

Microbiological Standards for Nasal Solutions

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Received 12 January 1976 and accepted 1 July 1976

In Great Britain there are no official standards for evaluating the preservation of nasal solutions. The development of a multi-dose nasal solution containing 2% (w/v) sodium cromoglycate provided an opportunity for devising suitable standards and for evaluating the selected preservative system.

IN GREAT BRITAIN there are no official standards for the preservation of nasal solutions. Furthermore, manufacturers are reticent to publish data concerning the preservation of such products. In 1974, a nasal solution containing sodium cromoglycate, packed in a conventional plastic squeeze type bottle for the treatment of allergic rhinitis, was introduced on to the British market. The development of this product provided an opportunity to set suitable standards and to evaluate thoroughly the selected preservative system. In reporting the standards we set and the methods used to demonstrate that preservation is adequate, we hope to provide a yardstick by which other manufacturers can assess the performance of their own nasal solutions, leading to official recognition as to what suitable standards and test methods should be adopted.

Many tests have been published for determining the efficiency of preservatives, e.g. Rdzok *et al.* (1955), Bassett *et al.* (1970), Bernard (1973), Desvignes *et al.* (1974). However, prior to the XVIII revision of the United States Pharmacopeia, USP XVIII (Anon. 1970), which describes an antimicrobial agent's effectiveness test, there were no meaningful official criteria for evaluating the efficacy of preservatives in pharmaceuticals. The USP XVIII test was designed to apply to parenteral and ophthalmic products; in the following edition of the Pharmacopeia, USP XIX (Anon. 1975), the test remained virtually unchanged but was extended to nasal and otic preparations. Although the USP test has certain limitations (Leitz 1972), it does provide some guidance whereby pharmaceutical manufacturers can assess the antimicrobial effectiveness of their products.

It is generally agreed that microbial challenges are the only way to evaluate preservative effectiveness in final products (Eriksen 1970; Raiman 1970; Bernard 1973). However, such tests are time consuming and not all parameters can be fully evaluated, thus a certain amount of rationalization is necessary.

Since the USP XIX test is the only compendial standard for nasal solutions, compliance with this test was considered to be necessary. However we believe that to protect adequately both product and patient from microbial contamination further tests and standards should be adopted.

It was anticipated that the average time between drug administration would be approximately 4 h, and it was thus considered desirable to achieve a 99.9% reduction of

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the initial concentration of viable bacteria within this period. The 14 days permitted by the USP XIX test to achieve this kill appears to be far too long.

Artificially maintained type cultures, recommended by the USP, do not necessarily relate to organisms with which the product is likely to be challenged in use. The major source of contamination of a nasal solution is likely to be the nose of the patient. A range of isolates from the noses of healthy human volunteers should, therefore, be tested against the criteria outlined above. Similarly, since the major health hazard from a contaminated nasal solution is likely to be infection of the respiratory tract, the preservative system should adequately control contamination with micro-organisms that are regularly implicated in nosocomial respiratory tract infections. Laboratory preservative efficacy tests can only be regarded as a guide of preservative adequacy. To ensure a product is adequately preserved in practice, it is necessary to examine microbiologically several packs after use. It was therefore decided that the selected preservative system would be considered satisfactory if no evidence of microbial proliferation was found in any unused solution, following patient usage during clinical trials.

In developing a nasal solution of sodium cromoglycate (SCG), we examined a multitude of preservatives, both alone and in combination. Many of these were rejected on grounds other than their antimicrobial effectiveness, e.g. anticipated toxicity or allergenicity. We report here our results on our final choice of preservative system, i.e. 0.01% (w/v) benzalkonium chloride (BKC) plus 0.4% (w/v) 2-phenylethanol (PE).

Materials and Methods

Challenge test organisms

Type cultures of *Candida albicans*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were as specified in the USP XIX.

Performance test organisms

Organisms isolated from respiratory tract infections of patients in hospital were supplied by Leicester Royal Infirmary. Organisms from healthy human volunteers were obtained by nasal swabs of Fisons' employees.

Preparation of suspensions

Tryptone soya agar (Oxoid) slants were used for all bacterial cultures (+ 10% (v/v) defibrinated horse blood (Oxoid) for the streptococci) and malt extract agar (Oxoid) for the fungal cultures. Bacterial cultures were incubated at 30 °C for 24 h, yeast cultures at 25 °C for 48 h and moulds at 25 °C for seven days.

Suspensions of the test organisms were prepared from freshly grown slants using sterile isotonic saline (+ 0.05% (v/v) Tween 80 (Hopkin-Williams) for the moulds). The suspensions were then washed by centrifuging at approximately 3000 g for 10 min and resuspended in sterile isotonic saline.

A suspension of ca. 10^8 cells/ml was prepared using opacity tubes (Wellcome Reagents Ltd) for the bacterial cultures and a haemocytometer for the yeast and mould cultures. Viable counts were determined retrospectively by the pour plate method.

Preparation of nasal solutions

Aliquots of 18 ml 2% (w/v) sodium cromoglycate B.P. (SCG) solution with (a) no added preservative, (b) 0.01% (w/v) benzalkonium chloride USP, (BKC; Winthrop), (c) 0.01% (w/v) BKC and 0.4% (w/v) 2-phenylethanol, B.P.C. 1963 (PE; Fisons Scientific Equipment) were sterile filled by membrane filtration through 0.22 μ m cellulose acetate filters (Millipore) into pre-sterilized (ethylene oxide treated) polythene nasal spray bottles.

Incubation of test solutions

The suspensions (0.1 ml) were inoculated into sterile samples of test solutions packed in conventional polythene nasal spray packs using a sterile 1.0 ml disposable needle and syringe through the shoulder of the pack. The needle was then sealed to the nasal spray pack with a molten mixture of 1:1 w/v white petroleum jelly (Gretna Laboratories) and liquid paraffin (Fisons). The inoculation containers were incubated with needle and syringe *in situ* at 25 °C ($\pm$ 0.25). 1.0 ml aliquots were removed after 1, 4 and 24 h; and 4, 7, 14, 21 and 28 days and viable counts by the pour plate method carried out. All media contained lecithin and Tween 80 to inactivate the preservatives present. Lethen agar (BBL) was used for the bacteria (+ 10% v/v sterile defibrinated horse blood for streptococci) and malt extract agar for fungi. Bacterial cultures were incubated at 30 °C for three days and fungal cultures at 25 °C for five days.

Examination of nasal sprays after patient usage

Clinical trials using 'preserved' and unpreserved nasal solutions were conducted under close medical supervision. Returned, used nasal sprays were microbiologically examined by removing aseptically 1.5 ml of the remaining solution. 0.5 ml volumes were inoculated into (a) 100 ml Tryptone soya broth (Oxoid) containing 0.1% (w/v) lecithin and 0.7% (w/v) Tween 80 inactivators and incubated at 25 °C for seven days; (b) 100 ml Thioglycollate Broth U.S.P. (Oxoid) containing the above inactivators and incubated at 30 °C for seven days; (c) 4.5 ml of sterile isotonic saline from which viable counts of bacteria, yeasts and moulds were carried out as described previously. A sample was considered to be 'sterile' if no growth was recorded in either broth after incubation. Isolates were typed using standard microbiological methods described by Cowan & Steele (1965).

Results

The data presented in Tables 1–4 are representative of the results obtained. Table 1 reports the results of the USP XIX test. Some antimicrobial activity was recorded when 0.01% (w/v) BKC was added to the preparation but *P. aeruginosa*, after an initial drop in viable numbers, multiplied within the solution. Similar results were recorded when organisms isolated from nosocomial respiratory tract infections and from the noses of healthy human volunteers were used (Tables 2 and 3). Effective antimicrobial activity was recorded when PE was included.

TABLE I
USP XIX antimicrobial agents—effectiveness test

Organism	Preservative	Inoculum	Viable count*								
			Contact period (h)				Contact period (d)				
			0	1	4	24	4	7	14	21	28
<i>A. niger</i>	None	5.65	5.52	5.52	5.56	5.54	5.56	5.48	5.54	5.49	5.60
	BKC	5.65	5.57	5.49	4.92	3.86	3.15	2.00	<2.00	2.00	<2.00
	BKC + PE	5.65	5.64	5.49	4.73	2.30	<2.00	<2.00	<2.00	<2.00	<2.00
<i>C. albicans</i>	None	5.63	5.57	5.49	5.62	5.59	5.62	5.57	5.42	5.59	5.56
	BKC	5.63	5.66	5.37	5.15	2.72	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.63	5.57	5.53	4.91	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>E. coli</i>	None	5.86	5.84	5.76	5.57	5.36	4.99	5.11	4.99	4.96	4.92
	BKC	5.86	5.43	5.23	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.86	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>P. aeruginosa</i>	None	5.63	5.68	2.49	3.97	4.71	6.79	5.57	5.63	6.04	5.69
	BKC	5.63	5.67	<2.00	<2.00	<2.00	2.76	3.61	2.04	<2.00	4.62
	BKC + PE	5.63	5.15	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Staph. aureus</i>	None	5.56	5.38	5.36	4.89	2.04	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC	5.56	5.18	3.89	2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.56	5.55	4.53	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00

* Counts expressed as log₁₀ organisms/ml.

BKC, benzalkonium chloride 0.01% (w/v); PE, phenylethanol 0.4% (w/v).

TABLE 2
Performance test using organisms recently isolated from nosocomial infections

Organism	Preservative	Inoculum	Viable count*							
			Contact period (h)				Contact period (d)			
			0	1	4	24	7	14	21	28
<i>P. aeruginosa</i>	BKC	5.65	5.08	2.18	<2.00	4.30	5.08	6.00	5.70	5.84
	BKC + PE	5.65	5.64	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>E. coli</i>	BKC	5.60	5.47	3.34	2.40	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.60	5.59	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Klebsiella pneumoniae</i>	BKC	5.60	2.85	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.60	5.72	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Proteus mirabilis</i>	BKC	5.70	5.50	3.50	3.15	3.00	3.36	2.54	2.70	<2.00
	BKC + PE	5.70	5.50	2.42	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Staph. aureus</i>	BKC	5.65	5.04	3.59	2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.23	4.97	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
β -haemolytic streptococcus	BKC	5.11	5.24	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.11	5.62	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>C. albicans</i>	BKC	5.11	5.16	2.95	2.78	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.11	5.05	4.67	3.78	<2.00	<2.00	<2.00	<2.00	<2.00

* Counts expressed as $\log_{10}$ organisms/ml.

BKC, benzalkonium chloride 0.01% (w/v); PE, phenylethanol 0.4% (w/v).

TABLE 3
Performance test using organisms isolated from the noses of healthy human volunteers

Organism	Preservative	Inoculum	Viable count*						
			Contact period (h)			Contact period (d)			
			1	4	24	8	15	22	29
<i>E. coli</i>	BKC	5.60	4.30	3.90	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.60	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Proteus</i> sp.	BKC	5.70	3.48	3.30	2.00	3.60	2.70	3.04	3.15
	BKC + PE	5.70	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Gram negative rod	BKC	5.72	4.18	3.40	3.40	4.18	<2.00	<2.00	<2.00
	BKC + PE	5.72	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Staph. aureus</i>	BKC	5.15	5.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.15	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Staph. epidermidis</i>	BKC	5.48	3.48	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.48	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Gram positive rod	BKC	5.85	4.70	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.85	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
Gram positive coccus	BKC	5.70	5.76	4.40	2.48	3.50	<2.00	<2.00	<2.00
	BKC + PE	5.70	4.00	<2.00	<2.00	<2.00	<2.00	<2.00	<2.00
<i>Candida</i> sp.	BKC	5.40	3.18	3.00	2.00	<2.00	<2.00	<2.00	<2.00
	BKC + PE	5.40	3.18	3.00	2.00	<2.00	<2.00	<2.00	<2.00

* Results expressed as log₁₀ organisms/ml.

BKC, benzalkonium chloride 0.01% (w/v); PE, phenylethanol 0.4% (w/v).

TABLE 4
Microbiological examination of 'used' nasal solutions: returned samples from clinical trials

Sample no.	Total viable aerobic counts*			Sterility test	Major contaminants
	Bacteria	Yeasts	Moulds		
No preservative					
1	<10	<10	<10	Sterile	—
2	9.0×10^7	<10	<10	Not sterile	<i>E. coli</i>
3	3.5×10^7	<10	<10	Not sterile	<i>Pseudomonas</i> sp.
4	4.0×10^5	3.7×10^1	1.0×10^1	Not sterile	Mixed flora
BKC + PE					
1	<10	<10	<10	Not sterile†	—
2	<10	<10	<10	Not sterile‡	—
3	<10	<10	<10	Sterile	—
4	<10	<10	<10	Sterile	—

* Counts as organisms/ml of sample.

† Gram positive spore forming contamination.

‡ *Penicillium* sp. contamination.

BKC, benzalkonium chloride 0.01% (w/v); PE, phenylethanol 0.4% (w/v).

Table 4 shows the results obtained when nasal sprays that had been used in clinical trials were examined microbiologically. When a preservative was not included the remaining solution was frequently highly contaminated especially with Gram negative bacteria, whereas all solutions preserved with both BKC and PE were either sterile or contaminated with low numbers (< 10/ml) of spore forming bacteria or fungi.

Discussion

The need to preserve 2% (w/v) SCG nasal solution is clearly demonstrated by performing a USP XIX antimicrobial effectiveness test (Table 1), *E. coli* surviving in high numbers and *P. aeruginosa* growing in the preparation possibly by utilizing SCG as a carbon source. The incorporation of 0.01% (w/v) BKC into the preparation demonstrates that the USP XIX criteria could not be met. With *P. aeruginosa* there was an initial drop in viable numbers, but re-growth occurred between one and seven days. The inadequacy of preservation is even more pronounced when 'wild type' organisms are used (Tables 2 and 3). Clearly this preparation was inadequately preserved. Richards & McBride (1971) reported that PE enhanced the activity of BKC. By incorporating PE into the nasal solution, the USP XIX test could be complied with (Table 1). Furthermore the solution met our tighter standard of 99.9% reduction of the initial inoculum within 4 h for the bacterial species.

The USP XIX test is designed to evaluate preservative efficacy of a product in the original unopened container in which it is distributed by the manufacturer. We consider compliance with the test does not necessarily mean that a product is adequately preserved. Whilst the range of organisms stipulated in the test is broad, only *P. aeruginosa* has been frequently implicated in the spoilage of pharmaceutical products. Furthermore

the organisms have been maintained for several years under freeze dried conditions. The greater resistance to preservatives by freshly isolated organisms is evident from Tables 2 and 3. When BKC was used as the sole preservative the 'wild type' organisms in general were more resistant to the preservative than their type culture counterparts. Nevertheless our standards for antimicrobial efficacy could be met when PE (0.4% w/v) was incorporated into the preparation.

Eriksen (1970) has noted that conventional plastic nasal packs have a tendency to suck back; to simulate this effect in the laboratory was considered to be difficult. Since nasal secretions not only contribute micro-organisms but could inactivate the preservatives present, there is a need to establish that after use the product is not highly contaminated.

Several packs returned after use from clinical trials were examined. The nasal solutions preserved with BKC and PE were not grossly contaminated with micro-organisms (Table 4), thereby complying with the standards we adopted. Thus, contamination of this preparation during use appears to be adequately controlled.

In conclusion, we have demonstrated that high standards of antimicrobial efficacy can be met and that 2% SCG containing BKC 0.01% (w/v) and PE 0.4% (w/v) for use as a multidose nasal solution is adequately preserved. This does not necessarily indicate that this combination of preservatives is adequate in all nasal solutions. The development of other nasal solutions would similarly require extensive assessment of antimicrobial efficacy.

The authors wish to thank Fisons Ltd for allowing publication of this work, Mr J. E. Tillman and Mr J. H. Bell for helpful discussions and Mrs S. M. Stevenson for valuable technical assistance.

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