THE TESTING OF PRESERVATIVES FOR OPHTHALMIC PREPARATIONS

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The preservation of ophthalmic preparations against bacterial contamination and growth is general and is often mandatory. Clear evidence is still needed concerning the efficiency of the compounds and concentrations used, alone and in combination with formulatory additives. Some commercially available contact lens solutions may be inadequately preserved (Norton, et al, 1974), and a simple, rational and reproducible challenge test is needed to study these effects.

The main test organism chosen was Staphylococcus aureus NCTC 6571, while Bacillus subtilis NCTC 3610, Escherichia coli NCTC 1157 and Pseudomonas aeruginosa NCTC 6750 were used in confirmatory tests. Chlorhexidine digluconate was the main test preservative. Cells were grown at 37° in a shaking water bath in a fully synthetic medium (Mah et al 1967) with glucose as the main energy source. Three subcultures were needed for full adaptation, and 24 hour-old stationary phase (SYN) cells from the third subculture were collected on 0.45μm membranes, washed with and adequately dispersed in M9 solution. They were diluted to approximately 10^7 viable cells.ml^{-1} and held at room temperature.

For the test, cells were added to prewarmed, assayed preservative solution held at 25° in a conical flask to a final concentration of 10^6 cells.ml^{-1}. Samples were withdrawn at timed intervals, immediately inactivated in thioglycollate/lecithin/polysorbate 80 quenching mixture and subsequently counted using M9 solution as the diluent. Aliquots were plated on Tryptone-soy agar, incubated at 37° for 24 hours or more and counted. From these survivor curves were constructed. The process was also used with cells grown in Tryptone-soy broth for comparative purposes (TS cells).

The significance of the variables in this system were examined in some detail. Growth curve determinations showed the need for at least three subcultures for full adaptation of SYN cells but not of TS cells. Optical density/viable count calibration curves were established for all the test organisms and the high inactivating capacity of the quenching medium for chlorhexidine demonstrated. Some regular variation in the behaviour of varying inoculum number and age was shown and needs further investigation.

With chlorhexidine at least, the true form of the survivor curve seems to be sigmoid, with a pronounced tail at low survivor levels. Quantitative analysis of such curves is difficult but probit transformation leads to linear responses which can be characterised by their slope and intercept values. Using this analysis the effect of experimental variables was investigated.

The SYN cells proved to be generally more resistant to chlorhexidine than TS cells, while showing similar responses to changes in chlorhexidine concentration, exposure temperature and pH. Of the additional buffer substances tested, TRIS and citrate reduced the activity of chlorhexidine below that of the diluted M9 at the same pH, citrate to a greater extent than TRIS. The system gave satisfactory results using chlorhexidine v. E.coli, Ps. aeruginosa and B. subtilis, and with benzalkonium chloride, chlorbutol and thiomersal v. Staph. aureus. The latter preservative appears to have little activity over the time scale used.

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