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VIEWPOINT

Increasing the probability of sterility of medicinal products

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Calculations show that official tests for sterility offer unacceptably low degrees of assurance of sterility. Some batches of heat treated articles with 10% contamination can pass the E.P. test on about 92% of occasions. It is proposed that viable counts be made on products immediately before the terminal inactivation procedure and that upper limits be set for the level of contamination. Samples of the product should be inoculated with spores of known resistance characteristics and also with samples of swabs from the production area. Such inoculated products should then be tested for absence of viable organisms, after being subjected to the terminal inactivation procedure. This test should be coupled with close environmental and process control and personnel education. It is recommended that these procedures replace the conventional test for sterility. The use of spores as direct and independent indicators of sterility, especially where the lethal conditions cannot be monitored instrumentally is recommended. A flexible approach is proposed for the use of the 'lethality factor' suitable for a terminal heat inactivation procedure; this would depend on the nature of the product and the standard of monitoring facilities and personnel. A change in the language is proposed. A medicinal product processed such that an acceptable probability of sterility exists, should be designated not as sterile but as safe.

There still exists disagreement about the value of Official Tests for Sterility and of the procedures for assuring the quality of pharmaceutical products that have passed through a process intended to produce sterility. One main source of confusion is removed by making a distinction between the absolute nature of the concept of sterility and the degree of probability with which it may be known that a product is sterile. The degree of probability is not absolute and the best that is possible is that it is acceptably high. This uncertainty is an inevitable consequence of two main factors. Firstly, not only might the sterility test detect organisms introduced to the product during testing, but this difficulty is coupled with the impossibility of proving the absence of all life. To overcome this uncertainty factor it would be necessary to devise a test that not only detected all possible species of micro-organisms, in all potential physiological states with absolute certainty, but that did this without disturbing or altering the product in any way. Secondly, the logarithmic nature of the kinetics by which a population of micro-organisms die under the physical stresses associated with a sterilization procedure implies that increasing the stress increases only the probability of no survivors. With autoclaving, the main concern of this paper, certainty of no survivors would be associated only with an infinite heating time.

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OFFICIAL STERILITY TESTS

Statistical considerations

Sterility may be defined as the state of absolute freedom from all living micro-organisms. Any direct test for sterility must therefore be destructive in that information obtained from such a single test relates only to the state of the object under test at the time of testing and not after it. In the assessment of the sterility of a large batch of articles, a significant proportion must be tested and the results obtained extrapolated to apply to the remainder, assuming that the sample was representative. The final analysis is therefore statistical, in that all that can be determined is the probability with which the remaining articles would pass or fail the test. The probability of rejecting a batch of articles as a result of a sterility test depends upon the frequency with which the batch is contaminated and the number of samples taken for testing (Davis & Fishburn, 1948; Knudson, 1949; Tattersall, 1961).

$$\text{Probability of rejection} = 1 - (1 - \rho)^n$$

where ρ is the proportion of contaminated containers and n is the number of containers tested.

In the United Kingdom the official test for sterility is that described in the European Pharmacopoeia (E.P.). In this test, sampling procedures vary for different batch sizes and for aseptically prepared and terminally sterilized products. With batch sizes of less than 100 articles, 10% or 4 of them must be tested, whichever is the greater. With batch sizes of between 100 and 500, only 10 articles need be tested, and with batches greater than 500, 2% or 20 must be tested, whichever is the least. For preparations treated in an autoclave at a temperature higher than 100°, the number of containers taken for the test may be reduced to 10, and when the quantity in each of these containers is greater than 250 ml, this number may be reduced still further to three. The probabilities of rejection of single batches of different sizes and varying frequencies of contamination are outlined in Table 1. These probabilities are calculated on the basis of a single test with no re-test facilities, and no account is taken of the problems encountered with culture media. The statistics speak for themselves, but their implications are grave, especially with products terminally sterilized by heat. Here contamination could occur routinely with a frequency of less than 1%, yet the probability of detection remains negligible.

Table 1 has been calculated on the basis of a single test. In practice there is a retest facility which aims at preventing the failure of a product due to chance contamination by the person making the test (Kelsey, 1972). A batch may be tested, in all, up to three times and passing at any stage allows the entire batch to be considered as sterile. The first retest is permitted only if growth is thought to have arisen from a single microbial species, and the second retest if growth is due to a different single microbial species. In practice it is difficult to decide whether growth in a liquid culture is due to one or more species, without making further microbiological tests. This is a matter of considerable consequence with terminally treated products where contamination, if any, is likely to be due to the survival of small numbers of organisms. Even if organisms are detected, a retest would probably be allowed thus giving the batch a second chance of passing. If the number of contaminated containers was small then there would be a greatly increased chance of the batch being passed as sterile. This point is illustrated in Table 2 for different batch sizes, and for aseptically prepared and terminally treated products.

These figures are calculated on the assumption that contamination is due to one or two similar types of organism. For a batch size of 100 and a 10% level of failure a single retest decreases the probability of rejection by 23% from 0·653 to 0·420; that is the batch will now be passed as sterile 58% of the time. For lower levels of contamination and for terminally treated products these changes are even more dramatic, and the effect of a second retest could be to decrease the probability of rejection even further. Table 2 also shows the possible effect of testing only a small part of each sample container. E.P. conditions state that when the volume of liquid preparations exceeds 20 ml then only 10% of each sample need be tested. Although this practice is not generally adopted, the effects on the probability of rejection can be dramatic, particularly, as shown here, where only one or two viable cells are present in each container. This condition could prevail if testing immediately followed 'sterilization'. The effect of each of these factors is cumulative and reduces the probability of rejection of a batch of articles to negligible levels on a statistical basis alone.

Table 1. *The probability of rejecting a batch of containers as non-sterile by a single E.P. sterility test.*

		Probability of batch rejections with % of containers in batch which are contaminated						
Batch size	SS	0·1	1·0	2·0	5·0	10·0	20·0	50·0
40	4	0·004	0·039	0·078	0·185	0·344	0·590	0·937
100–500	10	0·010	0·096	0·183	0·401	0·653	0·893	0·999
1000	20	0·020	0·180	0·330	0·640	0·878	0·988	0·999
1000*	3	0·003	0·029	0·059	0·143	0·271	0·488	0·875

SS—Sample size.
* Heat-sterilized.

Table 2. *Probability of batch rejection with E.P. single retest and proportionate sampling.*

Batch size	SS	Probability of batch rejection with % of contaminated containers in the batch 0·1	1·0	2·0	5·0	10·0	20·0	50·0
(1) Retest								
100	10	0·0001	0·0090	0·0330	0·1600	0·4200	0·8000	0·9980
1000	20	0·0004	0·0324	0·1089	0·4096	0·7744	0·9760	0·9980
1000*	3	0·000009	0·00084	0·0035	0·0204	0·0734	0·238	0·765
(2) 10% Vol. sampled								
100	10	0·0010	0·0100	0·0198	0·0489	0·0960	0·183	0·401
1000	20	0·0020	0·0200	0·0392	0·0953	0·1800	0·330	0·640
1000*	3	0·0003	0·0030	0·0059	0·0149	0·0290	0·059	0·143

SS—Sample size.
* Heat-sterilized.

Non-statistical considerations

In the calculation of these statistics the assumption has been made that if any single viable organism reaches the test media then growth will occur regardless of the organism's state or type. In practice this is not so. There are numerous reports in the literature of organisms failing to grow in typical sterility testing media (Kelsey, 1972; Bühlmann, 1971) and these are generally regarded as being far from universal growth conditions. Elaboration of the type and number of media would be necessary if a test were required to detect a wider range of species. The temperature of incubation will also affect growth, 30°–32° being a compromise for bacteria, in that it is aimed at detecting organisms with optimal growth temperatures of between 25°–35°. However, many thermophiles and psychrophiles will not be detected at these temperatures. A similar situation exists for the fungal media which are incubated at 22°–25°. The E.P. specifies that test samples be incubated for a period of seven days for fungi and two days for bacteria before readings are made. Generally this length of time would be adequate, however some types of spores and slow growing organisms, especially those starting from small inocula, might not give visible growth within that period.

Damaged micro-organisms often require meticulous conditions in which to survive and grow (Harris, 1963; Brown & Melling, 1971) yet might remain hazardous in a product. Many products are antimicrobial because of added preservatives or the activity of the agent itself, both must be inactivated before a test for sterility is made. Most commonly the agent is diluted out, but sometimes specific inactivators are added. These will have the effect of further reducing the variety of micro-organism capable of growing in the media. Yet this effect might not be detected by the test organisms which are supposedly picked for their sensitivity to the inactivated agent.

Thus the statistical and to a lesser extent the microbiological problems (Rawlins, 1977) embodied in official sterility testing procedures prevent these from offering an acceptable degree of quality assurance, especially for low levels of contamination. In practice the tests will probably detect only gross contamination which might result from total failure caused by broken equipment, faulty gauges or even omission of the sterilization procedure. If a test were designed specifically for the purpose of testing gross failure, it would certainly not take the form of the E.P. test for sterility since this is both inefficient and expensive. Indeed, the official test for sterility may be dangerously misleading in that a false sense of security may arise when a product has passed (Clothier, 1972). This will happen on as many as 92% of occasions for an E.P. test on heat sterilized loads of 1000 articles when 10% are contaminated and on about 23% of occasions when as many as 50% are contaminated (Tables 1 and 2). These criticisms are by no means unique to the E.P. test for sterility, and apply to varied extents to most pharmacopoeias. The United States Pharmacopeia accepts these limitations by interpreting the results of sterility tests with reservations, confidence in the results of the tests being based upon the knowledge that the batch has been subjected to an inactivation procedure of proved effectiveness. Added assurance in the quality of the product is gained here by the use of biological indicators in inoculated products and as inoculated carriers.

With aseptically prepared medicines the inadequacies of a sterility test has to be accepted since

there is no better alternative. However, with products that are terminally treated, alternative means of assessing the probability of sterility exist and must be considered. In such instances it is possible that some elaboration of control procedures could make the existing official sterility test superfluous.

PROCESS CONTROL

Quantification of microbial elimination

There seems to be general agreement that the death of a bacterial population under physical stress approximates to a logarithmic function (Brown & Melling, 1971). Decimal reduction times indicate the time taken under constant and defined lethal conditions for the viable population to be reduced by 90%. These times vary from species to species, but they allow the calculation of inactivation conditions for known bacterial populations. Any additional factor of inactivation will increase the probability of sterility being attained. Thus the advantages may be seen of reducing the contamination level in a product before a sterilization procedure, by preparation in aseptic areas with environmental control. Also, if the level and type of contamination is known then provided that the sterilization procedure itself can be adequately monitored, an inactivating dose sufficient to produce an acceptable probability of sterility in the final product can be selected and used. Indeed, if it is accepted that a process of reducing the probability of viable micro-organisms is the main concept then this process includes all procedures that reduce the microbial count before the final released product. It might therefore be useful to distinguish autoclaving, for example, as the most critical *procedure* among other procedures that constitute the entire *process* from raw materials to released product. The critical factors are the control and monitoring of the raw materials, especially water, of the environment and in-process control, of the terminal inactivation procedure and of the competence and continuing education of personnel (Fig. 1).

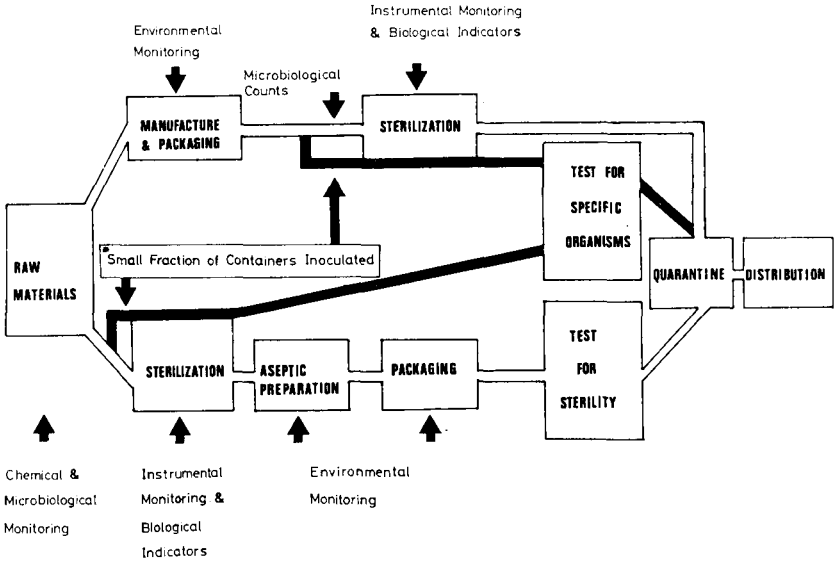

FIG. 1. Proposal for in-process control and monitoring of a typical production procedure. (* samples of the product inoculated with known spores and samples of typical known contaminants of the production environment).

In the United Kingdom the Guide to Good Manufacturing Practice already does much in specifying the type and degree of environmental control necessary for the production of heat sterilized fluids. These conditions go a long way in ensuring that the level of contamination in the product is kept to a minimum but they do not define that level. Neither do they ensure that the product is sterilized immediately after manufacture, yet storage greatly increases the potential level of contaminants.

We suggest that quality control procedures on raw materials ought to involve some form of microbiological counting and that the product itself should have a microbiological count immediately before

terminal inactivation. The treated product then ought to be kept apart to await the results of these tests. Upper limits should be set for the initial numbers of bacteria allowable in the products, in the knowledge of the effectiveness of the final inactivation procedure. This kind of microbiological monitoring would not suffer from the same drawbacks that occur with the official tests of sterility, since it would involve the assessment of the number and type of contaminants rather than the absolute detection of a single organism.

Monitoring the terminal inactivation procedure

Monitoring of the inactivating procedure is crucial. The critical lethal environmental conditions for microorganisms have been and continue to be ascertained in the research laboratory. The purpose in the production unit is to check if these predetermined optimal conditions have been achieved during the inactivation procedure. The problems of monitoring, particularly with autoclaving, are considered in a recent hospital technical manual (Manual, 1977). In general the critical lethal conditions for inactivation are as follows:—radiation—dose and time, wet heat—temperature, time and humidity; dry heat—temperature and time; chemical (solution)—concentration, time and temperature; chemical (gaseous)—partial pressure, time, temperature and humidity. Time, temperature and radiation dose can be measured instrumentally. Humidity cannot be monitored directly, but the Department of Health and Social Security (D.H.S.S.) recommended procedures (Manual, 1977), if adhered to, do much to eliminate any problems of steam quality. The partial pressure of lethal gases cannot be monitored. Biological indicators such as spore strips would therefore seem to have an important role where lethal gases are used and possibly a secondary role in autoclaving, especially for loads such as dressings.

Given, that official sterility tests detect only relatively gross contamination resulting from serious failure of the sterilization process, what, if anything, should replace them? Manufacturers do not rely exclusively on E.P. sterility tests as the sole indicator of quality assurance. However, we believe that for heat sterilized fluids adherence to the new rigorous control process recommended by the U.K. D.H.S.S. (Manual, 1977) and the U.S.A. Food and Drug Administration (Reports, 1976a, b) would inevitably reveal not only gross failure of the sterilization process but also relatively minor failings. The D.H.S.S. have recently relaxed the requirements for E.P. Sterility Tests under certain controlled conditions.

Microbial inactivation by radiation is well researched and lends itself to instrumental monitoring and control (Tallentire, Dwyer & Ley, 1971; Tallentire, 1973). It is significant that devices irradiated under controlled conditions are not normally required to pass a sterility test. Nevertheless if a *direct* and independent test of a terminal inactivation procedure is required by regulatory authorities perhaps for legal reasons, then the following would be effective and relatively inexpensive. Samples of the product should be inoculated with known spores and also with preparations of typical contaminants taken when routinely monitoring the microbial contamination of the production unit. After the terminal inactivation procedure products should be tested for absence of these micro-organisms. We therefore propose that the current official test for sterility be replaced by testing the sterility of products known to be contaminated with appropriate numbers of known spores and with samples of typical known contaminants from the production environment. (Fig. 1).

In the U.K. there is official reserve about spore preparations on the grounds of lack of reproducibility (Rosenheim, 1973). In our view, further work on spore strips is required but sufficient is already known (Cook & Brown, 1965a, b; Smith, Pflug & Chapman, 1976; Miller, 1971; Lee & Brown, 1975; Hodges & Brown, 1975) to justify the use of spore preparations. Firstly, they are useful where it is not possible to monitor lethal conditions instrumentally and here spore strips exposed, for example, in glassine envelopes can be used. Secondly, spore preparations could be used to inoculate samples of the product immediately before the terminal inactivation procedure with subsequent tests for sterility. Thirdly, spore preparations offer a *direct*, independent and simultaneous measure of all the critical lethal conditions.

An acceptable probability of sterility

What is an acceptable probability of sterility in the final product? A recent amendment to the Nordic Pharmacopoeia (1970) states that sterile drugs must be prepared and sterilized under conditions which aim at such a result that in one million units there will be no more than one living micro-organism. Discussion of this question has focussed mainly on the terminal inactivation procedure itself, and on various proposals made regarding numbers of decimal reductions of various

standard preparations of bacteria. There is perhaps a dubious assumption beneath the vexatious disputes between individuals and national regulatory authorities. The assumption seems to be that there is a standard number of decimal reductions of a potential population which constitutes safety. We deny the existence of such a number since the nature of safety is incorrigibly relative. Also, there has been dispute about suitable standard micro-organisms. There has been a tendency to use the most resistant species available. This has escalated as increasingly resistant strains are discovered; the possibility of spores trapped in crystals is another stage further.

We propose that the search be abandoned for the 'Holy Grail' of one standard number of decimal reductions of the most resistant micro-organism in its most resistant state. For medicine (as opposed to food) the following considerations seem relevant. Danger comes from pathogens. These are either vegetative cells or mesophilic spores. With vegetative cells the use of the data for *Pseudomonas aeruginosa* (Brown & Melling, 1971) heated at 50° in aerated broth and assuming a Z value of 10 shows that 1 min at 120° would reduce a theoretical population by about 10^6 log cycles. There is a well documented incident (Clothier, 1972) where about a third of a batch of infusion fluid was found contaminated with coliform organisms after 'autoclaving' and passing routine tests. It is hardly surprising that among many failures was one of monitoring and control. With pathogenic mesophilic spores 1 min at 120° for anaerobic *Clostridium botulinum* A and B and also for aerobic *Bacillus subtilis* would result in about 5 to 10 decimal reductions (Ingram, 1969). The U.S. Food and Drug Administration has recently established (Reports, 1976a, b) for large volume parenterals a 'sterilization procedure standard' described as a lethality factor (F_0) of 8. F means the equivalent amount of time in minutes at 121° (or 250°F) for which the product has been subjected to the sterilization procedure. In terms of inactivating the above pathogens the vegetative micro-organisms are irrelevent and the spores at 121° would have about 48 to 96 decimal reductions.

Clearly, solutions for intravenous injection are potentially more hazardous than other medicaments. Also, as spore resistance is influenced by many factors, some flexibility in official monographs is desirable. The FDA flexibly allows lower F_0 values than 8 under special circumstances such as drug instability. The U.S. Health Industry Manufacturers Association (Report, 1976b) has expressed the view that an F_0 of 8 is arbitrary and propose no standard lethality factor. The U.S. Pharmaceutical Manufacturers Association (Report, 1976b) has proposed an F_0 of 4. In our view the only rational approach is a flexible one. The model situation is that of irradiation where an accurate dose can be given and monitored as such. The available recorded evidence on failure shows clearly that both the monitoring of autoclaving and the training of personnel are crucial. In those situations where there is excellent monitoring of excellent facilities and trained staff are available, then a lethality factor of 4 on a relatively microbe-free product offers odds of about (10^{24}–10^{48}) to 1 against the survival of a mesophilic pathogenic spore. Surely the problem is not the 1 in 10^{24} risk of a pathogenic spore but rather the assurance that the conditions which produce such astronomic odds have actually occurred.

Perhaps a fraction of the money currently being wasted on the official sterility testing programmes could be spent where it is needed on monitoring the process, educating the personnel and better equipment.

The concept of sterility is absolute. Whether or not a medicinal product is sterile is inevitably a matter of probability. Consequently we propose that a product processed such that an acceptable probability of sterility exists, should be described not as sterile but as safe for its designated use.

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