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COMMUNICATIONS

A comparison of heat resistance in commercially available *Bacillus stearothermophilus* spore preparations used for monitoring steam sterilization

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Bacillus stearothermophilus spore preparations have been commercially available as biological indicators of heat sterilization processes for the last twenty years. During this period their use has increased and they have been recommended as adjuncts to, if not substitutes for, the conventional sterility testing procedures applied to medicinal products (Macek 1971; Brown & Gilbert 1977). This has resulted in their adoption as sterilization controls by the Nordic and United States pharmacopoeias. Before their inclusion in the U.S.P., Mayernik (1972) organized a collaborative study in which the spore preparations commercially available in the United States were compared. Of the five preparations examined only two were considered to be satisfactory although the performance characteristics of the autoclaves used by the participating laboratories varied substantially. In view of the recent discussions concerning the validation of autoclave cycles (Bain 1980) and the relative merits of spore preparations and a chemical temperature-time integrator (Line 1981), this present work was undertaken to determine whether the variations in heat resistance previously found to exist are still to be observed in the biological indicators currently available in Britain.

The spore preparations used are described in Table 1. These were obtained from the manufacturers who were unaware of their intended use, all were within the labelled expiry date and had been refrigerated before use. To avoid the danger of explosion of the glass ampoules resulting from excessively rapid temperature and pressure changes, the test pieces were heated in a conventional autoclave chamber rather than one designed specifically to minimize heating and cooling times.

Twenty test pieces of each type were simultaneously placed on a tray, covered within a thin layer of cotton wool and introduced into the empty chamber of a vacuum assisted autoclave. Copper-constantan thermocouples were placed around the tray and in the middle of the test pieces. The autoclave chamber was evacuated and steam rapidly introduced such that the temperature of 121 °C was achieved within 1 min and maintained throughout the 5 min exposure: the steam was removed from the chamber within 2 min. An identical temperature was recorded on each of the thermocouples throughout the cycle. These performance characteristics showed the autoclave to be of the type recommended by Meyernik (1972) for this purpose.

Where necessary the test pieces were transferred, within a laminar flow cabinet, to 10 ml (Oxoid) tryptone soya broth and incubated at 55 °C: after 7 days the number showing evidence of growth was recorded. 20 test pieces of each type were similarly exposed for 7, 9, 11, 13 and 15 min.

The proportions of test items showing evidence of survivors after the different exposure times are shown in Table 2: the test items bear a code letter so the order does not correspond to that in Table 1. It is evident that the spore preparations differ substantially in their heat resistance.

Those coded B appeared to be extremely heat sensitive and there were none showing growth even after only 5 min at 121 °C: unheated controls incubated at 55 °C nevertheless showed these to contain viable spores. The spores in preparation C were more heat resistant than the remainder although preliminary experiments (data not shown)

Table 1. Characteristics* of spore preparations examined.

Manufacturer and product name	Description	Test organism and nominal concentration (if stated)
Oxoid Spore Strips	Filter paper strips in glassine envelopes	NCIB 8919 (ATCC 12976)
Mast Stearothermophilus Spore Discs	Filter paper discs no envelope	NCIB 8919 (ATCC 12976)
American Sterilizer Co 'Spordi'†	Filter paper strips in glassine envelopes	$2{\cdot}9 \times 10^4$
3M Company 'Attest' Biological Indicator	Polypropylene vial + filter paper strip, culture medium with indicator	ATCC 7953 $< 10^5$
Becton Dickinson Co 'Kilit' spore strips	Filter paper strip with indicator in paper envelope	ATCC 7953
Beckton Dickinson Co 'Kilit' ampoules	Glass ampoule of spore suspension in culture medium with indicator	ATCC 7953

* Mast laboratories discs stated to have 50% showing survivors after 5 min at 121 °C and none after 10 min; the remainder stated to have survivors on all items after 5 min and on none after 15 min.
† Contains, in addition, spores of *B. subtilis var. niger.*

revealed no survivors after 20 min at 121 °C in several instances growth of survivors did not become evident until several days incubation had elapsed.

The standards of heat resistance claimed by four of the manufacturers are those recommended by the United States Pharmacopeia (U.S.P.) XIX for biological indicators. viz. all items having survivors after 5 min but none after 15 min exposure at 121 °C. The fifth manufacturer follows the recommendations of Kelsey (1961), i.e. 50% with survivors after 5 min and none after 10 min at 121 °C.

The results in Table 2 show that preparations A and E approach the U.S.P. specification but only F strictly conforms to it. All three, however, may be considered satisfactory in view of the sampling errors associated with low levels of survivors. An acceptable performance by three out of six preparations represents a slight improvement in reproducibility over that observed by Meyernik (1972), but it is clear that some spore preparations are still available which do not meet their own label claims, or alternatively, the U.S.P. specification, and thus cannot give adequate assurance of satisfactory sterilization.

Table 2. Number of test items* showing survivors after exposure to 121 °C for different times.

Exposure time min	Preparation A	B	C	D	E	F
5	20	0	20	4	19	20
7	20	0	20	0	16	20
9	20	1	20	0	16	20
11	10	0	20	0	0	18
13	2	0	16	0	1	0
15	2	0	14	0	0	0

* Out of a maximum of 20.

The problems associated with poor reproducibility of biological indicators are widely recognized and have led to a reluctance on the part of official bodies in Britain to support their widespread use (Rosenheim 1973; Guide to Good Manufacturing Practice 1977). Attempts to produce *B. stearothermophilus* spore suspensions of predictable and uniform heat resistance by growth and sporulation in both chemically defined (Lee & Brown 1975; Friesen & Anderson 1974) and complex media (Heinz et al 1976) have been reported. The results described here suggest that there is ample scope for further work to be conducted with these objectives in mind.

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Amitriptyline pharmacokinetics. Lentizol and ordinary amitriptyline tablets compared in a cross-over study of steady state plasma drug levels in depressed patients

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Lentizol, a sustained-release form of amitriptyline, was compared with ordinary tablets of the drug (Saroten) in a cross-over study of single doses in healthy subjects (Burch & Hullin 1981) the preparations being supplied by W. R. Warner (Pontypool, U.K.). Six depressed patients have now been treated with each of the formulations in turn and plasma drug levels in the steady state have been compared. Doses of 50 or 100 mg were given once daily, at 9 a.m. or 9 p.m. To avoid patients giving blood samples at frequent intervals during two days (or nights), neither the times of peak plasma drug levels, nor the heights of the peaks were measured. However, any slowing of the absorption of amitriptyline from the gut should result in higher plasma levels of the drug late in the interval between doses, provided that bioavailability is not reduced.

After 10 or more days administration of a constant daily dose, plasma levels of amitriptyline and of nortriptyline 12 h and 24 h after the dose were determined on several days for each patient on each formulation (Table 1).

Blood samples were treated and plasma concentrations of amitriptyline and nortriptyline were determined as described by Burch et al (1979). Values obtained on

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