The effectiveness of a three-step sterilization method for Goldmann tonometer prism: A cross-sectional study

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**Purpose:** To propose a three-step sterilization method for Goldmann tonometer prism (GTP) and to analyze the sterilization effects of each step. **Methods:** 120 patients (240 eyes) who underwent Goldmann applanation tonometry (GAT) IOP measurement were enrolled in this study. GTPs were used individually for each patient and wiped by swabs soaked with 75% ethyl alcohol, ofloxacin eye drops, and 75% ethyl alcohol for at least 5 s. GTPs were directly pressed onto the surface of agar plates before (W0) and after three-step sterilization (W1, W2, and W3). All the agars were sent to the laboratory in 2 h and incubated at 37°C for 48 h. Subsequently, the growth of microbial species was assessed through visual inspection of the colonies at the inoculation points on the agar surface. **Results:** *Staphylococcus epidermidis* was the most frequently isolated bacterium and was observed in 23.33% of all prisms. Most of the bacteria were eliminated at W3 except *Staphylococcus epidermidis* and *Kocuria roseus* in one case. The isolation rates of *Staphylococcus genus* and *Staphylococcus epidermidis* were significantly decreased (both with *P* < 0.001). The number of bacteria types isolated from prisms at time point W2 and W3 had a statistically significant difference compared with W1 and W (both with *P* < 0.05), while W2 and W3 had no significant difference. **Conclusion:** This three-step sterilization method for GTP proved to be effective and safe for repeated use. We recommend using ofloxacin to prevent the transmission of pathogens based on ethyl alcohol, which could also bring some economic benefits.

**Key words:** 75% ethyl alcohol, Goldmann tonometer prism, ofloxacin eye drops, sterilization

Intraocular pressure (IOP) measurement plays an important role in the diagnosis, clinical follow-up, and treatment of various ocular diseases.[1] Goldmann applanation tonometry (GAT) remains the gold standard for IOP measurement owing to its accuracy and consistency, with estimates of more than 122 million patients undergoing GAT annually worldwide.[2,3] However, GAT also had potential hazards; commensal bacteria could be directly transmitted from the ocular surface to the tonometer tip, which might result in the cross-patient infection upon the reuse of the tonometer tips, especially for patients with corneal epithelial injuries during the GAT examination. Nowadays, bacterial culture of conjunctival sac has become the routine procedure before intraocular operation and in the general population, *Staphylococcus*, *Corynebacterium*, and *Pseudomonas* have become the principal part of ocular surfaces. Also, there have been reports that infectious keratoconjunctivitis is transmitted from ocular equipment and GAT prisms act as a vector for infection transmission.[4,5] Walla et al.[6] proposed that GAT tips might play a role in the iatrogenic transmission of Creutzfeldt–Jakob disease due to insufficient sterilization, indicating that a standard sterilization method is required for clinical practice.

Currently, there appears to be no agreement on tonometer disinfection practices and guidelines that adequately ensures patients’ safety and prevent patients from keratitis around the world. A review of the literature revealed that at least 16 methods of tonometer disinfection have been proposed since 1987 in American Glaucoma Society (AGS) and the American Optometry Association (AOA),[7] and most of them use 70% isopropyl alcohol wipes or combine 70% isopropyl alcohol wipes with 10% hypochlorite as the disinfection method; few use 3% hydrogen or just soap and water. Briesen et al.[8] illustrated using and wiping with Sekucept 4% solution or isopropanol 70% to disinfect in developing countries. The United States Centers for Disease Control and Prevention (CDC) have recommended that tonometer prism is needed to disinfect for 5–10 min soaked either in 3% hydrogen peroxide, 5000 ppm chlorine, 70% ethyl alcohol, or 70% isopropyl alcohol.[2,8] However, not all groups choose to clean reusable tonometer tips immediately after use, and some only disinfect tonometer tips once a day.

In China, where the population is so large that sterilizing immediately after use makes a lot of sense, the abovementioned disinfection methods are not suitable for the wide application of medical institutions in China in terms of time-consuming and disinfection effect; 5–10 min of soaked disinfection will increase workload. Moreover, in developed countries, the use of disposable prisms frequently and effectively prevent...
cross-infection, however, from an economic perspective, disposable tonometer probe is not a good choice for the ophthalmology department in China with a large population because a report from England showed that the cost of disposable Tonosafe could reach five times or more when compared with disinfectant used in washing, immersing, and drying Tonosafe each year.

In the present study, we proposed a method for the sterilization of tonometer prism for instant reuse and to explore its efficacy in the perspective of bacterial growth. Furthermore, we also compared the effectiveness of sterilization among the different steps, aiming to provide a basis for the economic benefits in the clinical practice.

**Methods**

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Human Research and Ethics Committee. Written informed consent in Chinese version was obtained from each participant before enrollment.

**Patients**

In this study, patients were recruited from the outpatient department consecutively between August 2018 and April 2019. The inclusion criteria include a) patients who measured IOP using GAT, b) patients aged between 18 to 80. The exclusion criteria include: a) patients who had used systemic antibiotics or any topical use eye drops in the last 1 week; b) patients with inflammatory diseases of the external eyes; c) patients with obvious deformation of cornea which influenced the surface between tonometer prism and cornea; d) patients with HIV, hepatitis, syphilis, tuberculosis and other diseases that can be transmitted through body liquid; and e) pregnant or nursing mothers. Eventually, 120 patients (30 males and 90 females) were enrolled in this study. The mean ± SD age of patients was 41.3 ± 10.6 years (range: 23–73).

**Sample collection**

Samples were collected by the same ophthalmologist assistant who was wearing sterile gloves and mask. All the Goldmann tonometer prisms used in this study were sterilized with ethylene oxide. Sample collection was performed in the order described below. 1) After measuring intraocular pressure, the prism (W timing) was inoculated to the middle of the upper left quadrant of the agar plates (bioMérieux Biological Product Co. Ltd., Shanghai, China). 2) One sterile cotton swab was dipped in 75% ethyl alcohol and then used to wipe the surface of the prism in a clockwise direction, keeping the major axis of the cotton swab perpendicular to the major axis of the prism. The prism (W1 timing) was inoculated to the middle of the upper right quadrant of the agar plates. 3) Another cotton swab was dipped in ofloxacin eye drops (HangZhou Minsheng Medicine Co. Ltd., Hangzhou, Zhejiang, China) and then used to wipe the prism as previously described. The prism (W2 timing) was then inoculated to the middle of the bottom left quadrant of the agar plates. 4) Another cotton swab was used and dipped in 75% ethyl alcohol and the same procedure was repeated. The prism (W3 timing) was inoculated to the middle of the bottom right quadrant of the agar plates. Goldmann prism was wiped with different swabs for at least 5 s at each step and was drying in the air for 10 s before the incubation. Some steps of sample collection was shown in Fig. 1.

**Bacteria isolation and identification**

At four time points for each patient, Goldmann tonometer prism was directly inoculated to Columbia agar with sheep blood (bioMérieux Biological Product Co. Ltd., Shanghai, China). Then, the agar plate was transported to the laboratory immediately and incubated at 37°C for 48 h. The growth of bacteria colonies was observed every 4 h. Afterward, colonies of bacteria were separated and purified. Each colony of bacteria was tested in Gram’s staining, and the corresponded test kit was selected respectively according to the results of Gram’s staining for bacteria identification.

**Statistical analysis**

Statistical analysis was performed using SPSS 22.0 software. Isolation rates between different time points were compared overall and respectively with Chi-squared test. The number of bacteria types on each prism at four time points were compared using ANOVA. P < 0.05 was considered statistically significant.

**Results**

**Demographic characteristics**

The study comprised 120 patients (240 eyes). The mean age of the 30 males and 90 females was 41.3 ± 10.6 years (SD) (range: 23–73).

**Bacterial cultures at different sterilization steps**

Bacteria isolated from prisms after IOP measurement (W) are shown in Table 1. At time point W, most of the prisms showed no isolated bacteria (60.83%), and of those prisms with positive isolation results, S. epidermidis was the most frequently isolated bacterium and was observed in 23.33% of all prisms.
Identified isolated bacteria after IOP measurement were all Gram-positive, including *Staphylococcus*, *Kocuria*, *Micrococcus*, *Leuconostoc*, and *Enterococcus*. These results indicated that commensal bacteria in ocular surfaces could transfer to the prism during the IOP measurement, and sterilization of prisms is essential.

**Compositions of isolated bacteria at different time points**

The prisms with isolated bacteria descended gradually with each disinfection, especially compared with the time point W2 and W3, the isolated bacteria descended obviously in W and W1 [Fig. 2]. Most of the bacteria were eliminated at W2 and W3 except *S. epidermidis* and *Kocuria roseus*. Each type of bacteria isolated from Goldmann tonometer prisms at different time points was separately analyzed as well. As shown in Table 1, S. genus and *S. epidermidis* were significantly decreased (both with *P* < 0.001), and prisms with no isolation detected increased (*P* < 0.001). However, there were no statistical differences between W2 and W3 regarding the positive isolation rate (*P* = 1.000). Therefore, there were no obvious changes in sterilization between W2 and W3.

**Comparison of the number of bacteria types on prisms**

As shown in Table 2, the number of bacteria types isolated from all prisms decreased (*P* < 0.001). The number of bacteria types isolated from all prisms at time point W2 and W3 had a statistically significant difference compared with W1 and W (all with *P* < 0.001), while W2 and W3 existed no significant statistical difference (*P* = 0.341). As the rate of none bacteria at time point W was relatively high (60.83%), we analyzed the bacterial changes of 47 prisms with positive isolated bacteria at W; the results showed that the number of bacteria types at time point W2 and W3 had a statistically significant difference compared with W1 and W (all with *P* < 0.001). W1 and W also exhibited an obvious statistical difference (*P* < 0.001), while W2 and W3 showed no significant statistical difference (*P* = 0.065). Thus, the first step in this method took effect and isolated bacteria have been effectively sterilized already at time point W2 in this study.

**Discussion**

In this study, the bacterial composition of the Goldmann prism in a unicentral general population was explored. The efficacy of the proposed sterilization method for instant reuse was confirmed, and we proposed that a two-step sterilization method can be applied in the future, which could reduce medical costs.

Bacteria have been isolated in tears, conjunctiva, and cornea. Infectious organisms from the ocular surface of patients can be transmitted via medical procedures during daily practice, of which GAT might also play an important role. According to the results, most of the prisms showed no isolated bacteria, and *S. epidermidis* was the most frequently isolated bacteria after the direct contact of prisms with cornea. Additionally, other bacteria such as *Kocuria roseus* and *Micrococcus* were also detected. Culture results were consistent with previous studies in which cotton swabs were used to collect the bacterial specimen at the conjunctival sac in normal patients[10-13] in and patients with ocular surface diseases such as dry eye,[14] indicating that Goldmann prisms also greatly reflect the bacterial composition of the ocular surface and can be used as a tool to evaluate the commensal microbial conditions.

**Table 1: Bacteria isolated at different time points**

| Isolated bacteria                | W   | W1 * (6.67%)† | 1 (0.83%)** | 1 (0.83%)** | P    |
|----------------------------------|-----|---------------|-------------|-------------|------|
| *Staphylococcus* (G+)            | 36  | 8 (30.00%)    | 1 (0.83%)** | 1 (0.83%)** | <0.001|
| *S. epidermidis*                 | 28  | 8 (23.33%)    | 1 (0.83%)** | 1 (0.83%)** | <0.001|
| *S. aureus*                      | 2   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 0.248 |
| *S. capitis*                     | 3   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 0.661 |
| *S. hominis*                     | 1   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| *S. hemolyticus*                 | 1   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| *S. cohnii*                      | 1   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| *Kocuria roseus* (G+)            | 4   | 1 (0.83%)     | 1 (0.83%)† | 1 (0.83%)** | 0.175 |
| *Micrococcus* (G+)               | 3   | 1 (0.83%)     | 0 (0.00%)   | 0 (0.00%)   | 0.279 |
| *Micrococcus luteus*             | 2   | 1 (0.83%)     | 0 (0.00%)   | 0 (0.00%)   | 0.623 |
| *Micrococcus Kristinae*           | 1   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| *Leuconostoc mesenteroides* (G+)  | 1   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| *Enterococcus faecalis* (G+)      | 1   | 1 (0.83%)†    | 0 (0.00%)   | 0 (0.00%)   | 1.000 |
| Unknown                          | 2   | 0 (0.00%)     | 0 (0.00%)   | 0 (0.00%)   | 0.248 |
| None                             | 73  | 109 (90.83%)* | 118 (98.33%)** | 118 (98.33%)** | <0.001 |

*P*<0.05 compared to W; †*P*<0.05 compared to W1

**Table 2: Comparison of the number of bacteria types on each prism**

| Types of bacteria on each prism (mean±SD) | W    | W1 * (0.09±0.29)† | 0.03±0.16** | 0.02±0.13** | <0.001 |
|------------------------------------------|------|-------------------|-------------|-------------|------|
| Types of bacteria from designated 47 prisms (mean±SD) | 1.04±0.34 | 0.21±0.41* | 0.06±0.25* | 0.04±0.20* | <0.001 |

*P*<0.05 compared to W; †*P*<0.05 compared to W1; designated 47 prisms stands for the prisms which were detected with isolated bacteria at W.
Further studies to explore the correlation of the bacterial culture between the Goldmann prism and the swabs at the conjunctival sac are required.

Cross-patients infection caused by GAT has been gradually identified. A meta-analysis conducted by Alex Ragan et al. reviewed 19 primary-level studies for Goldmann tonometer prisms disinfection; the results revealed that the included studies were largely heterogeneous with regard to the pathogens and disinfectants, indicating that the present state of the disinfection does not permit a definitive conclusion; novel disinfection methods with the advantages of great efficacy, safety, and efficiency still have their markets. In our study, isopropyl alcohol, which was mostly recommended in CDC’s guidelines, was selected. Meanwhile, ofloxacin, which is a broad-sodium hypochlorite spectrum antibiotic, proved to be effective against most of the gram-positive and gram-negative bacteria and was also applied in the disinfection procedure. It was found that the positive rate of the tonometer cultures decreased from 39.17% before the disinfection to 9.17% after the first wipe, demonstrating the effectiveness of isopropyl alcohol in the disinfection. Akhtar et al. assessed the effectiveness of alcohol swabs and immerse prisms in peroxide and found a 64% reduction in log growth of epidemic keratoconjunctivitis when peroxide was used compared with alcohol swabs. We also found that a 76.6% reduction in bacteria in our experiment, which indicates that alcohol is not effective after a single wipe once and maybe alcohol is not the best disinfectant.

Then, after the second wipe, the positive rate of bacteria continued to decrease to 1.67%. According to previous research, a double-masked, randomized, controlled study confirmed that ofloxacin eradicated and controlled 85% of the Gram-positive and 89% of the Gram-negative organisms cultured; moreover, 98% of patients treated with ofloxacin got improvement in clinical signs. It was obvious that most of the bacteria were eliminated at W1 and W2, the first two steps.

The 75% ethyl alcohol used in the first and third step of our method is used nationwide as a conventional disinfectant and ofloxacin eye drops used in the second step is a new attempt and also plays an important role in sterilization. Through the cultures’ results of the tonometer tips, the use of ofloxacin reduced the positive rate of bacteria from 9.17% to 1.67%. The bactericidal effect of ofloxacin exhibited a bigger role based on ethyl alcohol use. However, there were no significant differences between W2 and W3 regarding positive isolation rate ($P = 1.000$) and the number of bacteria types (120 prisms: $P = 0.341$; 47 prisms which were detected with isolated bacteria at time point W: $P = 0.065$). In other words, the bacteria were not completely killed after the third step. This phenomenon can also be found in the literature. Cillino et al. compared various disinfection practices, including dry wipes, Minuten wipes, soaking in 3% hydrogen peroxide, and 0.5% benzalkonium chloride for 1, 5, and 15 min, and noted that B. subtilis required 5 min for disinfection and 1 min was not enough to kill the bacteria. Therefore, we suspect that it may take a long time for ofloxacin and alcohol to kill bacteria that are less sensitive to ofloxacin. Moreover, some patients may have used antibiotic eye drops; thus, the bacteria in ocular surfaces showed resistance to antibiotics.

Although the less disinfection times could prevent tonometer prism from shorting the life span, we cannot skip the third step in this method because the repeated use of ofloxacin may have some residual on the tip which might directly touch the ocular surface and increase the resistance of bacteria. Using alcohol for disinfection can reduce the antibiotics residual and guarantee the disinfection effect. Therefore, in our study, we first illustrated that the adoption of ofloxacin in sterilization may eliminate the remaining bacteria and help to achieve more effective sterilization. We demonstrate that the alcohol-ofloxacin-alcohol disinfection method is effective and we deduce that it is adequate for us to wipe three times in clinical work.

**Conclusion**

In this study, we first explored the efficacy of a three-step sterilization procedure in the hospital and compared the sterilization effect among each step. We recommend using ofloxacin to prevent the transmission of pathogens based on alcohol use. We made it clear that three times disinfection is acceptable, while other disinfection methods in CDC may be ineffective because of time-wastage and economic burden.

**Limitation**

There are some limitations of our study. First, we only determined bacteria as they account for about 98% of microorganisms on the ocular surface; more sensitive methods for microbe identification might be applied in future research. Second, this was only an observational research to demonstrate that alcohol-ofloxacin-alcohol disinfection method is effective. The Control group should be set in the next exploratory studies. Third, the sample size was small and it would be needed to replicate our findings in a large and multicenter study.

**Figure 2:** Composition of the bacteria at different time points

Note: The size of the area in the pie chart represents the positive isolation rate of each bacterium
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Conflicts of interest
There are no conflicts of interest.

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