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Effect of storage temperatures and time on the efficacy of multipurpose solutions for contact lenses

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Abstract

Purpose: To determine the effect of storage time and temperature on the efficacy of four multipurpose solutions for soft contact lenses.

Method: Aliquots of multipurpose solutions (OPTI-FREE Express, ReNu MultiPlus, COMPLETE and SOLO-care) stored at different temperatures over a 3-month period, were challenged with contact lens-related ocular pathogens, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Results: The results showed that OPTI-FREE Express had the best activity against *Ps. aeruginosa* at all temperatures; ReNu MultiPlus performed well at 25°C; COMPLETE barely achieved activity requirements at all temperatures, and lost efficacy after 2 months. SOLO-care maintained its activity best against *Ps. aeruginosa* at 30°C. Storage at fridge temperature reduced activity of all solutions. Regardless of storage temperature, activities of all solutions against *S. aureus* markedly decreased by 2 months. Only OPTI-FREE Express met FDA requirements against *C. albicans*.

Conclusion: Performance of multipurpose solutions is affected by time and temperature of storage. Contact lens users should be aware that the efficacy of opened solutions may not be sustained for as long as 3 months. Manufacturers should reconsider their recommendations to further safeguard the ocular health of contact lens wearers.

Keywords: *Candida albicans*, multipurpose contact lens solution, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, storage temperature, storage time

Introduction

Of all contact lens complications, microbial keratitis has received the most attention (Dart, 1988; Dart *et al*., 1991; Cheng *et al*., 1999; Seal *et al*., 2000), as it is associated with serious consequences including potential loss of vision or blindness. Contact lens wear and contaminated contact lens solutions and accessories are recognized as the main causes of microbial keratitis (Dart, 1999). Contact lens use increases the risk of corneal infection to about 80 times that of healthy non-contact lens wearers (Dart *et al*., 1991; Brennan and Coles, 1997). Although the risk of corneal infection with contact lenses may have been overdramatised (Brennan and Coles, 1997), when considering the large number of lens users worldwide, a change to a higher-risk pattern of lens wear can have significant impact.

Over the last decade contact lens users have increasingly changed to using disposable and frequent replacement soft lenses with multipurpose solutions. The preservatives in these solutions are high molecular weight materials such as polyhexamethylene biguanide and polyquaternium 1, which seem unable to penetrate into the lens matrix, preventing build up of toxic levels (Lowe *et al*., 1992; Christie and Meyler, 1997). Recently, a number of no-rub multipurpose solutions (no rubbing

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necessary during cleaning) have also been introduced (McLaughlin, 2001). Multipurpose solutions are convenient and simple to use, but unavoidably, represent a compromise of the cleaning and disinfecting functions (Greco, 1985). Non-compliance among contact lens wearers is well documented (Chun and Weissman, 1986; Collins and Carney, 1986; Donzis *et al*., 1987; Sokol *et al*., 1990) and is a major source of contact lens complications. Simplified care systems may lead practitioners and patients into a false sense of security, and the use of disposable or frequent replacement lenses may cause them to place less emphasis on the cleaning and disinfection of these lenses.

The incidence and morbidity of contact lens-associated microbial keratitis have shown little change compared with reports in the late 1980s (Cheng *et al*., 1999). Therefore, despite changes or claimed improvements to contact lenses and care products, and increased public awareness, microbial keratitis is still very much a threat in contact lens wear, particularly in extended wear. In Hong Kong, the extended wear modality of contact lens wear is not common, and incidence of microbial keratitis was reported as 3.4 per 10 000 contact lens wearers (Seal *et al*., 2000).

Proper lens care involves regular replacement of the solutions as the antimicrobial activity of the solutions deteriorates with time. Contact lens patients are advised to discard their solutions 1–3 months after breaking the seal of the solution bottle, 3 months being the standard manufacturers' recommendation. Home conditions and choice of storage place for contact lenses and accessories vary widely. Patients should be advised on how and where to store opened solutions, although this may not seem important and may be easily forgotten and/or ignored by both practitioners and patients. This may be especially relevant in areas which experience high temperatures. We identified three common storage conditions – at room temperature in a cool, dry room, in the bathroom, where the humidity and room temperature may fluctuate, and in the refrigerator.

There are numerous reports on the antimicrobial activities of contact lens solutions. The FDA Guidance Document for disinfection of contact lenses requires a solution to produce a 3-log reduction of viable bacterial cells and a 1-log reduction of viable fungal cells under the recommended disinfection conditions (Food and Drug Administration, 1997). Although newly opened bottles may meet these criteria, it is not clear if solutions maintain their antimicrobial activities after 1–3 months if the bottles are opened daily and if their prolonged activity is affected by storage temperature. We are unaware of published studies monitoring the efficacy of contact lens solutions over a period of time and stored under different conditions. This study aimed, therefore, to determine the effect of storage temperature and time on the efficacy of four multipurpose solutions for soft contact lenses.

Materials and methods

Contact lens solutions

Four commonly used multipurpose solutions for soft lenses, ReNu MultiPlus (Bausch & Lomb, Rochester, NY, USA), OPTI-FREE Express with ALDOX^{TM} (Alcon Laboratories, Fort Worth, TX, USA), SOLO-care (CIBA Vision Corporation, Duluth, GA, USA) and COMPLETE Multi-Purpose (Allergan, Irvine, CA, USA) were used in this study (*Table 1*). Three bottles of each solution from the same batch of manufacture were stored at each of three temperatures, 4, 25 and 30°C. Each bottle was opened daily and approximately 2 mL poured out to simulate daily use. At weekly intervals, a sample from each bottle was taken and challenged with three potential contact lens-related ocular pathogens: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Their disinfection activities were monitored for a period of 3 months.

Micro-organisms and culture conditions

One fungal and two bacterial strains were used in this study, namely, *Candida albicans* ATCC10231, *Pseudomonas aeruginosa* ATCC27853, and *Staphylococcus aureus* ATCC25923. 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.

Preparation of bacterial and fungal inocula

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

Table 1. Information concerning contact lens solutions used in this study

Contact lens solution	Antimicrobial agents	Manufacturer
ReNu MultiPlus	0.0001% polyaminopropyl biguanide	Bausch & Lomb, Rochester, NY, USA
COMPLETE	0.0001% polyhexamethylene biguanide	Allergan, Irvine, CA, USA
SOLO-care	0.0001% polyhexanide	CIBA Vision Corporation, Duluth, GA, USA
OPTI-FREE Express	0.001% polidronium chloride, 0.0005% myristamidopropyl dimethylamine	Alcon Laboratories, Fort Worth, TX, USA

peptone extract broth (2% peptone, 1% yeast extract, 2% dextrose), which was incubated at 37°C for 16–18 h. Each of the micro-organism preparations was diluted to a concentration of 10^6 CFU mL^{-1} in physiological saline.

Pilot study

To determine the effectiveness of killing of the organisms studied by the solutions chosen, a pilot study of time kill curves was conducted to determine the density of an initial inoculum which could be reduced by 3-log dilutions for bacteria or 1-log dilution for fungi. It was found that an initial cell density of 10^6 CFU mL^{-1} was suitable for all solutions for bacterial cultures. A suitable reduction in CFU could only be achieved for one solution for *Candida*.

Determination of antimicrobial activities of the lens solutions

This was carried out by mixing 0.2 mL diluted culture with 1.8 mL of each brand of lens solutions stored at different temperatures in a borosilicate glass bottle. The resulting mixtures were incubated at 25°C for 4–6 h (4 h for ReNu, SOLO-care and COMPLETE and 6 h for OPTI-FREE Express) according to the manufacturers' recommendations. Two samples were withdrawn from the mixture by means of an autopipette immediately after mixing and after 4 h (or 6 h) for viable microbial counts. For the first sample, 0.1 mL of the mixture was removed immediately and added to 10 mL Dey-Engley neutralizing broth (Difco, Detroit, MI, USA), 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 4-h (or 6-h) incubation, mixed with an equal volume of Dey–Engley neutralizing broth and spread over a second agar plate. Each culture was performed in duplicate and repeated for each bottle of solution at each temperature. All agar plates were incubated at 37°C for 16–18 h, and colonies were counted after incubation. If no growth of *Candida* was observed at 18 h, plates were re-incubated for a further 24 h. The average number of CFU from the duplicate plates was recorded. The procedures were repeated for 3 months, with 1.8 mL aliquots of lens solutions being withdrawn from the bottles weekly for the monitoring. Log reduction was calculated according to the following formula:

$$\log(\text{viable count at 0 hour} \times 1000) - \log(\text{viable count at 4th hour} \times 10).$$

The results were analysed using a trend analysis of the mean values of the log reductions for each month and by *post hoc* tests (Tukey) of differences in the monthly mean log reduction between solutions. *Post hoc* tests were also performed to compare the effects in differences in temperature over the entire 13-week period for each of the solutions.

Results

The log reductions for each organism tested with the multipurpose solutions which had been stored at various temperatures are shown in *Figures 1–3*. The results shown are the mean of those for the three bottles of each

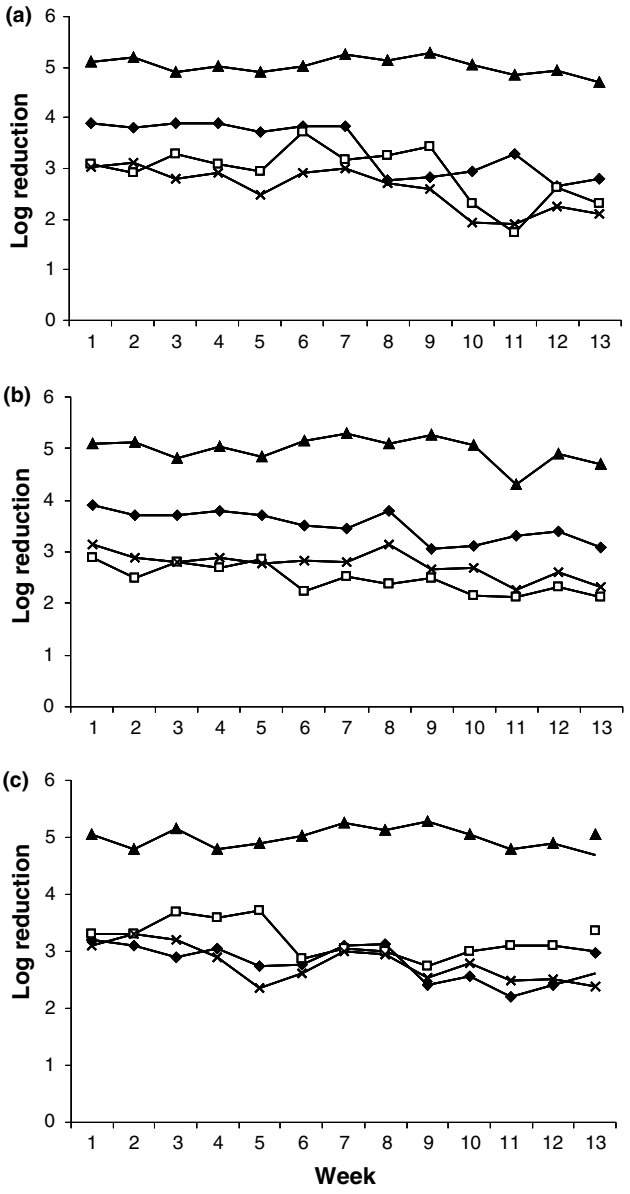

Figure 1. Log reduction of *Pseudomonas aeruginosa* following exposure to ReNu MultiPlus (♦), SOLO-care (□), OPTI-FREE Express (▲) and COMPLETE (×) stored at 4 (a), 25 (b) and 30°C (c).

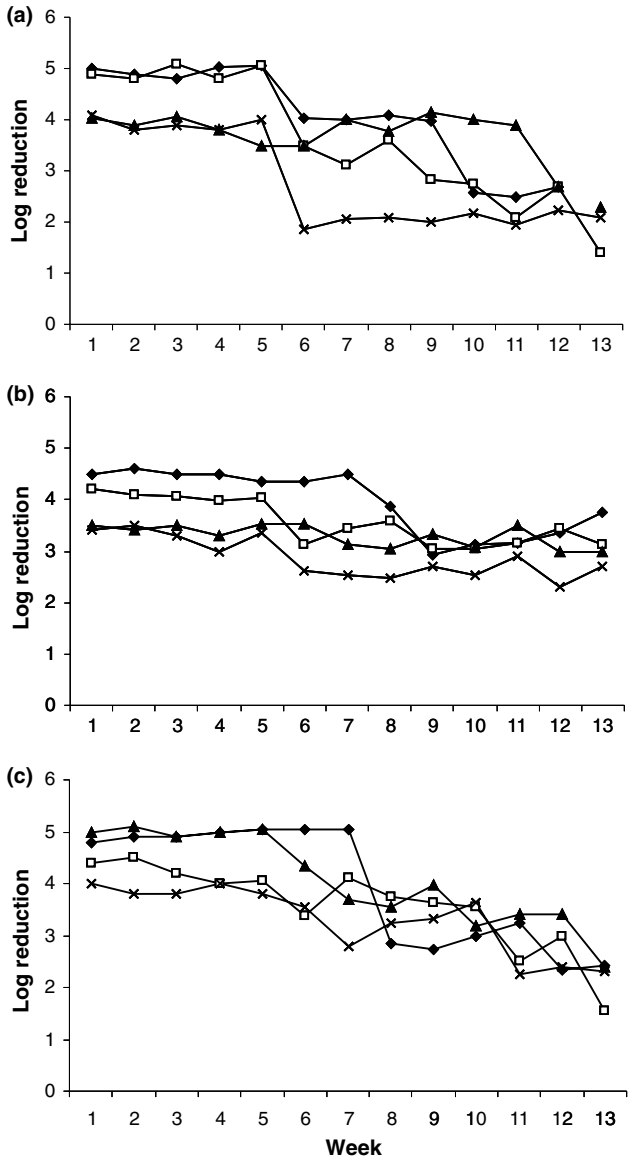

Figure 2. Log reduction of *Staphylococcus aureus* following exposure to ReNu MultiPlus (♦), SOLO-care (□), OPTI-FREE Express (▲) and COMPLETE (×) stored 4 (a), 25 (b) and 30°C (c).

brand of solution stored at each temperature. It can be noted that different effects on activity occurred due to storage temperature and that these differed between the organisms tested. The pilot study had shown that all solutions were able to achieve a ≥3-log reduction on a bacterial suspension of 10^6 CFU of either *Ps. aeruginosa* or *S. aureus*. However, only OPTI-FREE Express was able to achieve a ≥1-log reduction when mixed with a suspension of *C. albicans*. As the study aimed to investigate the effects of storage on maintenance of activity equal to FDA requirements, only OPTI-FREE Express was used against *C. albicans* in the extended trial.

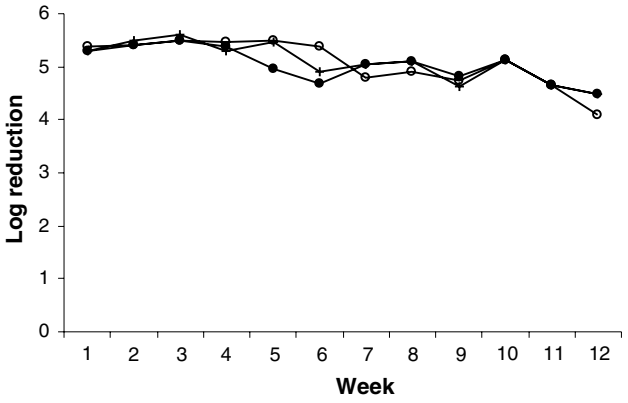

Figure 3. Log reduction of *Candida albicans* following exposure to OPTI-FREE Express stored at 4 (○), 25 (●) and 30°C (+).

Overall, OPTI-FREE Express had the best antibacterial activity against *Ps. aeruginosa* and this was maintained throughout the period of the trial, with log reduction never falling below the FDA requirement for this organism. Activity was well in excess of FDA requirements regardless of the storage conditions. Trend analysis showed no significant decline in activity over the 3-month period against any of the organisms at any temperature ($p > 0.05$) (*Table 2*).

Initial activity against *Ps. aeruginosa* was lower for the other solutions. ReNu performed well at 25°C, retaining activity throughout the trial period (*Figure 1b*), but activity was affected by higher temperature with activity being approximately 1-log reduction lower when stored at 30°C (*Figure 1c*). As a result, bactericidal activity fell below the FDA guideline during the storage period, dropping to below 3-log reduction after 2 months. A similar picture of reduced activity was seen for this solution when a storage temperature of 4°C was used, although FDA minimums were maintained (*Figure 1a*).

COMPLETE initially remained stable at 25°C but lost activity when storage was prolonged beyond 8 weeks. This pattern was also observed for storage at 4 and 30°C but activity loss was more pronounced at fridge temperature (*Figure 1a–c*).

Surprisingly, SOLO-care maintained its activity against *Ps. aeruginosa* best at 30°C, at which temperature FDA guidelines were achieved throughout the 13 weeks. However, by week 6 at room temperature or 9 in the fridge, activity levels had fallen below the requirements (*Table 2*). Trend analysis showed significant decline in activity against *Ps. aeruginosa* at fridge temperature for both ReNu and COMPLETE ($p = 0.023$ and 0.032 respectively) (*Table 2*) and there was a significant difference in activity between storage of SOLO-care at 25 and 30°C ($p = 0.028$). Significant differences in activity against *Ps. aeruginosa* between OPTI-FREE Express and other solutions were observed for all solutions at all temperatures after 2 months (*Table 3*).

Table 2. Trend analysis of effects on organisms by months and solutions

	ReNu						SOLO-care						OPTI-FREE-Express						COMPLETE					
	4°C		25°C		30°C		4°C		25°C		30°C		4°C		25°C		30°C		4°C		25°C		30°C	
Month	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Pseudomonas aeruginosa																								
1	3.88	0.04	3.78	0.39	3.07	0.30	3.10	0.02	2.73	0.23	3.48	0.33	5.06	0.23	5.01	0.51	4.95	0.05	2.96	0.13	2.93	0.26	3.13	0.22
2	3.55	0.32	3.62	0.12	2.94	0.02	3.28	0.18	2.50	0.10	3.16	0.25	5.08	0.26	5.10	0.88	5.08	0.63	2.78	0.31	2.89	0.12	2.74	0.19
3	2.90	0.01	3.19	0.38	2.44	0.02	2.48	0.16	2.23	0.20	2.99	0.01	4.96	0.35	4.85	0.01	4.95	0.65	2.16	0.19	2.51	0.10	2.55	0.36
Linear trend (p)	0.023*		0.478		0.197		0.103		0.347		0.294		0.851		0.729		0.994		0.032*		0.169		0.112	
Staphylococcus aureus																								
1	4.95	0.29	4.53	0.10	4.90	0.01	4.90	0.24	4.09	0.04	4.28	0.41	3.95	0.54	3.43	0.39	5.00	0.18	3.90	0.12	3.30	0.64	3.90	0.27
2	4.02	0.24	4.27	0.09	4.50	0.24	3.82	0.25	3.55	0.36	3.82	0.37	3.70	0.02	3.32	0.04	4.17	0.31	2.51	0.02	2.75	0.53	3.34	0.07
3	2.58	0.15	3.26	0.40	2.75	0.04	2.35	0.09	3.17	0.22	2.84	0.08	3.41	0.55	3.18	0.39	3.27	0.03	2.08	0.04	2.63	0.23	2.78	0.11
Linear trend (p)	0.084		0.105		0.010*		0.027*		0.125		0.103		0.613		0.140		0.055		0.038*		0.260		0.064	
Candida albicans																								
1	Not applicable						Not applicable						5.44	0.36	5.40	0.03	5.43	0.17	Not applicable					
2													5.15	0.07	4.95	0.15	5.12	0.05						
3													4.65	0.12	4.78	0.07	4.72	0.12						
Linear trend (p)													0.256		0.031*		0.182							

*Result significant. Mean = average of triplicate samples performed weekly in duplicate for 4 weeks (24 samples).

Activities of multipurpose solutions against *S. aureus* were somewhat different with a marked decrease in activity occurring in all solutions at approximately week 6 regardless of the storage temperature employed (*Figure 2a–c*). Decrease in activity was greatest if the solutions were stored in the refrigerator which led to all solutions failing to meet FDA requirements by the end of the trial, although this point was reached at week 6 for COMPLETE, weeks 9–10 for SOLO-care and ReNu, and not until the last week of the trial for OPTI-FREE Express. At room temperature, COMPLETE had significantly lost activity by week 6, SOLO-care and ReNu by week 8, and OPTI-FREE Express at week 12. A similar pattern was observed at 30°C. Trend analysis was significant for storage at fridge temperature for both SOLO-care and COMPLETE ($p = 0.027$ and 0.038 respectively), and for storage at 30°C for ReNu ($p = 0.010$) (*Table 2*). There was a significant difference in activity at 30°C between OPTI-FREE Express and all other solutions after 3 months and between OPTI-FREE Express and COMPLETE at both 2 and 3 months at fridge temperature (*Table 3*).

Activity of OPTI-FREE Express against *C. albicans* was well above requirements and remained high regardless of storage conditions used (*Figure 3*). Although trend analysis did show a significant decline in activity 25°C, the log reduction even after the trial period remained far in excess of FDA requirements (*Table 2*).

Discussion

Considerable differences in performance were noted for the multipurpose solutions under trial. Previous investigators had also reported on the differences in efficacy of these solutions, although they did not investigate the effect of storage temperature and time (Cano-Parra *et al*., 1999; Rosenthal *et al*., 1999; Miller *et al*., 2001). Although the activity of OPTI-FREE Express against *Ps. aeruginosa* was not affected by post-opening storage time or temperature, the other solutions were shown to be adversely affected by storage and failed to meet FDA requirements for one or more temperatures by the end of the 3-month trial. This suggests that their stability may be affected by temperature and presence of air in the bottle. These effects may be increased if temperature fluctuates during storage. SOLO-care and COMPLETE just met the FDA requirement at the beginning of the experiment, but the level of efficacy fluctuated and declined with time of storage. It was of particular concern that both solutions did not meet the FDA requirement even at the manufacturer's recommended storage temperature. As higher or lower storage temperature can reduce activity, the prolonged efficacy of these solutions under variable storage conditions may be further compromised and increase infection risks. Our

Table 3. Comparison of effectiveness of solutions against challenge organisms at different temperatures (*p* value using *post hoc* test (Tukey))

Month	Temp. (°C)	ReNu vs SOLO	ReNu vs OPTI-FREE	ReNu vs COMPLETE	SOLO vs OPTI-FREE	SOLO vs COMPLETE	OPTI-FREE vs COMPLETE
Staphylococcus aureus							
1	4	0.999	0.127	0.113	0.146	0.129	0.999
	25	0.678	0.134	0.100	0.406	0.296	0.986
	30	0.224	0.979	0.062	0.156	0.547	0.046*
2	4	0.681	0.372	0.003*	0.895	0.006*	0.008*
	25	0.264	0.133	0.032*	0.884	0.206	0.411
	30	0.201	0.646	0.044*	0.627	0.406	0.123
3	4	0.856	0.146	0.421	0.072	0.793	0.035*
	25	0.992	0.994	0.335	0.999	0.464	0.424
	30	0.603	0.008*	0.967	0.016*	0.823	0.010*
Pseudmonas aeruginosa							
1	4	0.015*	0.003*	0.008*	0.001*	0.744	0.001*
	25	0.137	0.088	0.236	0.011*	0.936	0.016*
	30	0.450	0.006*	0.994	0.014*	0.562	0.006*
2	4	0.759	0.017*	0.146	0.009*	0.378	0.004*
	25	0.203	0.096	0.466	0.015*	0.820	0.027*
	30	0.918	0.013*	0.940	0.019*	0.665	0.009*
3	4	0.342	0.002*	0.085	0.001*	0.516	0.001*
	25	0.040*	0.006*	0.113	0.001*	0.622	0.002*
	30	0.518	0.009*	0.989	0.021*	0.664	0.010*

*Result significantly different.

results with these two solutions were not in agreement with the results reported by previous investigators. Other investigators had reported initial activity for SOLO-care to be a little higher but experimental conditions may account for this (Cano-Parra *et al*., 1999; Miller *et al*., 2001). Similar differences were reported for COMPLETE (Cano-Parra *et al*., 1999; Rosenthal *et al*., 1999). Although initial activity of the solution is not disputed, it is the fall-off of efficacy during the period of storage that is of concern.

The loss of activity against *S. aureus* after 6–7 weeks which was observed for all solutions needs further investigation. This effect may be related to the Gram-positive cell wall structure. *Staphylococcus aureus* is an important causative organism of eye infections and this loss of activity against a common constituent of the normal flora of the hands is a cause for concern. It may be appropriate for practitioners to recommend that lens solutions are discarded after 2 rather than 3 months. This may be especially important in patients with poor technique as they are more likely to contaminate their contact lenses and accessories.

The failure to meet FDA guidelines for ability to kill *C. albicans* of three of the solutions is a worrying result, as fungi can be a cause of serious corneal infection. Rosenthal *et al*. (1999) had shown both OPTI-FREE Express and ReNu to fulfil FDA requirements against *Candida*. Cano-Parra *et al*. (1999) however, reported the failure of ReNu and COMPLETE to meet FDA guidelines, which agrees with our findings. Although fungi are not part of the normal ocular flora, non-compliance in contact lens wear can lead to contaminated lenses. Challenges were performed in borosilicate glass bottles and this may have altered adsorption of active ingredients to the surface compared with plastic lens cases. Most studies do not state whether plastic or glass materials were used in the challenges reported.

Donzis *et al*. (1987) reported microbial contamination in the lens care systems of about 50% of their patients. Contamination of the lens case is also a commonly reported problem in contact lens wear (Devonshire *et al*., 1993; Gray *et al*., 1995; McLaughlin-Borlace *et al*., 1998). Mowrey-McKee *et al*. (1992) also reported that lens handling by patients is a significant source of microbial contamination. Although levels of contamination may not be equal to those of the challenge cultures, it is important that the efficacy to the full range of organisms likely to be introduced into the eye is sustained for the full period of recommended use. The effect of storage temperature was marked for efficacy against the organisms tested in several cases. In general, best activity was maintained at room temperature which is usually recommended for storage by the manufacturer. However, this is not a point usually stressed by practitioners and contact lens users may be inclined to store products in the refrigerator thinking this is safer or that this may prolong the period of effectiveness of the solution.

This study did not include the effect of storage conditions on Acanthamoeba and it is recommended that further study is performed to determine the long-term

effectiveness of multipurpose solutions against this organism. The study can also be extended to investigate the effects of fluctuating temperatures and humidity.

Conclusion

The performance of multipurpose solutions varies against different bacterial and fungal species. Activity is affected by storage temperature and time. Stability in general appeared to be highest if the solutions were stored at room temperature but differences in stability were noted between solutions. Failure to meet FDA guidelines for activity against *C. albicans* was observed for three solutions. The marked fall-off in activity against *S. aureus* at 6–7 weeks coupled with reduced activity of several solutions against *Ps. aeruginosa* after this length of storage suggest that recommendations for earlier replacement of contact lens solutions may be appropriate. Appropriate storage temperature (place) of care systems should also be recommended to patients.

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Conflict of interest statement

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

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