LABORATORY SCIENCE

Optimizing the antisepsis protocol: Effectiveness of 3 povidone–iodine 1.0% applications versus a single application of povidone–iodine 5.0%

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Purpose: To determine the minimum effective concentration of povidone–iodine that reduces the bacterial load by 3-$\log_{10}$, the U.S. Food and Drug Administration requirement for antiseptic agents, and to study alternative dosing schedules of povidone–iodine to optimize its bactericidal effect.

Setting: Microbiology Laboratory, Evanston Hospital, Evanston, Illinois, USA.

Design: Experimental study.

Methods: A standard 0.5 McFarland solution of *Staphylococcus epidermidis* was applied to blood agar plates. The plates were treated with a single application of povidone–iodine solutions from 10.0% to 0.1% to define the range of interest. Another set of plates received 3 applications of various povidone–iodine solutions. Microbial growth was evaluated after 24 hours. Standard deviations with 99.0% and 99.9% confidence intervals for each concentration were estimated and used to estimate the minimum concentration that reduced the colony counts by at least 3-$\log_{10}$.

Results: Povidone–iodine at 2.5% and higher concentrations was effective in eliminating *S epidermidis* with a single application. Three 30-second applications of povidone–iodine at concentrations of 0.7% and higher resulted in at least a 3-$\log_{10}$ reduction of colonies.

Conclusions: Povidone–iodine 5.0% has been the standard of care for preoperative ocular antisepsis for 3 decades. Povidone–iodine 0.7% was as effective as a bactericidal agent when applied multiple times. This suggests povidone–iodine 1.0%, applied in three 30-second applications for preoperative surface disinfection might be as effective for preoperative antisepsis.

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Although cataract surgery is typically a safe and effective operation, endophthalmitis remains a rare but serious postoperative complication. The incidence of endophthalmitis after cataract surgery was reported in a Swedish review of 2002 to 2004 data at 0.48 per 1000 rate of infection and a retrospective analysis of all 2004 United States Medicare claim data at 1.11 per 1000.[1,2] Visual outcomes worse than 20/200 were seen in one third of endophthalmitis cases in another recent Swedish study.[3]

The organism most often responsible for postoperative endophthalmitis is coagulase-negative staphylococci (CoNS) (*Staphylococcus epidermidis*) followed by *Staphylococcus aureus*, and *Streptococcus* species.[4,5] These organisms reflect the bacterial flora of the eyelids and conjunctiva.[6] These pathogens can enter the anterior chamber through clear corneal incisions, thus exposing the eye to potential endophthalmitis.[7] Prophylaxis against endophthalmitis via antisepsis of the eyelids and conjunctiva has therefore been an important area of study.

Perioperative povidone–iodine use is not without risk; povidone–iodine 10.0% and 5.0% solutions have been shown to be toxic to the corneal epithelium when placed topically, and povidone–iodine 1.0% placed intracamerally is toxic to the corneal endothelium.[8,9] In addition, povidone–iodine 5.0% and 2.5% cause edema and irritation in rabbit corneas, while 1.0% and 0.5% concentrations do not.[10] Because of such experimental results, an effort has been made to apply more dilute concentrations of povidone–iodine before or during surgery. Shimada et al.[6]

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have shown that povidone–iodine 0.25%, when used as a surface irrigation agent throughout the procedure in addition to usual preoperative surface preparation, can reduce the number of bacteria found in aqueous sampling to zero at the completion of surgery.

The importance of administering povidone–iodine at a concentration that is both safe and effective is critical.[A] In addition to the toxicities, higher concentrations of povidone–iodine cause burning and irritation while lower concentrations cause less discomfort.[11,12] Most patients can tolerate low concentrations of povidone–iodine solution without prior use of anesthetic agents.[12] Using lower concentrations of povidone–iodine would minimize the number of insults to the ocular surface and the potential for a barrier effect caused by some forms of topical anesthetic.[13,14]

MATERIALS AND METHODS

Study Design

A 0.5 McFarland suspension, approximately 1×10^8 colony forming units (CFU)/mL, of (CoNS) (*S epidermidis* RP62A) was prepared. A 0.25 mL of the 0.5 McFarland suspension was used to inoculate each 5.0% sheep blood agar plate by evenly flooding the surface. The fluid was allowed to soak into the agar for more than 2 minutes. This represents approximately 2.5×10^7 bacteria on the agar surface (0.25 mL of a 1×10^8 CFU/mL suspension), the typical bacterial load of infected conjunctiva.

In the first exploratory experiment, 24 agar plates were covered with 0.25 mL of the 0.5 McFarland suspension as described above. As the bacterial solution was drying on the agar, povidone–iodine 10.0% (Betadine) was used to create the following diluted solutions by the following serial dilutions with Balanced Salt Solution (Alcon Laboratories, Inc.): 10.0%, 5.0%, 2.5%, 1.0%, 0.5%, 0.25%, and 0.1%. After all the solutions were made, 2 mL of each povidone–iodine solution was applied to the agar surfaces of 3 plates. After a 30-second exposure, excess povidone–iodine was poured off into a biohazard container. The 3 remaining plates were used as controls and were not treated with any solution.

A second exploratory experiment was performed using 15 plates prepared as above. Three plates were covered with 2 mL of povidone–iodine 1.0% for 30 seconds. Another 3 plates received 6 mL of povidone–iodine 1.0% for 30 seconds. Three additional plates were treated with 2 mL of povidone–iodine 1.0% for 90 seconds. A final set of 3 plates was treated with 3 applications of 2 mL of povidone–iodine 1.0%, with each application lasting 30 seconds and with over 120 seconds between applications. The 3 remaining plates were used as controls.

A final set of 55 plates inoculated with 0.25 mL of a 1:1000 dilution of the initial 0.5 McFarland suspension was applied to the surface of the agar. This dilution was used to better represent the bacterial load of a noninfected preoperative ocular surface. Given the starting bacterial suspension of 1×10^5 CFU/mL and inoculum volume of 0.25 mL, 2.5×10^4 CFU were inoculated to each plate prior to povidone–iodine application. Therefore, a 3-$\log_{10}$ reduction should lead to fewer than 25 colonies. The povidone–iodine 10.0% stock solution was serially diluted with a balanced salt solution to create a range of povidone–iodine solutions with concentrations of 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, and 0.1%. After all the solutions were made, each plate was treated with 2 mL of povidone–iodine for 30 seconds and the process was repeated with each plate receiving a total of three 30-second applications of the specific povidone–iodine solution with at least 120 seconds between each application. The experiment was repeated 5 times using the same process to prepare the bacterial solution and povidone–iodine dilutions, thus each dilution was tested on 5 agar plates. The remaining 5 plates were used as a control and were exposed to 2 mL of the balanced salt solution 3 times for 30 seconds.

All plates were incubated at 36°C for 24 hours and then evaluated for growth. For the first 2 experiments, bacterial growth was categorized as heavy growth (confluent surface colonies), intermediate growth (>100 colonies), light growth (<100 colonies), or no growth. Because these experiments were performed to optimize the protocol for the final experiment, the exact number of bacterial colonies on each plate was not quantified. In the final experiment, a masked microbiology technician manually counted the colonies.

A third experiment was used to further explore the effect of multiple application of povidone–iodine solution. For this study, a diluted McFarland inoculum was used to better replicate the bacterial load of a noninfected ocular surface.

Statistical Analysis

The basic mean and standard deviation was determined for each concentration using the 5 replicate samples. Confidence intervals of 99.0% and 99.9% were estimated, and the upper limit was used in the analysis for the concentration. An upper limit provides the worst-case result at the 0.01% and 0.001% level. The 0.01% cutoff exceeds the 0.05% *t* test values and the 0.001% level exceeds the 0.01% *t* test values, indicating statistical significance at the respective level. A regression line of upper limits was used to determine the intersection of the 3-$\log_{10}$ reduction in colony counts.

RESULTS

Single Application of High Concentrations of Povidone–Iodine

The first experiment showed a dose-response relationship between the concentration of povidone–iodine solution used and the amount of bacterial growth (Figure 1). Single applications of highly concentrated povidone–iodine

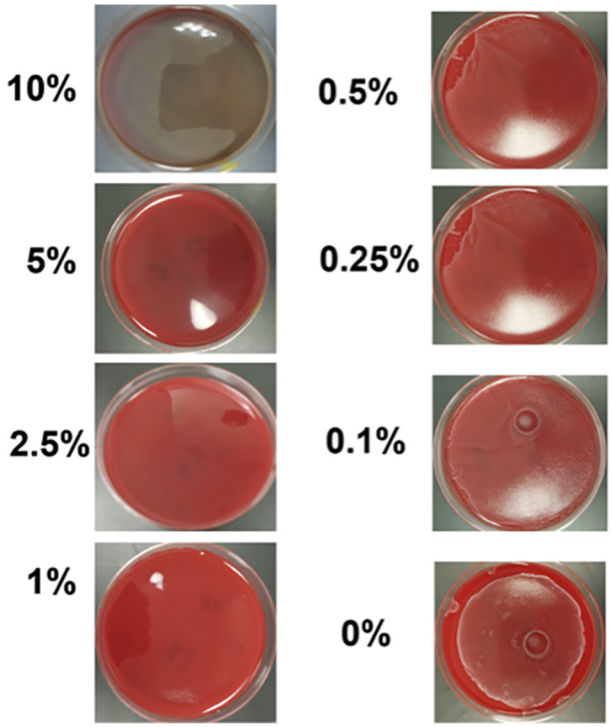

Figure 1. Bacterial growth on plates inoculated with 2.5×10^7 CFU and then treated with a single application of various dilutions of povidone–iodine solution. A control plate is shown to show the effect of the diluent alone.