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An 'in-production' method for testing the sterility of infusion fluids

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SUMMARY

The addition of dehydrated broth powder to a random selection of bottles from each batch of infusion fluids before sterilization, followed by incubation of the bottles after sterilization, provides a method of sterility testing which possesses many advantages over the traditional method of culturing small samples from bottles after sterilization.

INTRODUCTION

The discovery of contaminated intravenous fluids at Plymouth in 1972 (Meers, Calder, Mazhar & Lawrie, 1973) focused attention on the need for scrupulous care in the supervision of production and sterilization techniques, and to a lesser degree on the need for sterility testing of the final product before it is released for use.

The Rosenheim Committee (Department of Health and Social Security, 1973) recommended that the recently introduced technique of membrane filtration in which a whole bottle of fluid is filtered, or the alternative method of injecting into the bottle a concentrated broth solution, should be considered by the Medicines Commission as offering more certain ways of detecting contamination than the traditional method of taking a small sample from a bottle and culturing it.

Whichever of these methods of sterility testing is used, a considerable amount of work is likely to be demanded from hospital bacteriology departments, wherever perfusion fluids are manufactured on a large scale in hospital pharmacies. To provide a method of sterility testing which (*a*) would not involve much extra work in terms of technicians' time or materials, (*b*) could be carried out by quality control staff in the pharmacy, (*c*) would not involve opening the test bottle or any other procedures by which contamination could be accidentally introduced, the following scheme was evolved.

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MATERIAL AND METHODS

Just before a batch of fluid was autoclaved, a number of bottles (determined according to the batch size) were selected and a weighed amount of dehydrated broth powder was added to each (14·75 g. of Thioglycollate Medium U.S.P. Oxoid CM 173 to 500 ml. of fluid). The bottles were then resealed and placed in the autoclave with the rest of the batch, in various positions in the load, the position being varied from batch to batch in accordance with a rotational pattern. A bottle immediately adjacent to each test bottle was specially marked. After the autoclaving process was completed, the test bottles were retrieved and incubated immediately. The incubated test bottles were inspected daily for 7 days, and any showing signs of turbidity referred immediately to the bacteriologist. Should any bacterial growth be detected, the marked bottle which stood next to the test bottle in the autoclave is available for examination. During 18 months' use of this technique only 1 bottle was referred to the laboratory for further examination, and this proved to be sterile.

Investigations

The main point which required investigation was whether the composition of the fluid to be tested, particularly with respect to sodium chloride and dextrose concentrations, would affect the growth-promoting properties of the broth when the mixture had been autoclaved. A short preliminary trial was conducted in 1973, in which organisms 1–9 (Table 1) were used to test distilled water, compound sodium lactate injection (B.P.), 0·9% saline, 4·3% dextrose-saline, and 5% dextrose solution, and organisms 1, 3, 6 and a 'wild' strain of *Proteus mirabilis* to test solutions of dextrose from 1 to 50% and solutions of sodium bicarbonate from 1·7 to 8·4%. This gave such encouraging results that it was decided to adopt this method of sterility testing in the main hospital pharmacy, while continuing the traditional methods for the products of a smaller pharmacy in another hospital, pending a full-scale trial. This was conducted early in 1974.

Full-scale trial

Twelve 500 ml. samples were taken of the intravenous fluids, or perfusion fluids for blood transfusion processes, normally made in the pharmacy in relatively large amounts. The necessary amount of broth powder was added and the bottles were autoclaved in the normal way. The resultant broth mixture then received an inoculum of a dilute suspension of each test organism, controlled by colony counts on blood agar plates (Miles & Misra, 1938). Each fluid was tested with 12 different organisms some of which were wild strains of common pathogens recently isolated from clinical specimens and some were strains isolated in Plymouth from the contaminated bottles. The organisms used are shown in Table 1.

Method of preparation of the suspensions

Overnight broth cultures of the test organisms were diluted 10^{-5} or 10^{-6} according to the results of preliminary tests. Three separate drops of each suspension were

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Table 1. *Code numbers and origins of the organisms used*

Code no.	Organism	Origin
1	*Staphylococcus aureus*	Wild strain
2	*Escherichia coli*	Wild strain
3	*Strep. faecalis*	Wild strain
4	*Bacteroides* sp.	Wild strain
5	*Clostridium welchii*	Wild strain
6	*Pseudomonas aeruginosa*	Wild strain
7	*Erwina herbicola*	H3516 Plymouth
8	Irregular *Enterobacter*	H3586B Plymouth
9	*Pseudomonas thomasii*	H03587B Plymouth
10	*Klebsiella aerogenes/oxytoca*	H3517C Plymouth
11	*Bacillus* sp.	Wild strain
12	*Neisseria catarrhalis*	Wild strain

delivered onto the surface of a blood agar plate for counting. The suspensions were diluted 1/5, and 5 drops of each suspension were added to each test bottle. In this way the number of organisms introduced into each bottle could be calculated. The aim was to produce an inoculum of approximately 5–10 organisms per bottle.

Preparation of test bottles

Perfusion fluids are prepared in the Southend Hospital pharmacy in a purpose built unit, recently modified to comply essentially with the requirements of the Guide to Good Pharmaceutical Manufacturing Practice (Department of Health and Social Security, 1971). Manufacturing is in a clean environment, in a positive pressure filtered air unit, final filling into pre-washed and steamed bottles being done within a laminar flow cabinet, the solutions being terminally filtered through 0·45 μm or 1·2 μm disposable filter pads. Pyrogens are kept within limits by use of distilled water from a thermal decompression still; total impurities of the water are not allowed to exceed 5 parts per million, and all solutions are made, bottled and sterilized within 4 hr. of the water distillation.

For the purpose of this series of tests, at the filling unit, bottles were filled as usual, plugged and capped, crated, and the crates placed in an autoclave loading trolley ready for the British Sterilizer spray cooled autoclaves. Each load consisted of 12 crates of 10 bottles. At this stage, autoclave tape was fixed to each crate, bearing batch and production identification references, and according to a rotational pattern, which sequentially monitors all regions of the autoclave, bottles were selected and marked for quality control and sterility tests, permanent storage for retrospective testing, and heat penetration (Browne's indicator tube inserted). Now 12 bottles were randomly selected, opened, and the requisite amount of thioglycollate medium added. These bottles were immediately re-plugged and capped, shaken, and placed back in the autoclave trolley after marking for identification. Viscose caps were fitted as usual over each bottle neck, acting not only as dust excluders during subsequent storage, but also as 'umbrellas' over the bottle necks to minimize the risk of the autoclave cooling fluid being drawn into the bottles via the neck seal at the early critical stage of the cooling process.

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Table 2. *Growth produced by test organisms in each fluid/broth solution*

	Code number of organisms used											
	1	2	3	4	5	6	7	8	9	10	11	12
Inoculum (colonies)	4	14	15	10	6	12	11	9	12	6	4	16
	Maximum growth* achieved and time taken in days											
Distilled water	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +1	+ +3
0·3% sodium chloride	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +3	+ +3
0·45% sodium chloride	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +1	+ + +2	+ +7	+ + +2	+ + +2	+ +3
0·9% sodium chloride	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +3	+ +3
1·8% sodium chloride	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +3	+ +3
1·4% sodium bicarbonate	+ + +7	+ + +2	+ + +2	+ + +3	+ + +3	+ + +7	+ +7	+ + +3	+ +7	+ + +2	+ +3	+ +3
2·7% sodium bicarbonate	+ + +7	+ + +2	+ + +2	+ + +3	+ + +3	+ + +7	+ +7	+ + +7	+ +7	+ + +3	+ +3	+ +3
8·4% sodium bicarbonate	+ +7	+ +7	+ +7	+7	+7	+3	+7	+ +7	+7	+ +7	+7	±7
5% dextrose	+ + +7	+ + +1	+ + +2	+ + +7	+ + +3	+ + +3	+ + +3	+ + +3	+ +7	+ + +2	+ +7	+ +3
10% dextrose	+ + +7	+ + +2	+ + +3	+ +7	+ +7	+ +7	+ +7	+ + +7	+ +7	+ + +2	+3	+7
20% dextrose	±7	+2	+3	±7	±7	+7	–	+7	– ±7	+2	±7	–
50% dextrose	–	–	±3	–	±7	–	–	–	–	–	–	–
4·3% dextrose/0·45% saline	+ + +7	+ + +2	+ + +2	+ + +7	+ + +3	+ + +3	+ + +3	+ + +3	+ +7	+ + +2	+ +7	+ +3
4·3% dextrose/0·18% saline	+ + +7	+ + +2	+ + +2	+ + +7	+ + +3	+ + +3	+ + +3	+ + +3	+ +7	+ + +2	+ +7	+ +3
5% mannitol	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +2	+ +3
10% mannitol	+ + +7	+ + +1	+ + +2	+ + +3	+ + +3	+ + +3	+ + +2	+ + +3	+ +7	+ + +2	+ +3	+ +3
20% mannitol	+ + +7	+ + +2	+ + +3	+ +7	+ + +3	+ +7	+ + +7	+ + +7	+7	+ + +3	+ +3	+ +3
5% laevulose	+ +7	+ + +2	+ + +3	+ + +7	+ + +3	+ + +3	+ +7	+ + +3	+ +7	+ + +2	+ +7	+ +7
25% laevulose	+7	+2	+7	±7	+7	+7	–	±7	–	+7	±7	–
Sodium lactate Co	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +2	+ + +2	+ +7	+ + +2	+ + +7	+ + +7
M/6 lactate	+ + +7	+ + +1	+ + +2	+ + +3	+ + +2	+ + +3	+ + +3	+ + +2	+ +7	+ + +2	+ + +7	+ + +7
Acid citrate/dextrose	+ + +7	+ + +1	+ + +3	+ + +3	+ + +3	+ + +7	+ + +7	+ + +2	+ +7	+ + +2	+ +3	+ +7
Glycerol sorbitol saline	–	–	+3	–	–	–	–	–	–	–	–	–
Sorbital saline	+ + +7	+ + +2	+ + +2	+7	+ + +7	+ + +7	+ + +3	+ + +7	+7	+ +3	+3	+3

* –, no growth; ±, growth just visible; +, light growth; + +, moderate growth; + + +, complete turbidity.

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After cooling, the load was removed from the autoclave, the main batch being sent on to the finishing area for normal processing – checking, quality control, bacteriology, labelling. The twelve test bottles for these investigations were extracted for the necessary laboratory procedures.

Incubation

The bottles were incubated at 36° C. and inspected at 18, 24, 48 and 72 hr. and at 7 days. The amount of growth at each inspection was recorded. Typical appearances of different degrees of growth are illustrated in Plates 1–3.

RESULTS

The results are shown in Table 2. The only solutions in which growth either did not occur or was seriously impaired were 50% dextrose, 20% dextrose, 25% laevulose, 8·4% sodium bicarbonate and glycerol/sorbitol/saline. It is therefore recommended that if strong dextrose or laevulose solutions are tested by this technique, a suitable volume of the solution be discarded from the test bottle and replaced by an equal volume of distilled water to give a final concentration of 10%. The particular formula of glycerol/sorbitol/saline used appears to be a self-sterilizing solution and therefore cannot be tested by this method. When 8·4% sodium bicarbonate is to be tested it is recommended that half the test bottles in each batch should be diluted to half strength.

DISCUSSION

After allowance is made for the large sampling error inherent in any method where a small percentage of any batch of bottles is tested for sterility, this method offers at least as good a safety margin as conventional methods in which a whole bottle is cultured. It also controls to some extent the sterilization process, in that most commercial dehydrated media may contain a varying number of bacterial spores and a sterile test bottle therefore shows that the time/temperature exposure was adequate to destroy these. The manufacturers of the dehydrated broth recommend that when it is reconstituted for normal use, the mixture should be allowed to stand for 15 min. before autoclaving. During this period any spores present would begin to germinate. However, when the medium is used for sterility testing the bottles should be autoclaved immediately, since any spores present in the original solution would be likely to germinate much more rapidly in the bottles containing broth than in those without broth.

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REFERENCES

Department of Health and Social Security (1971). *Guide to Good Pharmaceutical Manufacturing Process*. London: H. M. Stationery Office.

Department of Health and Social Security (1973). *Report on the Prevention of Microbial Contamination of Medical Products* (Chairman: Rosenheim). London: H. M. Stationery Office.

Meers, P. D., Calder, M. W., Mazhar, M. M. & Lawrie, G. M. (1973). The infusion of contaminated dextrose solution, the Devonport incident. *Lancet* ii, 1189.

Miles, A. A. & Misra, S. S. (1938). The estimation of the bactericidal power of the blood. *Journal of Hygiene* **38**, 732.

EXPLANATION OF PLATES

Plate 1

(Left) Irregular *Enterobacter* (8) in 5% mannitol, 18 hr. growth. (+, light growth.)

(Right) *Erwinia herbicola* (7) in 0·3% sodium chloride, 18 hr. growth. (+, light growth.)

Plate 2

The same two bottles as in Plate 1, positions reversed, after 48 hr. growth. (+ + +, complete turbidity.)

Plate 3

(Left) *Streptococcus faecalis* (3) in 10% dextrose, 48 hr. growth. (+ +, moderate growth.)

(Right) *Streptococcus faecalis* (3) in acid citrate dextrose, 48 hr. growth. (+ +, moderate growth.)

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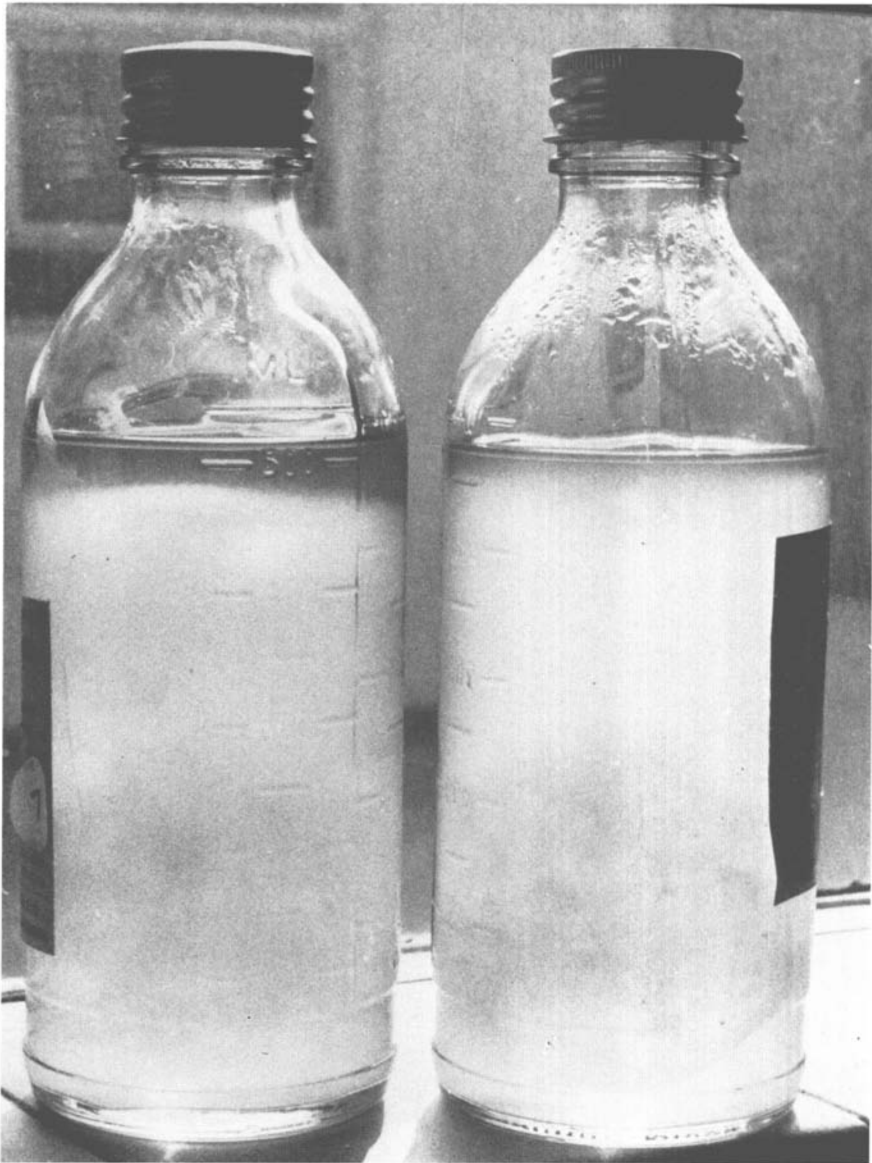

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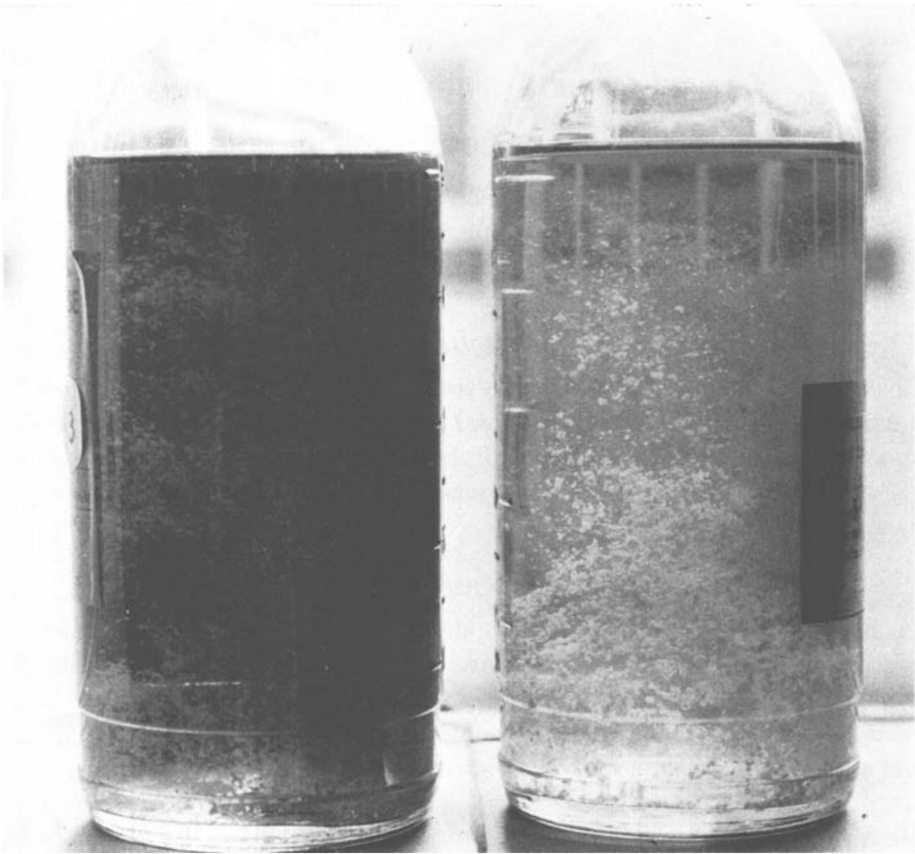

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