0.08)</td>
<td>3.8 (0.20)</td>
<td>1 (0.21)</td>
<td>1.1 (0.45)</td>
</tr>
</tbody>
</table>

The table shows the mean value of the log reduction obtained with the three bottles of solution tested and the (standard deviation).
Prolonged incubation of resuscitation cultures in RCM and subsequent sub-culture failed to reveal the presence of any VBNC organisms.

Discussion

This study aimed to determine the effects of storage time and conditions on solutions recommended for use with RGP lenses. There have been no previous reports on the keeping quality of solutions for rigid lenses. The method used was a modification of the FDA recommended method to allow testing to be performed over a period of 12 weeks and included daily opening of the bottles to simulate patient use.

Although the use of RGP lenses has declined, they remain the best contact lens option for patients with irregular astigmatism, such as keratoconus, or for those with high visual demands (Griffins et al., 1998; Leung, 1999; Looi et al., 2002). An additional reason for the evaluation of solutions designed for rigid lenses is that the same solutions are recommended for disinfection of orthokeratology (ortho-k) lenses. In Hong Kong and some other countries such as Singapore, ortho-k is primarily used for myopic reduction and control, and children are involved (Cho et al., 2002, 2005). The current wearing modality for ortho-k lenses is overnight wear, where oxygen access and tear flow are reduced (Nichols et al., 2000; Tahhan et al., 2003). Adequate daily disinfection is therefore even more important than for conventional RGP lens wearers (Boost and Cho, 2005). The risk of keratitis is increased by the use of contact lenses (Brennan and Coles, 1997; Cheng et al., 1999; Dart, 1999) and there have been several case reports of infections associated with ortho-k use (Chen et al., 2001; Hutchinson and Apel, 2002; Lau et al., 2003; Young et al., 2003; Sun et al., 2006). However, levels of infection can be minimized by good compliance with contact lens care routines (Rah et al., 2002; Boost and Cho, 2005; Cho et al., 2005). It is important that solutions for these patients have good disinfection capacities and stability to help minimize development of eye infections.

This study has shown that most RGP solutions were able to meet FDA guidelines for disinfection capacity both initially and after a 12-week storage period for all organisms tested. This is important because of the need for efficient disinfection not only for patients using RGP lenses, but especially for patients undergoing ortho-k treatment. Although *E. coli* is not included in the Stand Alone Guidelines (FDA), disinfecting capacity against this organism was also measured in the current study as it commonly contaminates contact lens accessories stored in the bathroom (Boost and Cho, 2005). It was particularly reassuring to note that neither storage nor ambient conditions affected the activity against *Pseudomonas*, which is generally considered the most important...
contact lens pathogen. Although activity against Serratia, C. albicans and F. solani was affected by prolonged storage, the resulting drop in activity was not enough to render the solutions unsuitable with reference to the FDA guidelines. There were more noticeable effects of temperature on activity against S. aureus, with activity falling off significantly more rapidly during the first 2 months, but once again, the loss of activity was not severe enough for killing capacity to fall below the recommended three log reductions in numbers of viable cells. It was interesting to note that sunlight exposure did not adversely affect activity in any of the solutions tested.

The failure of one of the solutions, Menicon O₂, to meet FDA guidelines is of concern. When initial tests showed that the required reduction in viable cells was not achieved (Table 1), the tests were repeated with two further batches of the product with similar outcome. Although this solution is not labelled to be used as a ‘disinfecting’ solution, nor is it claimed to have disinfecting ability, it is labelled as a cleaning and ‘soaking’ solution. A solution for soaking RGP lenses is generally assumed to have disinfecting capability especially as this product labelling does not state that disinfection should be done separately.

It was observed that Menicare Plus was able to meet the FDA Guidelines within 5 min for five of the tested organisms, with a three-log reduction not being achieved for S. aureus. Although this performance is impressive, its failure to effectively reduce numbers of S. aureus within the suggested time does not allow a recommendation to reduce disinfection time to 5 min for this product. In addition, this short disinfection period may be further shortened by patients who underestimate the 5-minute period, possibly leading to survival of other pathogens.

The ability of the RGP multipurpose solutions to achieve and maintain activity over a 12-week period in various storage conditions is in contrast to the results obtained with soft lens multipurpose solutions (Leung et al., 2004), most of which displayed reduced activity falling below the FDA guidelines over the storage period. However, the adverse effects of refrigerator storage seen in that study was once again observed with two RGP solutions, which lost activity more rapidly when stored at 4°C.

The study attempted to determine the presence of VBNC organisms in the lens solutions, which had been in daily use for 12 weeks. However, in spite of prolonged culture in recovery media, no organisms grew on sub-culture suggesting that organisms had been killed rather than just inhibited. It is possible that the recovery methods employed were not sufficient to revive VBNC damaged by disinfectant effects and further work including the use of other disinfectant neutralising agents (Rompre et al., 2002), or investigation of physiological activity of bacterial or fungal cells after exposure to the disinfectant by flow cytometry (Nebe-von-Caron et al., 2000), is needed.

The change from individual solutions (each with its own specific function) to multipurpose solutions does not appear to have compromised the activity of RGP solutions against ocular pathogens. Although more toxic substances, such as phenylmercuric nitrate, which had good disinfecting abilities, are no longer used in disinfecting solutions, currently used antimicrobials (Table 1) appear to have adequate disinfecting power. The effectiveness of polyaminopropyl biguanide has previously been demonstrated (May et al., 1995). None of the currently available formulas uses either chlorhexidine or benzalkonium chloride as the single active agent, which had been shown to be ineffective against some ocular pathogens in former RGP solutions (Keven et al., 1995; May et al., 1995; Modi et al., 1995).

In general, the agents used in multipurpose solutions for RGP lenses are largely similar to those in multipurpose solutions for soft lenses. However, the concentrations used tend to be higher than in soft lens solutions, and so, the change to less toxic antimicrobials in RGP solutions does not appear to have affected their efficacy. In contrast, the level of antimicrobials in soft lens solutions is not always adequate to maintain FDA standard activity for a 3-month period (Leung et al., 2004). Higher concentrations are appropriate in RGP solutions as these lenses do not absorb or accumulate the disinfectants, which could have adverse effects on the eye. It should be noted that rinsing of the lenses with saline before use is no longer recommended by the manufacturers, and it is possible that the disinfecting agents may have toxic effects on the eye or affect patient comfort. This is however, beyond the scope of this study, and should be further investigated.

It must be noted that the effectiveness demonstrated in this study related to organisms in suspension, and organisms adhering to the surface of the lens case may be more difficult to kill (May et al., 1995), as disinfectants may not penetrate well into biofilms. The importance of the lens case as a source of contaminants has been shown previously, and is well recognised (McLaughlin-Borlace et al., 1998; Boost and Cho, 2005). The inclusion of lauryl sulphate or other detergents into all-in-one formulas, as recommended by an earlier study, does help the ability of the solutions to detach adhering bacteria (Landa et al., 1998).

Although it is important that a lens care solution can achieve sufficient disinfecting capacity which is stable over the recommended period of use, it must also be appreciated that multipurpose solutions are intended to be introduced into the eye, and should not adversely affect the cornea (Begley et al., 1991, 1992; Mowrey-
Mckee et al., 2002). Therefore, solutions should not be rated ‘better’ solely based on the fact that their disinfecting capacity is higher. As long as sufficient ability to kill major pathogens can be achieved and maintained over the relevant time period, a solution with a lower capacity may have less toxic effects on the eye and may thus be preferable. Further studies on the effects of the newer RGP solutions on cell viability are needed.

This study has provided evidence that recent improvements in formulations of RGP multipurpose solutions, such as use of combined disinfecting agents, have improved their efficacy. This is particularly important with the increasing popularity of ortho-k for which these solutions are used for disinfection of lenses.

Nevertheless, it remains important for patients to be reminded to dry their lens cases after use, periodically disinfect their cases, and replace the case at regular intervals. In addition, these solutions should be stored at room temperature rather than in the fridge, and discarded after 3 months.

**Statement**

The authors have no proprietary or commercial interest in any of the solutions used in this study.

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