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Microbiological contamination of eyedrops. Part 2: A novel method to evaluate proper and improper administration

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Commercial eyedrops may become contaminated with micro-organisms during patient, practitioner or clinic use. Manufacturers and practitioners routinely warn the user never to touch the tip of the eyedrop dispenser to any surface while administering the eyedrops. This article presents preliminary results from a new test model designed to evaluate the level of contamination that can occur inside eyedrop containers after appropriate and inappropriate use of the dispensers. This method represents a novel approach to the traditional microbial challenge tests that can determine the robustness of preservative systems of multidose eyedrops in the real world. A vulnerable, non-preserved saline solution with no added preservatives was chosen as the test formulation to represent a "worst-case" scenario and help estimate the level of antimicrobial preservative needed effectively to protect these products. This model considers single and multiple administration of a non-preserved test formulation, *ie* non-preserved saline. Two studies using human volunteers showed that touching the eyedrop dispenser to the conjunctiva, cheek or hand, one or more times, increased the contamination rate over proper administration (*ie* without touching the tip to any surface). Not surprisingly, the levels of contamination were the greatest when the facial cheek was touched multiple times over a 5-day period during administration. The studies were quantitative and found these levels ranged from 0 to 10,000 colony-forming-units per eyedrop dispenser. Nevertheless, in general, most of the data indicate that very few organisms contaminated the non-preserved saline used in these studies. The conclusion is that this challenge approach is a reasonable way of assessing microbial contamination of multiple-dose products. Also, the levels of contamination required by the compendial preservative efficacy tests seem to be inordinately high and unrealistic in light of these data from this model.

Key words: Microbiological contamination, eyedrops, eyedrop dispensers, testing, appropriate or inappropriate administration, preservatives

Introduction

Although the eye is open to the environment, it rarely gets infected and can withstand numerous microbial assaults before an infection takes place.[1] One modern assault is the administration of contaminated eyedrops. Commercial eyedrops have been developed to alleviate, cure or prevent many ocular diseases, but they can become contaminated with micro-organisms during patient, practitioner or clinic use.[2] To prevent eyedrops from becoming grossly contaminated, many multiple-dose ophthalmic products contain antimicrobial preservatives (*eg* benzalkonium chloride, benzethonium chloride, chlorobutanol, polyquaternernium-1, thimerosal, cetrimide, phenylmercuric salts, benzyl alcohol, phenylethyl alcohol, chlorhexidine, parabens) to act as a secondary safety barrier to protect these products and reduce or eliminate the risk of microbial contamination during use.[3-7] The current European, Japanese, United States and earlier pharmacopoeias (*eg* British Pharmacopoeia, Deutsches Arznei Buch) require multiple-dose eyedrops to be preserved properly.[8-10]

Antimicrobial preservative efficacy tests are ways to demonstrate the power and robustness of antimicrobial preservative systems within multidose products. Typically, in these tests, formulations are inoculated with high numbers (*ie* millions) of micro-organisms, whose survival, growth, reduction or death are measured at various times throughout specified storage periods.

High concentrations of preservative agents within the product are often required to provide the level of preservative activity necessary to satisfy the official compendia. In contrast to this, there is increasing interest in minimising the exposure of patients to high levels of relatively toxic preservative agents.[11-19] A good preservative system must effectively kill some cells (*ie* microbes) and leave other cells (*ie* host ocular tissues) alone. Single-use, non-preserved products avoid this disadvantage, but are not always practical as a means to avoid multiple-dose preserved formulations, because of price considerations, safety or environmental reasons.[19-22] Great debates rage internationally as to which antimicrobial preservative efficacy standards are reasonable and which are required to assure the public is not placed at undue risk.[4,5,23]

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Manufacturers and practitioners routinely warn the user never to touch the tip of the eyedrop dispenser to any surface while administering the eyedrops. Appropriate use during ophthalmic administration would be to:

1 remove the cap from the eyedrop dispenser,
2 administer one to two drops of fluid into the eye without touching the tip of the dispenser to the skin, eye, hand or face, and
3 replace the cap onto the dispenser without touching the tip. If this process is followed, it is probable that antimicrobial preservatives would never be needed.

Nevertheless, some consumers ignore these precautions and misuse the product by unintentionally touching the tip of the eyedrop dispenser to the eye, face or hand.

The purpose of the studies included in this report was to determine the level of microbiological contamination that can occur after appropriate or inappropriate administration of a vulnerable eyedrop product (*ie* non-preserved saline) from an eyedrop dispenser. Single (Study 1) and multiple (Study 2) abuses were studied. Healthy volunteers contaminated the products by touching the eyedrop dispenser tips to their conjunctiva, cheek or hand. These latter situations, called "inappropriate use", were compared to an "appropriate use" in which the product was administered, according to the manufacturer's instructions, through the air without contacting the eyedrop dispenser tip to anything as a source of possible contamination.

The overall goal was to determine the amount of contamination that occurs when a simple non-preserved formulation is administered appropriately or inappropriately. Coupled with the findings from the extensive literature review,[2] the resulting level of contamination from these studies puts a realistic perspective on the levels of inocula chosen and specified in the standardised preservative efficacy tests in official pharmaceutical compendia. This report designs and provides preliminary results for a new, practical, challenge test for use in developing multidose ophthalmic products to assess the robustness of preservative systems in the real world.

A new method for determining eyedrop contamination

Three studies simulated the actual use and abuse of an ophthalmic product. They evaluated the level of contamination occurring inside eyedrop containers after appropriate and inappropriate use during single and multiple administration of the dispenser. A vulnerable, non-preserved saline solution with no added preservatives was chosen as the test formulation. Knowing a realistic level of microbial contamination that occurs in multiple-dose eyedrops, we can determine the level of antimicrobial preservative needed effectively to protect these products and provide a perspective on the risk-to-benefit relationship of having strong preservatives with antimicrobial power and irritation potential. This investigation supports efforts to minimise the microbiological and toxicological risks associated with unnecessarily strong preservative systems in new drug products. This new approach offers a practical way to assess the robustness of a multidose ophthalmic product and determine its ability to withstand normal contamination during use.

Microbial challenge Study 1 (single-use contamination)

The purpose of this human volunteer study (10 subjects) was to determine the contamination level in eyedrop dispensers containing non-preserved saline after a single appropriate or a single inappropriate administration.

Microbial challenge Study 2 (multiple-use contamination)

The purpose of this human volunteer study (20 subjects) was to determine the contamination level in eyedrop dispensers containing non-preserved saline after repeated appropriate or repeated inappropriate administration.

Microbial challenge Study 3 (gross contamination by Pseudomonas aeruginosa)

The purpose of this *in vitro* study was to determine the extent of contamination of non-preserved saline solution in eyedrop dispensers when the tips of the dispensers were grossly challenged by immersing them into various suspensions of *P. aeruginosa* (10^2, 10^4, 10^6 colony-forming units [CFU]/ml).

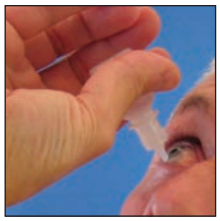
Volunteer enrolment

Studies 1 and 2 were conducted at the Klinikum de Albert-Ludwigs Universität in Freiburg, Germany under the direction of Prof. F. Daschner. For these studies, healthy female and male volunteers were asked to follow specific protocols and administer sterile saline from eyedrop dispensers under appropriate and inappropriate conditions of use. Volunteers were excluded from these studies if:

a they had any irritation or infections of the eyes, skin, hands or cheeks within the past week;
b they had used any antibiotics within the past week;
c they were contact lens wearers; or
d they were participating in any other microbiology or clinical trial.

Patients were informed that the test articles were being used for investigational purposes and that an Institutional Review Board had reviewed and approved the study protocol. They were also informed of their rights and obligations as trial participants, the possible side-effects from the study and the insurance coverage for the study. Immediately before the trial, each volunteer was given the following colour-coded bottles: Test A (blue), Test B (red), Test C (green), Test D (yellow). The samples were handed out to each volunteer individually and in succession so that the technical conductor could supervise the simulated administration of the drops. After each bottle was opened and drops administered, the bottle was closed, shaken and marked with a cross to indicate that it had been "used". The name of the volunteer was also

Table 1. Administration simulations used in Study 1 (single application) and Study 2 (multiple applications).

Simulation		Actions to be taken once (Study 1) or twice per day for 5 consecutive days (Study 2)			
Test A: Appropriate use		Open eyedrop dispenser	Administer dispenser in air (no contact with anything)	Close dispenser	Shake dispenser
Test B: Inappropriate use (conjunctiva)		Open eyedrop dispenser	Touch the tip of dispenser to the conjunctiva and at the same time administer one or two drops from the dispenser	Close dispenser	Shake dispenser
Test C: Inappropriate use (cheek)		Open eyedrop dispenser	Touch the tip of dispenser to the cheek and at the same time administer one or two drops from the dispenser	Close dispenser	Shake dispenser
Test D: Inappropriate use (hand)		Open eyedrop dispenser	Touch the tip of dispenser to the hand and at the same time one or two drops from administer the dispenser	Close dispenser	Shake dispenser

marked on the bottle. Ten volunteers were used for Study 1 and 20 for Study 2. No volunteers were involved in Study 3.

Administration protocols

The test formulations for Study 1 and Study 2 were 5ml multiple-dose containers (eyedrop dispensers) filled with sterile, non-preserved physiological saline solutions provided by Alcon Laboratories (Puurs, Belgium). Each volunteer was given a set of colour-coded eyedrop dispensers, which they used to simulate appropriate (Test A) and inappropriate (Tests B, C, D) usage, as indicated in **Table 1**.

Study 1 determined the contamination level after a single administration of the eyedrop dispenser using the four test systems described in **Table 1**. This simulation was performed once on a single day. Ten volunteers repeated this study six times and provided the 60 evaluable data sets for this study. Therefore 60 occurrences were measured for each test (*ie* 60 As, 60 Bs, 60 Cs and 60 Ds). A total of 240 bacteriological filters (0.2μ) were required for Study 1. Each volunteer administered his or her four eyedrop dispensers once for this study. The entire contents of each dispenser were then filtered through a 0.2μ filter and counted for micro-organisms. Therefore, each dispenser was administered (used or abused) only once in this study.

Study 2 determined the contamination level after multiple administration over the course of 5 days of use. The four test systems described in **Table 1** were used twice on each of the five consecutive days of investigation at the beginning and at the end of the working day. Twenty volunteers provided 20 evaluable data sets for this study. There were 20 data points measured for each test (*ie* 20As, 20 Bs, 20 Cs and 20 Ds). A total of 80 bacteriological filters (0.2μ) were required for the tests and controls for Study 2. Each volunteer administered his or her four eyedrop dispensers twice per day for 5 consecutive days. After 5 days of administration the entire contents of each dispenser were filtered through a 0.2μ filter and counted for micro-organisms. Therefore each dispenser was used or abused ten times over a period of 5 days.

Microbiological evaluation of eyedrop dispenser contents

Each eyedrop dispenser was subjected to qualitative and quantitative determination of microbial contamination. After use, the entire contents of each dispenser were filtered through a 0.2μ filter and counted for micro-organisms at the Hygiene Clinic Laboratory. The number of CFU per dispenser was determined and all aerobic bacteria and fungi were identified. Media growth controls were used to show that low levels (10–100 CFU) of known micro-organisms (*eg Staphylococcus aureus*) could be detected on the bacteriological filter. Also, a

sterility control was used to show that there were no organisms isolated from the initial eyedrop dispensers containing sterile, non-preserved saline. The total CFU for each dispenser for each volunteer were recorded and analysed. Since the counts ranged from 0 to over 10,000 CFU per dispenser, the data were normalised using a log base10 transformation. The actual transformation of the data followed the formula: transformed CFU per dispenser = log (CFU per dispenser + 0.5). The overall contamination levels present in each of the four simulations (A, B, C, D) were determined by averaging the transformed CFU data (Study 1: n=60; Study 2: n=20) and converting to actual counts (*ie* antilogarithm transformed CFU per dispenser). In addition, for each simulation (A, B, C, D) the numbers of CFU were rank ordered, high to low, to determine the 90% frequency distribution of counts.

Study 3: Special Pseudomonas challenge

Study 3 was an *in vitro* simulation of a worst-case or a grossly abused situation for eyedrop administration: inappropriate use of a dispenser on patients with eyes infected with *P. aeruginosa*. Eyedrop dispensers (5ml) containing sterile non-preserved physiological saline were dipped into various suspensions of *P. aeruginosa*. Ten eyedrop dispensers each were dipped into one of three solutions containing 10^2, 10^4 or 10^6 CFU/ml. Sterile non-preserved saline (containing no *Pseudomonas*) was used as a control for this study.

No. 1 = *P. aeruginosa* (10^2 CFU/ml)
No. 2 = *P. aeruginosa* (10^4 CFU/ml)
No. 3 = *P. aeruginosa* (10^6 CFU/ml)
No. 4 = Sterile non-preserved saline (control; no *Pseudomonas*)

This simulated the case of the most massive contamination (ie contact with a conjunctiva or cornea infected with P. aeruginosa). No volunteers were used in Study 3. After dipping the sample eyedrop dispenser tips into the solutions of P. aeruginosa, the entire contents of each dispenser were filtered through a 0.2μ filter and counted for viable pseudomonads. A total of 40 bacteriological filters (0.2μ) were required for this study.

Results

Study 1 (single administration) (Tables 2 and 3)

Test A (appropriate use): After a single administration from an eyedrop dispenser in an appropriate manner, only 4 of the 60 samples were contaminated (6.7%). Most of the dispensers were sterile (56/60 or 93.3%). For Test A, the average count of microbes was 0.1 CFU per dispenser.

Tests B, C, D (inappropriate use): In the cases of incorrect or inappropriate use (Test B, conjunctiva; Test C, cheek; or Test D, hand), the contamination rates were also very low. Contamination after contact with the cheek (Test C) yielded the highest contamination rate (44 out of 60 or 73%; 23.9 microbes per dispenser). The predominant contaminants were normal skin flora organisms, *eg Staphylococcus epidermidis*. The pathogen, ocular *S. aureus*, was isolated in only one case following contact with the conjunctiva. The results indicate that contamination of the eyedrop containers can be virtually discounted as a result of opening and closing the dispenser (appropriate use).

Contamination of the dispensers was minimal even in the case of incorrect or inappropriate use. In these cases the contaminating micro-organisms were predominantly normal skin flora. Contacting the dispenser tip to the patient's cheek provided the most contamination. Nevertheless, very little contamination enters the dispensers after a single administration, even if they are misused by contacting the tip to contaminated surfaces such as the conjunctiva, cheek or hand.

Study 2 (multiple administration) (Tables 2 and 3)

As expected, the contamination rates after multiple administration from the eyedrop dispensers were higher than those in Study 1 (single administration), but they were still quite low overall.

Test A (appropriate use): After multiple administration from the eyedrop dispenser appropriately, the average contamination rate was 5% (1/20). Like the single administration study (Study 1), the average count was only 0.1 micro-organisms per dispenser.

Table 2. Micro-organisms isolated from Study 1 and Study 2

	Study 1 (Single administration)		Study 2 (Multiple administration)	
	Occurred CFUs	Total	Occurred CFUs	Total
Acinetobacter lwoffi	1	5	4	,175
Aerobic sporeformer	4	9	10	18
Candida colliculosa			1	1
Corynebacterium spp.	1	1	1	35
Micrococcus	1	2	6	1,252
Micrococcus varians/roseus			1	1
Moraxella sp.	4	15	1	1
Pasteurella haemolytica	1	1		
Pasteurella multocida			1	7
Pseudomonas maltophilia			3	76
Pseudomonas paucimobilis			1	3
Pseudomonas vesicularis			1	32
Rhodotorula glutinis			1	15
Rhodotorula rubra			1	10,000
Staphylococcus aureus	1	33	4	56
Staphylococcus epidermidis	71	1,687	38	34,420
Staphylococcus saprophyticus	7	64	4	1,235
Streptococcus viridans	1	2	1	30

Table 3. Administration studies

	Study 1 (Single administration) Contamination					Study 2 (Multiple administration) Contamination				
	Pos/ total	%	Avg CFUs Arith	Log	90% Freq count	Pos/ total	%	Avg CFUs Arith	Log	90% Freq count
Appropriate use	4/60	7%	1	1	0*	1/20	5%	0	1	0*
Inappropriate use (conjunctiva)	11/60	18%	5	1	1	17/20	85%	92	19	90
Inappropriate use (cheek)	44/60	73%	33	6	82	16/20	80%	1,595	54	10,000
Inappropriate use(hand)	18/60	30%	18	2	13	17/20	85%	61	7	72

* Sterile

Tests B, C, D (inappropriate use): In the case of multiple incorrect usage, the number of microbes per eyedrop container increased to an average of 60-90 CFUs (conjunctiva and hand) or 1600 CFUs (cheek), respectively. Pathogenic bacteria (*eg S. aureus*) were isolated more frequently during this study, but were always in very small numbers. Again, contact of the dispenser tip to the patient's cheek offered the greatest chance of microbial contamination of the dispenser contents.

Study 3 (Table 4)

During this *in vitro* study, the tips of eyedrop dispensers were immersed in suspensions of 10^2, 10^4 or 10^6 *P. aeruginosa*/ ml. This represents an extremely gross contamination of the dispensers. Despite this, only relatively small numbers of *Pseudomonas* were isolated from the contents of the dispensers: 0.1, 8.5 and 313 bacteria per dispenser for the 10^2, 10^4 and 10^6 tests, respectively.

Discussion

The results of our three challenge studies indicate that, even under abused or misused situations, products are not easily contaminated. Few investigations of eyedrop contamination in the literature are quantitative[2] and do not offer an estimate of actual counts of contaminating microbes. Our present studies do count organisms and allow us to give exact levels of bacteria or fungi contaminating the test samples.

The greatest source of contamination was by touching the eyedrop dispenser tip to the cheek while administering the non-preserved saline. Initially this is surprising, as conventional wisdom might indicate that the hands would offer a richer source for contamination than the face or conjunctiva. But hands are generally washed many times per day, whereas the face may be washed only once per day. More microbial bioburden build-up is likely on the face than on the hands.

Our current studies represent a reasonable and relevant approach for estimating contamination rates of ophthalmic products. Our studies with a non-preserved formulation show similar contamination rates to those reported in the literature[2] for actual marketed products.

Table 4. Study 3 (*in vitro* gross contamination by *Pseudomonas aeruginosa*): contamination results

Set	10^2 Sterile	10^4 CFU/ml	10^6 CFU/ml	CFU/ml
1		7		70
2		5		3,500
3		6		110
4		4		90
5		2		60
6	1	7		150
7		4		2,600
8		110		1,100
9		5		120
10		20		70
Sum of all CFU	0	1	170	7,870
Avg CFU	0.0	0.1	17.0	787.0
Sum of non-zero values	0	1	170	7,870
Total no. of non-zero values	0	1	10	10
Avg CFU in non-zero values	-	1.0	17.0	787.0
Log sum of all counts	0	0	8.7139	23.7197
Avg log CFU of 10 samples	0.0000	0.0000	0.8714	2.3720
Avg CFU based on log CFU	1.0	1.0	7.4	235.5
Incidence	0/10 = 0.0%	1/10 = 10.0%	10/10 = 100.0%	10/10 = 100.0%

Initial contamination	Contamination rate Incidence	%	Average counts Calculated arithmetically	Calculated on log basis
0 CFU	**0/10**	**All sterile**	**0**	**1**
10^2 CFU	**1/10**	**10%**	**0**	**1**
10^4 CFU	**10/10**	**100%**	**17**	**7**
10^6 CFU	**10/10**	**100%**	**787**	**235**

Multiple contacts with facial skin increase the contamination rates. In this study, eyedrop dispensers containing sterile, non-preserved physiological saline were subjected to abuse by volunteers. Subjects were instructed to handle and administer the eyedrop dispensers properly as directed by the manufacturer and not touch the tip of the dispenser to anything. Subjects also handled and administered the dispensers inappropriately by touching three types of surfaces that could lead to microbial contamination: the conjunctiva, the hand and the cheek.

After single (Study 1) and multiple administration over a 5-day period (Study 2), the entire contents of the eyedrop dispenser were evaluated for microbial contamination. Bacteria and fungi isolated from the dispensers were counted and identified. There was no antimicrobial preservative agent in the saline in the dispensers, so contamination of the solution could proceed without interference from or inhibition by any antimicrobial agent or preservative system. Therefore the design of these studies represents a worst-case situation: ie multiple contaminations, touching tips to contaminated surfaces and no antimicrobial preservative in the solution. Contamination rates for solutions containing any level of antimicrobial preservative agent would certainly be less than those found in these studies. Nevertheless, the present studies also demonstrate the importance of teaching patients to administer their ophthalmic medications properly.

These studies show that under actual conditions of single and multiple administration over a 5-day period, the level of contamination is quite low, even if the eyedrop dispensers are used inappropriately under the worst conditions. Appropriate administration of the dispensers provided little risk to the interior contents; *ie* the levels of contamination were minimal. This affirms and reinforces the good practice and labeling recommendations by manufacturers never to touch the tip of the dispenser to any surface while administering the eyedrops. Even in the case of inappropriate usage, only minimal levels of typically non-pathogenic organisms accumulated in the contents of the eyedrop dispensers. Even the highest contamination levels were far lower than the levels of bacteria and fungi required as inoculations for ophthalmic formulations in the current pharmacopoeial preservative efficacy tests.

The results of these studies support the premise that, even if products have measurable preservative activity, but do not meet the rigid standards of antimicrobial preservative efficacy set out by the pharmacopoeia, they would probably succeed in overcoming most microbial assaults reasonably expected to occur during patient misuse of the eyedrop dispenser. It is probable that a patient can tolerate a certain level of contamination by his own microflora. Large numbers of resistant micro-organisms can overwhelm any antimicrobial system, but extremely powerful preservative systems also contribute considerably to the ocular irritation and sensitivities of the patients using the product.[13,18] A good, safe and effective ophthalmic product can be made useless because of an overly powerful and irritating preservative system. The results of this study seem to indicate that extremely powerful preservative agents are not necessary under realistic conditions of misuse and that current ophthalmic products in eyedrop dispensers are effectively protected during limited usage.

Recommendations

Based on the literature reviewed previously, plus the data from the contamination studies performed, it is apparent that improper administration of eyedrops from containers is the major cause for product contamination. Clear instructions to the patients on the appropriate techniques for administration of eyedrops will avoid product contamination. The antimicrobial preservative systems in these products offer a significant level of safety and assurance, but are only secondary barriers to contamination. The microbiology challenge model proposed and explored in this report can be a useful tool to assess the robustness of the antimicrobial preservative system in eyedrop products under development in real-world conditions.

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References

1. Evans DJ, McNamara NA, Fleiszig SMJ. Life at the front: dissecting bacterial-host interactions at the ocular surface. *The Ocular Surface* 2007; **5**(3): 213–227.
2. Schlech BA. Microbial contamination of eyedrops. Part 1: Review of the literature. *Eur J Parenter Pharm Sci* 2007; **12**(4): 103–108.
3. Moore SL, Payne DN. Types of antimicrobial agents. In: *Russell, Hugo and Ayliffe's principles and practice of disinfection, preservation and sterilization,* 4th edition. Fraise AP, Lambert PA, Maillard J-Y, eds. Blackwell Publishing, Malden, MA, 2004, pp 8-97.
4. Matthews BR. Preservation and preservative efficacy testing: European perspectives. *Eur J Parenter Pharm Sci* 2003; **8:** 99–107.
5. Sutton SVW, Porter D. Development of the antimicrobial effectiveness test as USP chapter <51>. *PDA J Pharm Sci Technol* 2002; **56:** 300–311.
6. Sklubalova Z. Antimicrobial substances in ophthalmic drugs. *Ceska Slov Farm* 2004; **53**(3): 107–116.
7. Yamada A. Preservative effectiveness tests. *JP Pharm Forum* 2002; **11:** 2002.
8. JP 14 2001. Preservatives-effectiveness tests. In: Supplement I, pp. 1616-8. *The Japanese Pharmacopeia,* 14th edition. Society for Japanese Pharmacopeia, 2003.
9. Ph Eur 2005. 5.1.3 Efficacy of antimicrobial preservatives, pp. 447-9. In: *European Pharmacopoeia,* 5th edition. European Directorate for the Quality of Medicines within the Council of Europe, Strasbourg, 2004.
10. USP 30. 2007 <51>. Antimicrobial effectiveness testing, pp. 79-81. In: *United States Pharmacopeia*, 30th revision. US Pharmacopeial Convention, Inc, Rockville, MD, 2006.
11. Boles-Carenini B, Boldrini E, Brogliatti B. Real advantages of preservative-free preparations in special containers for long-term glaucoma therapy. *Acta Ophthalmol Scand* 2002; Suppl **236:** 57–59.
12. Furrer P, Mayer JM, Gurny R. Ocular tolerance of preservatives and alternatives. *Eur J Pharm Biopharm* 2002; **53**(3): 263–280.
13. Gasset AR, Ishii Y, Kaufman HE, Miller T. Cytotoxicity of ophthalmic preservatives. *Am J Ophthalmol* 1974; **78:** 98–105.
14. Pisella PJ, Pouliquen P, Baudouin C. Prevalence of ocular symptoms and signs with preserved and preservative free glaucoma medication. *Br J Ophthalmol* 2002; **86:** 418–423.
15. Debbasch C, Brignole F, Pisella P-J, *et al*. Quaternary ammoniums

and other preservatives' contribution in oxidative stress and apoptosis on Chang conjunctival cells. *Invest Ophthalmol Vis Sci* 2001; **42:** 642–652.
16. De Saint Jean M, Brignole F, Bringuier A-F, *et al*. Effects of benzalkonium chloride on growth and survival of Chang conjunctival cells. I*nvest Ophthalmol Vis Sci* 1999; **40:** 619–630.
17. Sklubalova Z. Adverse effects of preservatives in eyedrops. *Folia Pharm Univ Carol* 2003; **29-30:** 87–94.
18. Tripathi BJ, Tripathi RC, Kolli SP. Cytotoxicity of ophthalmic preservatives on human corneal epithelium. *Lens Eye Toxic Res* 1992; **9:** 361–375.
19. Abelson MB, Washburn S. The downside of tear preservatives. *Rev Ophthalmol* 2002; **09:** 05. www.revophth.com
20. Qureshi MA, Wong R, Robbie SJ, *et al*. Contamination of single-use Minims eyedrops by multiple use in clinics [Letter to Editor]. *Hosp Infect Soc* 2005; 245–247.
21. Seal DV. Multiple use of single use solutions: a dangerous practice. *Br J Ophthalmol* 2005; **89**(6): 783.
22. Wilson LA. To preserve or not to preserve, is that the question? *Br J Ophthalmol* 1996; **80**(7): 583–584.
23. Spooner DF, Davison AL. The validity of the criteria for pharmacopoeial antimicrobial preservative efficacy tests. *Pharmaceut J* 1993; **251:** 602–605.