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The use of spore strips for monitoring the sterilization of bottled fluids

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SUMMARY

A bacterial spore test has been developed which enables the efficacy of the sterilizing cycle recommended by the British Pharmaceutical Codex (1973) for bottled fluids to be accurately monitored. During a 14-month period this test detected faults in 3·3% of the sterilizing cycles, representing five distinct episodes of sterilization failure that passed unnoticed by the conventional controls of physical measurements and sterility testing. There were no failures of sterilization as detected by conventional techniques which were not indicated by the spore test.

INTRODUCTION

The large sampling error inherent in any of the currently recommended methods for testing the sterility of a sample of a sterilized batch of fluids (European Pharmacopoeia, 1971) limits such procedures to detecting gross contamination only. Thus, in testing a 2% random sample of a batch of 1000 articles there is a 1 in 8 chance of failing to detect contaminants even when 10% of the articles are heavily contaminated (Rubbo & Gardner, 1965). Furthermore, the failure of a sterility test to detect viable bacteria does not mean that an adequate sterilizing cycle has been achieved. For example, there may not have been any bacterial contamination, or any bacteria that were initially present may have been heat-sensitive. Consequently, sterility tests cannot be used as a method of ensuring that the terminal sterilization procedure used was satisfactory. At present, control of the sterilization procedure is mainly dependent upon physical measurements which rely on the accuracy of thermocouples and the associated recording machinery. Unfortunately, it has been our experience over many years that, on occasions, the routine temperature recording charts have failed to demonstrate unsatisfactory sterilization cycles. In view of the limitations of existing techniques the feasibility of using bacterial spores to monitor the efficacy of sterilization of bottled fluids was investigated. The technique is described here, together with our experience using this test during a 14-month period.

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MATERIALS AND METHODS

Preparation of spore papers

As the temperature–time cycle used to sterilize bottled fluids was 115 °C for 30 min and appropriate spores were not available commercially, it was necessary to select a strain of *Bacillus stearothermophilus* of suitable heat resistance. Oxoid *B. stearothermophilus* spore strips (batch no. 3460223) were cut into three pieces, each of which was placed in 10 ml of tryptone soya broth in screw-capped bottles. One bottle was incubated at 56 °C directly, to act as a positive control, one was exposed to 115 °C for 20 min and the other to 115 °C for 25 min, and both were then incubated at 56 °C. The broths were examined after 7 days incubation. The spores that failed to grow after being subjected to 115 °C for 25 min but which grew after being subjected to 115 °C for 20 min were selected as those most likely to be of suitable heat resistance for the purpose of the study.

The positive control broth of each spore strip shown to have contained suitable spores was then plated out for single colonies on blood agar and incubated at 56 °C overnight. The strain was cloned twice, and an overnight broth culture in 10 ml tryptone soya was used to inoculate one Roux bottle containing the 'BS medium' of Williams *et al.* (1957). Each Roux bottle was plugged loosely with cotton-wool and incubated at 56 °C for 7–10 days. When a stained film showed good sporulation (over 70%) the growth was harvested by the method of Kelsey (1961) and stored at 4 °C until required.

The spore suspension was diluted to produce 10^5–10^6 spores per ml and was used to impregnate a small number (about 50) of Whatman's antibiotic assay disks, 6 mm in diameter, each of which absorbed 0·033 ml of water. The disks were placed in petri dishes in shallow layers and carefully flooded with the suspension. Excess liquid was removed, the disks (hereafter referred to as spore papers) were dried at room temperature and stored at the same temperature in the dark.

Preliminary testing of spore papers consisted of demonstrating that none survived 115 °C for 25 min, whereas at least 75% survived 115 °C for 20 min, and all papers incubated directly at 56 °C gave heavy overnight growth. If the spore papers appeared too heat-sensitive (or resistant), a second small batch was made using a heavier (or more dilute) suspension of spores.

If the primary batch of spore papers appeared to be suitable a large batch (about 500) of spore papers was made as before and similarly tested for suitability. If satisfactory the spore papers were stored at room temperature in the dark until required. Every month, each batch of spore papers was re-tested to check that it still fulfilled the above criteria.

Test of the sterilization procedure

Each morning spore papers were placed in sufficient bottles containing 1 l or 500 ml tryptone soya broth to allow the day's sterilization cycles to be monitored. Bottles of the same size as the product being autoclaved were selected and were sealed in the same way with rubber bungs and viscaps. With each sterilization

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cycle, two spore bottles were placed in the autoclave in positions which previous multi-channel thermocouple recordings had indicated attained the lowest temperatures. When special investigations of the autoclave were being undertaken a larger number of bottles, sometimes up to 6, were placed at different points. At the end of the day when all the sterilization cycles had been completed, the bottles of media containing the spores were incubated at 56 °C for 7 days. They were examined daily macroscopically for the presence of growth. The absence of visible growth by the seventh day was taken to indicate satisfactory sterilization.

Sterility tests

Sterility tests were carried out by aspirating 50 ml of the product and adding this to 500 ml of tryptone–soya broth. These were then incubated at 30 °C for 7 days and examined macroscopically for growth. If growth was observed, the organism was isolated and identified.

RESULTS

During the 14 months 1027 sterilizing cycles were carried out in four autoclaves and these were monitored using 2548 spore tests. Of the 2548 tests a total of 84 (3·3%) from 56 different sterilizing cycles, yielded positive results which could be grouped into five distinct episodes, as shown in Table 1. In each of these a failure of the cycle was finally demonstrated, there were no failures of the spore test which could not be explained.

The sterility tests which were carried out on the products being sterilized in these 56 cycles yielded a positive result in only one instance, this was of a *Bacillus* species and was from a bottle which was in episode 3 in Table 1.

DISCUSSION

Our experience using the bacterial spore test during a 14-month period is summarized in Table 1 which is largely self-explanatory. It is useful, however, to consider episode I in some depth as this proved to be the most complex encountered. Many failures of sterilization were indicated by the bacterial spore test, yet the temperature charts and even the 6-channel recordings (which are not standard practice) seemed satisfactory. Thus it appeared that both autoclaves were maintaining 115 °C for at least 30 min, therefore the spore papers were rechecked to ensure that they were not too heat-resistant. When these proved to be satisfactory and there were more failures indicated by the spore test, it was suspected that the 6-channel recorder might not be giving accurate readings. On recalibration by the manufacturers it was discovered to have been reading 2 °C too high. When it was returned both autoclaves were shown not to be attaining 115 °C.

The temperature-chart recordings proved to be quite inadequate on several occasions. In episode 3 the temperature charts used were so small that accurate interpretation was very difficult. On close scrutiny after the spore test failed several cycles the charts did seem erratic, and investigation of the autoclave

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Table 1. *Summary of spore tests yielding positive results*

Episode	Spore papers	Temperature charts	Fault
1	Failed 37 of 324 cycles		
	Failed many cycles over several weeks	Appeared quite normal: showed both autoclaves holding 115 °C for at least 30 min on each run	6-channel recordings on both autoclaves showed they were holding 115 °C for at least 30 min
	Spore papers re-checked and found to be quite satisfactory		
	Failed more cycles	Still appeared satisfactory	6-channel recorder found to be reading 2 °C high. After re-calibration, autoclaves shown to be holding only 113 °C 30 min
2	Failed 5 batches out of 6 from autoclave 1, in 2 days	Showed each run at 115 °C for 30 mins. at least	6-channel recording showed timer cutting in at 110 °C and significant temperature difference between top and bottom
3	Failed 12 batches out of 15 from autoclave 2	Very small charts (recording line spanned 2 °C) all seemed very borderline or erratic	6-channel recording showed timer was cutting in too soon, and temperature charts had ± 6 °C error
4	Spore bottle at bottom back of autoclave was positive	Normal	Sticking valve
5	Spore in 1 litre bottle on bottom shelf positive	Normal for 600 ml bottles	Heating up period allowed was correct for 600 ml but 4 min too short for the 1 litre bottles being sterilized

using the 6-channel recorder showed that there was ± 6 °C error on the temperature chart compared with the more accurate recorder and that the autoclave timer was cutting in too soon. In episodes 2 and 4 the charts were normal, although after the spore test indicated sterilization failures autoclave faults were quickly detected. The precise reasons for the misleading temperature recordings were not established. Episode 5 resulted from operator error which should have been detected had the temperature chart been scrutinized more closely in conjunction with the knowledge that the load was of 1-litre bottles.

It is obvious from our experience that the use of a biological indicator gives a more sensitive control over the sterilization process than sterility testing, the temperature-recording charts provided with the autoclaves and even the 6-channel

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recorder where the interpretation of the results depends entirely on the accurate calibration of the machine. Over 14 months the spore test has detected a variety of errors which would otherwise not have been noticed, with possibly disastrous consequences. Only one sterility test was positive from 1027 sterilizing cycles and, whilst this may reflect that the risk of serious contamination is very low, even with incomplete sterilization, there is no room for complacency when bottle fluids are being produced.

The spore test largely overcomes the problems of random sampling, accidental contamination and ineffective culture techniques inherent in sterility testing. It has the further advantage of using less labour and consequently is cheaper to perform.

There remains the possibility of the bottled fluids being contaminated after sterilization by the cooling water used in rapid-cooling autoclaves (Phillips, Eykyn & Laker, 1972). In this hospital, the cooling water of one autoclave is held at 80–90 °C until just before use and the other autoclaves utilize steam condensate in their cooling cycles. As an additional control, samples of cooling water from each autoclaves are monitored for sterility every week.

Since the development of this technique, the sterilization cycle has been altered to 121 °C for 15 min in accordance with present-day practice. Suitable spore strips are commercially available and we are currently using Oxoid BR 23 spore strips. These are tested before being issued to the pharmacy to ensure that they can withstand 121 °C for 12 min, as experience here shows that a considerable number of batches fail to meet these requirements.

In the *Guide to Good Pharmaceutical Manufacturing Practice* (1977) it is stated that 'the inclusion in the sterilizer load of containers of inoculated medium, which are incubated after sterilization, is not an adequate substitute for a test on the Finished Product'. Our results have demonstrated the insensitivity of sterility testing as an indicator of satisfactory sterilization of the product, and therefore the validity of this statement is questionable. We feel that the spore test represents a more reliable control of the sterilizing cycles and is a test that could easily become standard procedure and supersede the laborious and less-sensitive method of sterility testing the product.

REFERENCES

British Pharmaceutical Codex (1973). Prepared in the Department of Pharmaceutical Sciences of the Pharmaceutical Society of Great Britain and published by direction of Society's Council. London: Pharmaceutical Press.

European Pharmacopoeia (1971). **11**, 53.

Guide to Good Pharmaceutical Manufacturing Practice (1977), p. 47. London: H.M.S.O.

Kelsey, J. C. (1961). The testing of sterilizers. 2. Thermophilic spore papers. *Journal of Experimental Biology* **14**, 313.

Phillips, I., Eykyn, S. & Laker, M. (1972). Outbreak of hospital infection caused by contaminated autoclaved fluids. *Lancet* i, 1258.

Rubbo, S. D. & Gardner, J. F. (1965). *A Review of Sterilization and Disinfection*. London: Lloyd-Luke.

Williams, D. J., Franklin, J. G., Chapman, H. R. & Clegg, L. F. L. (1957). Methods of assessing the sporicidal efficiency of an ultra-high-temperature milk sterilizing plant. 1. Experiments with suspensions of spores in water. *Journal of applied Bacteriology* **20**, 43.

https://doi.org/10.1017/S0022172400025882 Published online by Cambridge University Press