RATIONAL APPROACH TOWARDS IMPROVING THE REPRODUCIBILITY AND PREDICTIVE EFFECTIVENESS OF 'OFFICIAL' ANTIMICROBIAL PRESERVATIVE CHALLENGE TESTS

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The United States Pharmacopoeial (USP) (XIX), 1975, Antimicrobial Agents. Effectiveness Test intends to provide a basis for routine prediction of 'in use' efficiency of antimicrobial preservatives in multidose parenteral, otic, nasal and opthalmic solutions. A broadly similar test is proposed for the 1980 British Pharmacopoeia, but for a wider range of formulations. Moore (1978) has reported on the poor reproducibility between laboratories and low predictive efficiency of the USP test. We consider that the suggested test organisms, methods of culture maintenance and the wide latitude given for choice of growth media for inoculum preparation, will result in tests of poor reproducibility and of low predictive efficiency with respect to 'in use' conditions. The USP tests were performed upon test formulations comprising nutrient broth (Oxoid CM1) containing varying concentrations of thiomersal, benzalkonium chloride, or chlorhexidine. The formulations were challenged with *Staphyloccous aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027, grown in complex media or synthetic media deficient in carbon substrate, phosphate, or ammonium ions. The test formulations either passed with ease or failed depending on the inoculum used. MIC determinations were conducted in parallel to the USP test and were able to detect the variation in preservative sensitivity of the inoculum and were predictive of the USP test result. A complex inter-relationship was observed between the micro-organisms, conditions for growth of the inocula, type of preservative and degree of effectiveness.

Many isolates of *Pseudomonas, Citrobacter, Acinetobacter, Moraxella, Alcaligenes, Aspergillus, Saccharomyces, Cladosporium* and *Penicillium* species have been obtained from various environments and spoiled pharmaceutical and cosmetic formulations which aggresively degraded preservatives, disinfectants, surfactants, lipids, polymers and medicaments in simple mineral salts media or the formulations themselves. Within two subcultures on standard laboratory media (e.g. nutrient agar, potato-dextrose agar) this spoilage aggressiveness had, in a large number of instances, been irreversibly lost or dramatically diminished.

In order to obtain greater reproducibility of product testing between laboratories we suggest the need for the growth medium and method of inoculum preparation to be rigidly defined. MIC determinations should be included in the protocol for the test inocula. Confidence in the end result of the test could then be gained when the sensitivity of the test strains (MIC) falls between set limits for each preservative and test organism. We further recommend that if these tests are to be of significant value in predicting the behaviour of formulations to 'in use' challenges, then realistic contaminants and spoilage organisms should be used. They should be maintained in media designed to retain their spoilage potential. The behaviour of these organisms in preservative free formulations should be included in the test and consideration given to the inclusion of mixed inocula for challenges (e.g. see Yablonski 1972; Cowen and Steiger 1976; Beveridge 1975).

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