

Sterility of Non-Preservative Eye Drops

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Objectives: To assess microbial contamination of common non-preservative eye drops stored at 4 °C and non-preservative fortified antibiotic eye drops used in a hospital inpatient setting.

Material and Method: A prospective study of the sterility of non-preservative eye drops was examined by dividing the patients into 2 groups. Group 1 composed of 5 samples of each ophthalmic preparation, which consisted of fortified antibiotics (vancomycin, cefazolin, gentamicin, and amikacin), non-preservative steroid eye drops (1% methylprednisolone and 0.01% dexamethasone), and 20% autologous serum stored at 4 °C. Group 2 was composed of 28 samples of fortified vancomycin and 23 samples of fortified amikacin used in an inpatient setting for 1 week and kept in 4 °C for 28 days. The contamination was assessed at the day of preparation and then at day 4, 7, 14, 21, 28 of all medications except autologous serum was done up to day 84. The sterility was examined by the culturing on tryptic soy broth and thioglycolate broth.

Results: In group 1 and 2, no contamination was detected in any samples within 4 weeks and up to 12 weeks for autologous serum.

Conclusion: Non-preservative topical eye drops can be safely kept at 4 °C without contamination for 4 weeks and 12 weeks for 20 % autologous serum drop. For topical antibiotics, under a strict protocol for preparation and usage might be used without significant risk of bacterial contamination for 4 weeks.

Keywords: Sterility, Non-preservative topical eye drop, Fortified antibiotics, Non-preservative steroid, Autologous serum

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Preservatives as benzalkonium chloride and thimerosal are commonly used in ophthalmic preparations to keep the medications sterile from microorganisms and increase the usage time of eye drops⁽¹⁾. However, the important side effects are toxicity to corneal and conjunctival epithelia depending on the dosage of preservatives, frequency and duration of usage⁽²⁻⁵⁾. In diseases requiring vigorous usage of topical eye drops, such as corneal ulcer, severe dry eye, glaucoma, the frequent applications of preservative topical eye drops commonly cause the toxicity⁽⁶⁻⁸⁾. The abnormal ocular surface conditions such as delayed tear clearance, aqueous tear deficiency, limbal stem deficiency, burn, Stevens-Johnson disease,

multiple surgeries, are more sensitive from the preservative substances⁽⁸⁻¹⁰⁾. It induces wound healing problems, corneal epithelial erosion, persistent epithelial defect, stromal edema, corneal infiltration, conjunctival injection and symblepharon^(1,3).

Therefore, non-preservative eye drops are mandatory in those kinds of patients. The common use of non-preservative topical eye drops are tear, antibiotic, steroid and autologous serum. However, artificial tear is the only non-preservative topical eye drop that is commercially available in Thailand. The rest have to be prepared in the hospital. To the best of our knowledge, there is no previous study to evaluate the period of sterility of each eye drop in Thailand.

It is wondered for how long those eye drops can be kept. So, it is very interesting to assess the microbial contamination of those non-preservative eye drops stored at 4 °C while keeping and using for patients.

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Material and Method

A prospective study was performed to study the sterility of commonly used non-preservative eye drops in Siriraj Hospital, Mahidol University from December 2004 - July 2005.

Study groups

The non-preservative eye drops were divided into 2 groups. Group 1 was the topical eye drops stored at 4 °C. Group 2 was the eye drops used in an inpatient setting.

Group 1 composed of 35 samples of 7 ophthalmic preparations including 4 samples of non-preservative fortified antibiotics (vancomycin, cefazolin, gentamicin, amikacin), 2 samples of steroids (1% methylprednisolone, 0.01% dexamethasone) and autologous serum eye drops stored at 4 °C. Five samples of each preparation were collected at day 0, 4, 7, 14, 21, 28 except autologous serum eye drop which was extended to day 56 and 84.

Group 2 composed of antibiotic of fortified vancomycin and fortified amikacin. Twenty eight samples of fortified vancomycin and 23 samples of fortified amikacin were included. All of the eye drops were stored at 4 °C and used for patients in a hospital inpatient setting with the frequency ordered by attending doctors and applied by hospital nurses for 7 days. After 7 day, those eye drops received the imitative situation of usage by exposure to the outside and opened the bottle was opened for 5 minutes twice a day while being kept at 4 °C. The specimens were collected for culture at day 0 (before applications), 7, 14 and 28.

Preparation of nonpreservative eye drops

Antibiotic eye drops of fortified vancomycin (50 mg/ml), fortified cefazolin (50mg/ml), fortified gentamicin (14 mg/ml), fortified amikacin (20 mg/ml) were prepared by hospital nurses by mixing parenteral forms of antibiotics with BSS solutions.

Steroid eye drops of 1% methylprednisolone were prepared by pharmacologists by mixing the parenteral form of methylprednisolone with NSS/2 solution. 0.01% Dexamethasone was prepared by hospital nurses by mixing the parenteral form of dexamethasone with BSS solutions.

20% autologous serum eye drop was prepared by venesection of 100 ml blood by hospital nurses. After blood was sent to the blood bank unit, it was left to clot for 4 hours and centrifuged at 3500 rpm for 5 minutes, then pure serum was separated. Those pure serum bottles were kept frozen at -20 °C in the

ward. Each bottle was thawed and diluted with BSS solution to be 20% of concentration by pharmacologists.

Techniques of cultivation

Three drops from each sample were collected for culture by taking each drop into the center of the culturing bottles. They were tryptic soy broth for aerobic bacteria, facultative bacteria and fungus, and thioglycolate broth for anaerobic bacteria and facultative bacteria. When the result was negative it was reported as "no growth". If the result was positive, the culture was repeated at the microbiology department. The rest of the sample of 0.5 ml would be sent for the second culture of tryptic soy broth, thioglycolate broth, blood agar, chocolate agar and sabouraud dextrose agar under laminar flow cabinet. The contamination would be concluded when the second culture reported any organisms (Diagram).

Results

In study group 1, the culture results were no growth in all specimens of antibiotic eye drops of fortified vancomycin, fortified cefazolin, fortified gentamicin, fortified amikacin and all specimens of steroid eye drops of 1% methylprednisolone, 0.01% dexamethasone at day 0, 4, 14, 21, 28. The culture results were also finally negative in all specimens of 20% serum eye drops at day 56 and 84, even the first culture result was found coagulase negative staphylococcus in one sample at day 56 but the repeated culture was no growth.

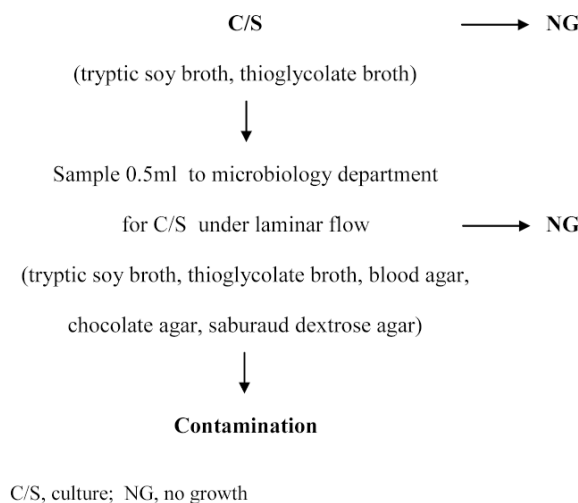


Diagram. Process of microbial identification

In study group 2, the culture results were no growth in all specimens of fortified vancomycin and fortified amikacin eye drops at days 0, 7, 14, 28.

Discussion

Three groups of non-preservative fortified antibiotics, steroid and serum eye drops are commonly used in ophthalmology. Those topical eye drops can get the higher efficacy by using them every hour without toxicity of preservative to the ocular surface⁽¹¹⁾. But as far as the authors know, there has been no previous study of sterility of those preparations in Thailand and only a few studies in the literature⁽¹²⁻¹⁵⁾.

Fortified antibiotics are very useful in the treatment of bacterial keratitis. They give a higher concentration than commercial antibiotic eye drops by mixing the parenteral form of antibiotics with BSS solutions. The present study demonstrated that there was no contamination for 4 weeks of fortified antibiotics stored at 4 °C. This result was the same as Arici et al⁽¹²⁾ previously reported that there was no significant change in the potency and stability for 3-4 weeks of fortified vancomycin, fortified cefazolin and fortified gentamicin and no contamination after being for 4 weeks at 4 °C. The result in group 2 was no growth for 4 weeks even being used in the infected eyes for 1 week and periodic exposure to room temperature while being kept in a fridge at 4 °C for 3 weeks. This implies that if the procedure of application topical eye drops is restricted to sterile technique, it might be possible to use the non-preservative antibiotic up to 4 weeks in real situations for patients.

Topical steroid eye drops are beneficial and widely used in the ophthalmological field. It reduces the inflammation of the diseases and postoperative ocular conditions⁽¹⁶⁾. Concerning non-preservative steroid eye drop, as far as the authors know, there is no previous study about sterility in the literature although Prabhasawat et al⁽¹⁷⁾ and Marsh et al⁽¹⁸⁾ previously mentioned it for the benefit of using non-preservative steroid for delayed tear clearance and aqueous tear deficiency, respectively. The present study also showed no contamination for 4 weeks of non-preservative steroid stored at 4 °C, the same as the result of fortified antibiotic even though the steroid itself can increase the risk of infection⁽¹⁶⁾.

Regarding autologous serum, the present study proved that there was no contamination after keeping at 4 °C for 12 weeks after being diluted to 20%. This sterility result was longer than Tseubota et al⁽¹⁴⁾ who reported no contamination for 2 weeks.

Auto-logous serum trend to safely keep longer by reducing the temperature as Tatananuvat et al⁽¹³⁾ reported of keeping 20% autologous serum in -20 °C for 2 months. Because of the presence of growth factors, vitamin A, fibronectin, cytokine and immunoglobulin, autologous serum is more effective than artificial tear for a severe dry eye, Stevens-Johnson syndrome, ocular cicatricial phemphigoid, persistent epithelial defect^(13-15,19).

In summary, the present study showed that non-preservative topical eye drops could be safely kept at 4 °C without contamination for 4 weeks and 12 weeks for 20% autologous serum drop. For topical antibiotic, under a strict protocol for preparation and usage might be kept and used without significant risk of bacterial contamination for 4 weeks. This might give physicians more convenience to give the non-preservative eye drops to the patients and advise them to extend the period of keeping and probably using for each bottle of topical eye drop and help in saving cost of treatment. However, sterility of the other topical non-preservative eye drops while using in the inpatient and outpatient settings for a longer period of time needs to be further studied.

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การศึกษาภาวะการปลอดเชื้อของยาหยอดตาที่ไม่มีสารกันเสีย

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วัตถุประสงค์: เพื่อศึกษาภาวะการปลอดเชื้อของยาหยอดตาที่ไม่มีสารกันเสียที่ใช้บ่อยทางจักษุวิทยาที่ผสมใช้ในโรงพยาบาลศิริราช เมื่อเก็บยาไว้ที่อุณหภูมิ 4 °C รวมถึงศึกษาหยอดตาปฏิชีวนะที่ไม่มีสารกันเสียที่ใช้ในหอผู้ป่วย

วัสดุและวิธีการ: แบ่งกลุ่มศึกษาเป็น 2 กลุ่ม กลุ่มที่ 1 ได้แก่ ยาหยอดตาที่ไม่มีสารกันเสียเก็บไว้ที่อุณหภูมิ 4 °C โดยแบ่งเป็น 3 กลุ่ม คือ fortified antibiotic (vancomycin, cefazolin, gentamicin, amikacin), topical steroid (1% methylprednisolone, 0.01% dexamethasone) และ 20% autologous serum drop ชนิดละ 5 ตัวอย่าง. กลุ่มที่ 2 ได้แก่ ยาหยอดตาที่ไม่มีสารกันเสียที่ใช้กับผู้ป่วยในหอผู้ป่วยเป็นเวลา 7 วัน และเก็บไว้ที่อุณหภูมิ 4 °C คือ fortified vancomycin 28 ตัวอย่าง และ fortified amikacin 23 ตัวอย่าง โดยส่งตรวจเพาะเชื้อในวันที่ 0 (หลังผสมยาเสร็จ), 4, 7, 14, 21, 28 ยกเว้น 20% autologous serum จะส่งตรวจเพิ่มอีกในวันที่ 56 และ 84 วิธีการเพาะเชื้อ จะหยอดยาลงในขวดเพาะเชื้อ tryptic soy broth และ thioglycolate broth

ผลการศึกษา: ทั้งในกลุ่มที่ 1 และกลุ่มที่ 2 ไม่พบการปนเปื้อนในทุกตัวอย่างตรวจเป็นเวลา 4 สัปดาห์ และ autologous serum เป็นเวลา 12 สัปดาห์

สรุป: ยาหยอดตาที่ใช้บ่อยที่ไม่มีสารกันเสีย สามารถเก็บไว้ที่อุณหภูมิ 4 °C ได้นาน 4 สัปดาห์ และ autologous serum ได้นาน 12 สัปดาห์ และยาหยอดตาปฏิชีวนะอาจใช้ในหอผู้ป่วยได้นาน 4 สัปดาห์ หากหยอดอย่างถูกวิธี และระมัดระวังการปนเปื้อน
