Evaluation of Thermal Disinfection Procedures for Hydrophilic Contact Lenses

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The kinetics and efficacy of moist heat disinfection for hydrophilic contact lenses were investigated by using representative microorganisms of ophthalmic concern and several heat-resistant species. In replicate challenges, 80°C for 10 min and 75°C for 5 min were proven efficacious for moist-heat disinfection.

A moist-heat disinfecting unit for hydrophilic contact lenses has been shown to kill pathogenic microorganisms in both laboratory tests using known inocula and clinical use situations (2, 8). The objective of this study was to evaluate time-temperature limits that provide effective disinfecting action against microorganisms of ophthalmic concern (11) by using a standardized procedure of thermal death analysis. This analysis is considered essential in establishing specifications for moist-heat disinfection of hydrophilic contact lenses.

All microorganisms used were isolated from clinical specimens or from sources indicated in Tables 1 and 2, which included bacterial and fungal species of ophthalmic concern plus Streptococcus faecalis, a relatively heat-resistant species (3), an Aspergillus fumigatus isolate that reportedly survived boiling (10), and a clinical isolate of Pseudomonas aeruginosa. Broth and agar media were obtained from Baltimore Biological Laboratories and Difco Laboratories. Bacteria and yeasts were transferred for 3 consecutive days on soybean casein digest agar at 35°C and Sabouraud dextrose agar at 25 to 30°C, respectively. Cells from the final culture were harvested, washed three times, and suspended in sterile 0.85% saline (7). Final concentrations were estimated by optical density and confirmed by dilution pour plate counts. Medium for the cultures of Moraxella sp. and Streptococcus pyogenes was supplemented with 5% sheep blood, and medium for Haemophilus sp. was supplemented with 5% rabbit blood. Neisseria gonorrhoeae was grown on chocolate agar and Propionibacterium acnes was grown in reduced brain heart infusion broth. These five microorganisms were suspended only once in saline to avoid damage to the cells. Bacillus cereus and B. subtilis spore suspensions were prepared from growth on soybean casein digest agar that contained 0.02% manganese sulfate. Cells were suspended and washed in sterile distilled water before heating to 100°C for 1 min to kill vegetative cells. Surviving spores were quantitated by the dilution pour plate method. Molds were grown on Sabouraud dextrose agar at 25 to 30°C. Mold spores were harvested from 7-day cultures and suspended in 0.85% saline. The suspensions were agitated with glass beads to minimize clumping, filtered through sterile gauze to remove hyphal debris, and plated for enumeration.

For kinetic analysis, physiological sodium chloride solution was sterilized by autoclaving, and 99.0-ml quantities were dispensed into sterile three-necked distillation flasks. One side neck of the flask held a centigrade thermometer and the other held a gently spinning stirrer. The center neck was used as the port for inoculation and withdrawal of test samples. After assembly, the apparatus was equilibrated in a circulating water bath to test temperatures ranging from 46 to 65°C. A 1-ml portion of a standardized inoculum of a single species was added to each flask resulting in a final concentration of approximately 10⁵ colony-forming units (CFU) per ml. Portions of 1 ml withdrawn at 0.5- and 1-min intervals for 15 min after inoculation were mixed immediately with 9 ml of recovery medium held at 26 to 30°C. The recovery media used were: Sabouraud liquid broth modified for Candida albicans, A. niger, and A. fumigatus; fluid thioglycolate broth for Serratia marcescens, Proteus vulgaris, and P. acnes; and soybean casein digest broth for the remaining test microorganisms. Sterile defibrinated sheep blood (0.1 ml) was added to each tube of liquid medium used for recovery of S. pyogenes. Liquid recovery media were incubated at 35°C for bacteria and

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at 25 to 30°C for fungi for a maximum of 14 days. To determine the number of surviving microorganisms, appropriate dilutions in 0.1% peptone-water were plated in duplicate from the recovery medium. When testing *P. acnes*, a 2-ml portion of sample was withdrawn at each interval, and 1 ml was mixed with each of two 9-ml portions of recovery medium. One tube was incubated directly and the second was used for pour plate preparations. Soybean casein digest agar was used to prepare pour plates for all the organisms except *C. albicans*, *A. niger*, and *A. fumigatus*, which were plated on Sabouraud dextrose agar. *S. pyogenes* was grown on soybean casein digest agar supplemented with sheep blood. Agar plates of aerobic bacteria were incubated 48 h before counting colonies, whereas yeast and mold cultures were examined throughout 14 days of incubation. Plates inoculated with *P. acnes* were held for 7 days at 35°C in anaerobic jars (GasPak, BBL).

For the microorganisms tested, *D* values and a summary of the associated statistical parameters derived from linear regression analysis of log survivors versus time are listed in Table 1. *S. faecalis* was the most heat-resistant vegetative species, whereas cells and spores of *Bacillus* yielded typical biphasic responses, e.g., at 55°C in saline *B. cereus* yielded no further reduction in CFU after 2 min of heating. The logs of the *D* values were plotted versus their respective test temperatures, and a best-fit straight line was computed by linear regression analysis. Reciprocals of the absolute value of the slopes, *z* values, are listed in Table 1. Assuming a linear relationship beyond the experimentally sampled

### Table 1. *D* and *z* values for microorganisms heated in saline

<table>
<thead>
<tr>
<th>Organism*</th>
<th>Temp (°C)</th>
<th><em>D</em> value (min)</th>
<th><em>z</em> value (°C)</th>
<th>Organism</th>
<th>Temp (°C)</th>
<th><em>D</em> value (min)</th>
<th><em>z</em> value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. faecalis</em> 85W1000, Ward's National Science Establishment Inc., Rochester, N.Y.</td>
<td>55.0</td>
<td>9.16</td>
<td>52.5</td>
<td>0.90</td>
<td>55.0</td>
<td>0.50</td>
<td><em>S. marcescens</em> ATCC 14756</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>50.0</td>
<td>1.91</td>
<td>52.5</td>
<td>0.90</td>
<td>55.0</td>
<td>0.50</td>
<td><em>Escherichia coli</em> ATCC 8739</td>
</tr>
<tr>
<td><em>B. cereus</em> vag ATCC 14579</td>
<td>50.0</td>
<td>2.12</td>
<td>55.0</td>
<td>0.45</td>
<td>60.0</td>
<td>0.13</td>
<td><em>P. vulgaris</em></td>
</tr>
<tr>
<td><em>B. subtilis</em> vag ATCC 6633</td>
<td>46.0</td>
<td>8.20</td>
<td>47.8</td>
<td>3.10</td>
<td>49.9</td>
<td>1.92</td>
<td><em>P. aeruginosa</em> ATCC 9027</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td>50.0</td>
<td>4.99</td>
<td>55.0</td>
<td>0.65</td>
<td>60.0</td>
<td>0.18</td>
<td><em>A. fumigatus</em> ATCC 16424</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td>50.3</td>
<td>38.83</td>
<td>55.5</td>
<td>4.21</td>
<td>57.5</td>
<td>2.56</td>
<td><em>A. niger</em> ATCC 16404</td>
</tr>
<tr>
<td><em>C. albicans</em> ATCC 10231</td>
<td>50.0</td>
<td>15.26</td>
<td>52.1</td>
<td>5.37</td>
<td>54.0</td>
<td>2.87</td>
<td><em>F. solani</em> ATCC 16372</td>
</tr>
</tbody>
</table>

* Organisms are either clinical isolates or from sources indicated. Statistical parameters of *D* values expressed as the average (range) were: correlation coefficient = 0.9678 (0.8121–0.9999); standard error = 0.1941 (0.4160–0.6131); level of significance = *P* < 0.014 (<0.01 to <0.10).

* veg. Vegetative cells.
temperature range, straight lines were extrapolated to the 75 and 80°C coordinates on the log \( D \) value plot. At 75°C, the extrapolated \( D \) values ranged from \( 1.19 \times 10^{-7} \) to \( 6.46 \times 10^{-2} \) min. At 80°C, the \( D \) values ranged from \( 3.00 \times 10^{-9} \) to \( 1.76 \times 10^{-2} \) min.

For study purposes, the reduction of the number of CFU of each species by a factor of nine logs was adopted as the minimal acceptable disinfection treatment for hydrophilic contact lenses. The nine-log reduction times for \( S. \) faecalis were calculated as 0.581 min at 75°C and 0.158 min at 80°C. To verify that a nine-log reduction of each microorganism occurred within the proposed disinfecting periods, efficacy tests were performed in which approximately \( 10^9 \) CFU of each microorganism was heated at 75°C for 5 min and at 80°C for 10 min.

Hydrophilic contact lenses were added to vials containing 9.8 ml of 0.85% saline and sterilized by autoclaving. Cooled vials were equilibrated at 75 and 80°C in a circulating water bath and monitored by a thermocouple sealed in one vial. After equilibration, 0.2 ml of inoculum containing approximately \( 1 \times 10^9 \) organisms was added to the contents in each set of 20 vials. The cell concentration exceeded \( 1 \times 10^9 \) CFU for all microorganisms tested except \( P. \) acnes (3.2 \( \times \) \( 10^5 \)), \( A. \) niger (6.4 \( \times \) \( 10^5 \)), \( F. \) solani (3 \( \times \) \( 10^7 \)) and \( N. \) gonorrhoeae (1.5 \( \times \) \( 10^7 \)). After 5 and 10 min of exposure, sets of 10 vials were wiped dry, and the fluid contents were transferred to 90 ml of appropriate liquid recovery medium, as described for the kinetic analysis. Defibrinated rabbit blood and Difco supplement B were added to soybean casein digest broth for \( H. \) influenzae, and sheep blood was added for \( M. \) axellae, \( N. \) gonorrhoeae, and \( S. \) pyogenes. Lenses were transferred to 15 ml of broth medium. Broth media prepared with blood were
subcultured to blood agar after 2, 7, and 14 days. With the exception of the bacterial sporeformers, no positive cultures were recovered for any of the test organisms, after either 10 min at 80°C or 5 min at 75°C (Table 2). To test for the presence of survivors when heat-protecting organic loads were added to the meniscum, approximately 10⁶ cells of 12 of the more resistant microorganisms were exposed to the heat treatments in the presence of either 1.6 × 10⁶ heat-killed cells of C. albicans per ml or 10% horse serum. All inocula were killed by the treatment (Table 2).

The D values of many thermally treated species have been shown to be dependent on strain differences, nutritional conditions of growth, conditions of sporulation, composition and pH of the heating meniscum, concentration and age of the cells, and conditions of recovery (1, 5). In our experiments, the heating meniscum, saline, and saline-containing organic load were at pH 6.7, close to the pH which was reported to be the point of maximum heat resistance for S. faecalis (13). This represents a maximum challenge condition in the kinetic study of contact lens disinfection. Spores of the mycotic organisms were relatively heat sensitive when compared with cells of S. faecalis. Although the D values of A. fumigatus are greater than those of S. faecalis at respective assay temperature ranges, their rate of decrease is also greater, indicating greater sensitivity at higher disinfecting temperatures. Analysis of P. aeruginosa did result in a slight scatter of D values at the higher temperature range. However, at 60°C 2.2 × 10⁵ cells were killed in less than 30 s. This is equivalent to a D₀ value of less than 0.09 min, which is within the range of values extrapolated by linear regression analysis.

Previously reported data indicate that the z value is not as dependent as the D value on the composition of the heating meniscum and other variables connected with the heating. The z values have been reported to be 4 to 6°C for non-spore-forming bacteria, 3 to 5°C for yeasts, and 5°C for A. niger (5). In the present study, z values were 4 to 9°C for bacteria, 3 to 5°C for yeasts and 3.4°C for A. niger.

Since earlier studies have shown that non-sporing bacteria, yeast, molds of ocular concern, and herpes simplex were rapidly killed by exposure to moist heat temperatures of less than 80°C (4, 6, 8, 9, 12), a process of 80°C for 10 min was adopted to assure an adequate margin of safety for the disinfection of hydrophilic contact lenses (2, 8). In the present study, treatment at 80°C for 10 min provides a margin of safety 63 times the theoretical 0.158 min required to produce a nine-log reduction of S. faecalis. Theoretical calculation of a nine-log reduction time of 0.581 min for 75°C equates to 8.6 factor for the margin of safety for a 5-min treatment. The lack of recovery of the non-spore-forming cells in the efficacy test proved the disinfecting reliability at either of the time-temperature combinations. Kill was achieved even with the most heat-resistant, non-spore-forming species, S. faecalis, after exposure at 75°C for 5 min in the presence of an organic load and hydrophilic contact lenses. Therefore, it can be concluded that disinfecting systems designed to operate at 80°C for 10 min assures an adequate margin of safety for achieving disinfection of hydrophilic contact lenses.

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LITERATURE CITED