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Microbial Contamination on Disposable Hypodermic Syringes Prior to Sterilization by Ionizing Radiation[1]

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A large number of syringes were taken from the production lines of three independent manufacturers; the numbers and types of microorganisms contaminating these randomly sampled syringes were assessed in the laboratories maintained by each of these manufacturers for routine sterility testing, according to a standard protocol devised by the Research Committee of the UK Panel on Gamma and Electron Irradiation, which coordinated the investigation and analyzed the results. Items produced by a manufacturer were assessed for microbiological contamination both in their own laboratories and in the laboratories of the other manufacturers. The level of "false-positive" results was determined independently for each laboratory by the testing of "known sterile" items which had been subjected to the radiation-sterilization process. Both the percentage of syringes initially sterile and the average number of organisms per contaminated syringe differed among the three manufacturers. When corrected for interlaboratory differences, the number of syringes initially sterile ranged from 16 to 48%, and the mean number of organisms per contaminated syringe was 20 to 70. Of 964 syringes tested by all three laboratories, only one contained over 1,000 aerobic organisms (1,133). The most common organisms found were coagulase-negative, gram-positive cocci. Two manufacturers assessed contamination by anaerobic organisms; of 610 syringes, 1 contained 4,275 organisms and 3 more had 100 to 1,000 organisms, but 488 (80%) were uncontaminated by anaerobes. The results are discussed in the context of the choice of radiation dose necessary for the sterilization of medical products manufactured under controlled hygienic conditions.

The usefulness of conventional microbiological sterility testing of products *after* they have been exposed to a sterilizing process has been seriously challenged in the past (2, 3, 5, 6, 9, 10). It has been pointed out that more useful information about the margin of safety inherent in any sterilizing process, whether steam, gas, or radiation, must be obtained from: (i) knowledge of the level and type of microbial contamination on the items to be sterilized *before* they have been exposed to the sterilizing process; (ii) the physical control of the parameters of the sterilizing process itself; and (iii) the information obtained in model systems of the efficiency of the sterilizing process for the various microorganisms found on the items to be sterilized, under various environmental conditions.

The present study was undertaken to ascertain whether reliable information on the level and type of presterilization microbial contamination on plastic disposable hypodermic syringes could be obtained economically within industrial microbiological laboratories accustomed to routine sterility testing. The syringe was chosen as a model test object because two of its components, the barrel and the plunger, are sterile when they emerge from the high temperature of the moulding process but are then subjected to accidental contamination from handling during assembly and packing, as well as to possible

[1] A publication of the Research Committee of the UK Panel of Gamma and Electron Irradiation. Members of the Committee are as follows: A. M. Cook (Chairman), R. J. Berry (Technical Secretary), R. W. H. Cook, C. G. Crawford, J. F. B. Dealler, J. O. Dawson, R. S. M. Frohnsdorff, G. E. Gale, J. C. Kelsey, F. J. Ley, P. J. Lindop, and K. Tattersall. The manufacturers paricipating in this study (who are all Full Members of the UK Panel on Gamma and Electron Irradiation) were Gillette Surgical Ltd., Reading, Berks.; Johnson and Johnson (Great Britain) Ltd., Slough, Bucks.; and Smith and Nephew Research Ltd., Harlow, Essex.

contamination from the material of the grommet which seals the plunger in the barrel.

Strict control of the physical parameters (irradiation dose) for the sterilization of disposable medical products by ionizing radiation has been required for products acceptable to the UK Ministry of Health since this process was first used commercially in the UK in 1960. In addition, dose-response data for survival of microorganisms of many types irradiated under a wide variety of environmental conditions have been reported by many authors (e.g., 1, 8). It was hoped that the present study would contribute information which would have a significant bearing on the validity of the somewhat arbitrary choice of sterilizing dose which has been found to be satisfactory for a wide range of medical products on the basis of the extensive practical experience gained over the past 7 years in the UK.

The participating laboratories were those of three large industrial firms manufacturing disposable medical products. These firms bore all the costs of the microbiological examinations performed. The experimental protocol was drawn up by, and the results were collated and analyzed by, the Research Committee of the UK Panel on Gamma and Electron Irradiation.

MATERIALS AND METHODS

The following experimental protocol was adopted; the microbiological techniques were modified from those recommended in 1962 by Pashley (7).

Sampling. Syringes were drawn from the production line at random immediately after packing but before sterilization. Two samples were taken each time, and a minimum of 10 samples were taken per week from each participating manufacturer. The samples were identified on the package as to time and date at which they were taken. Samples thus drawn were mailed to the respective microbiological laboratories.

Microbiological techniques. Wide-mouth screw-capped bottles or boiling tubes containing 30 ml of sterile one-fourth strength Ringers solution plus 0.1% Tween 80 (culture grade) were used. Oxoid Tryptone Soya Broth (CM 130) plus Oxoid Agar No. 3 (CM 49) was prepared from tablets and dispensed in 9-ml amounts (double strength). Oxoid Reinforced Clostridial Agar was prepared from granules (CM 151) or tablets (CM 152) and dispensed in 9-ml amounts (double strength).

The media, bottles, petri dishes, etc., were sterilized by autoclaving (10 psi, 20 min).

Syringes to be tested were disassembled in the pack, and the components were transferred aseptically to bottles or boiling tubes containing the Ringers solution (one syringe per bottle or tube). The rubber grommet on the syringe plunger was not separated from the plunger. Bottles or boiling tubes containing the syringes were shaken vigorously for a minimum of 2 min. Two 9-ml portions of the Ringers solution recovery medium were taken from each bottle and transferred to sterile petri dishes. A 9-ml amount of double-strength molten agar medium at 50 C was added to each petri dish; the plates were mixed by rotation, and care was taken to prevent solidification of the agar before adequate mixing had been achieved. The plates were dried for 40 min and then incubated at 32 C. Counts were made of the plates after 24 and 48 hr of incubation. In two of the participating laboratories, a third 9-ml portion of the Ringers solution recovery medium was used for the preparation of an anaerobic culture; the anaerobic cultures were incubated similarly to the aerobic cultures, but in appropriate anaerobic jars. The remaining Ringers solution recovery medium was stored at 4 C overnight, so that diluted pour plates could be made if the 24-hr growth on the full-strength pour plates was excessive.

Gross colony morphology was noted, and Gram stains were performed on surface colonies of each morphological type seen. Colonies of gram-positive cocci were subcultured on Mannitol Salt Agar (Oxoid CM 86) or tested for coagulase production by any of the standard methods.

Preparation of heavily contaminated test syringes. In addition to the routine microbiological assessment of presterilization contamination levels described above, on at least four occasions within a period of 1 year, each participating firm placed three or more syringes (size, 2 to 5 ml), taken at random from the production line and unwrapped, in cardboard trays with the barrel and plunger separated. These were left in the factory in a location which was thought to have the highest level of dust (e.g., window-sills, etc.) for 1 week. They were then sealed in clean polythene bags, identified by time and place of collection, and marked "dust, heavily contaminated." At the same time, an additional three or more syringes were hung in the lavatories for 1 week, and the staff was instructed to handle them before washing their hands. These were then packed as above and identified as "lavatory, heavily contaminated." All syringes were then sent for microbiological examination.

RESULTS

Over a period of 15 months, 964 syringes taken from production lines after packing but before sterilization were examined; the cumulative data are shown in Fig. 1. Of these syringes, 857 were assessed by the laboratories of their own manufacturer; 225 (23.4%) were found to be sterile and on the remainder a total of 30,247 colonies of aerobic microorganisms were counted, a mean of 40.9 organisms per contaminated syringe. Only one syringe was contaminated with more than 1,000 aerobic organisms (1,133), 16 (1.7%) had 301 to 999 organisms, and 48 (5.0%) had 100 to 300 organisms. Thus, 93.3% of all syringes, and 91.4% of those syringes accidentally contaminated during the process of manufacture had fewer than 100 aerobic organisms on them before being subjected to the radiation-sterilization process.

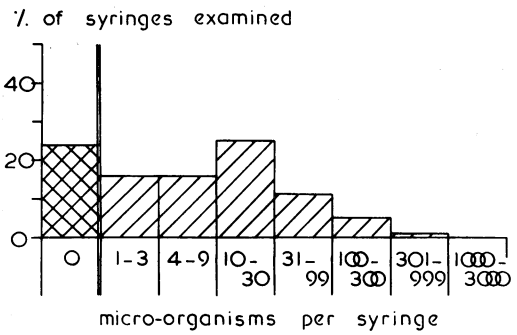

FIG. 1. *Microbial contamination of 964 syringes taken from the production lines of three manufacturers over a 15-month period.*

Two manufacturers also assessed contamination by anaerobic organisms. Of 610 syringes examined, one was contaminated with 4,275 organisms; however, only three more syringes (0.5%) had 100 to 1,000 anaerobic organisms, and 488 (80%) were totally uncontaminated by anaerobes.

Sampling. The number of syringes taken from the production lines each month for microbiological examination was somewhat inconstant through the 15-month experimental period. Sufficient data were available, however, from two of the three participating manufacturers, to assess the effect of seasonal variation upon the proportion of syringes initially sterile, and upon the magnitude of contamination observed (Fig. 2). The only point of similarity in the variation of percentage of syringes initially sterile (Fig. 3) is occurrence of the minimum in both laboratories during the spring (April-May), and the occurrence of the maximum during the summer months. The mean numbers of organisms per contaminated syringe, however (Fig. 3), show markedly similar seasonal trends, with high counts in the winter months (when the incidence of upper

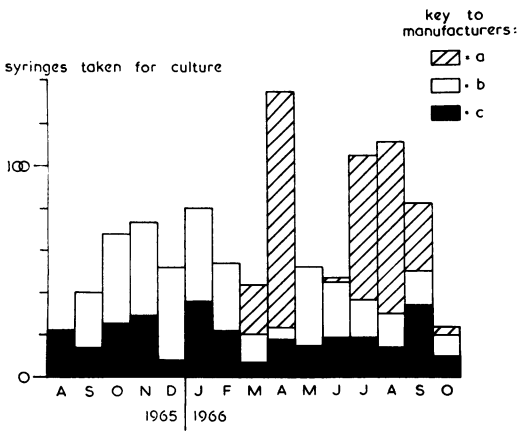

FIG. 2. *Syringes taken for culture during the 15-month experimental period.*

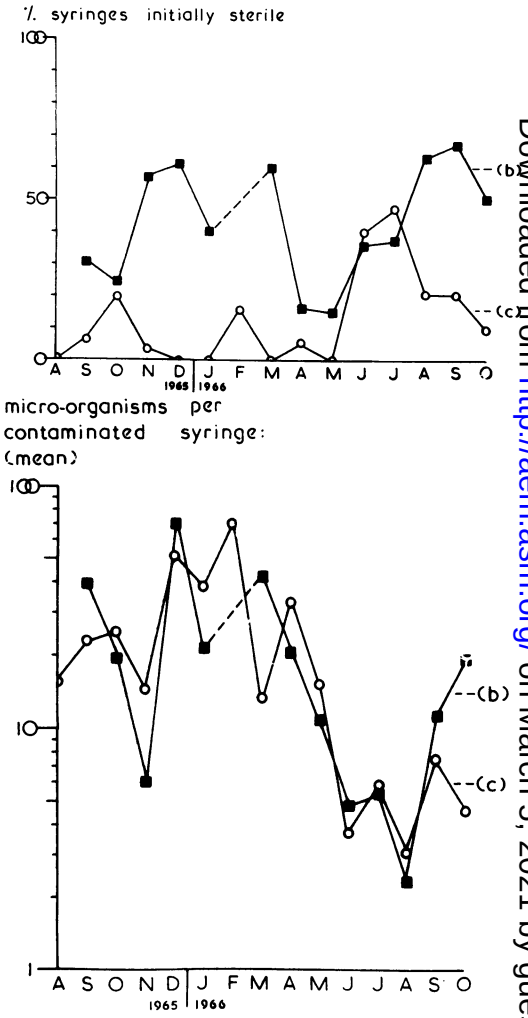

FIG. 3. *Seasonal variation in the proportion of syringes initially sterile and in the mean number of organisms per contaminated syringe.*

TABLE 1. *Microbiological examination of "known sterile" syringes*

Laboratory	No. of syringes examined	No. of syringes sterile	No. of colonies counted	Mean no. of microorganisms per contaminated syringe
a	30	26	5	1
b	31	27	8	2
c	52	22	87	3

TABLE 2. *Percentage of syringes initially sterile*

Manufacturer	Laboratory	Percentage of syringes initially sterile	
		Uncorrected	Corrected
a	a	14.0	16.2
b	a	27.3	31.5
b	b	42.0	48.4
b	c	20.4	48.2
c	b	33.3	38.3
c	c	14.4	33.9
All	All	23.4	32.8

TABLE 3. *Occurrence of contaminating microorganisms on syringes*

Type of organism	Manufacturer b		Manufacturer c	
	Laboratory b	Laboratory c	Laboratory b	Laboratory c
Gram-positive cocci				
Coagulase-negative				
Colony type: White	18[a]	42	27	21
Yellow	3	7	0	3
Buff	31	7	18	10
Total	52	56	45	34
Coagulase-positive				
Colony type: White	7	0	3	0
Yellow	1	0	0	0
Buff	20	0	3	0
Total	28	0	6	0
All gram-positive cocci	80	56	51	34
Rods				
Gram-negative	3	0	3	1
Gram-positive				
Spores present	4	7	21	10
Spores absent	6	4	3	14
Total	10	11	24	24
Diphtheroids	2	11	6	6
Fungi and yeasts	3	4	10	8
Anaerobes	3	20	6	26

respiratory disease is high) and dramatically lower numbers of organisms per contaminated syringe during the "healthy" spring and summer months. For these data, each laboratory provided its own control over differences in microbiological technique.

Interlaboratory variation. Each participating laboratory examined a group of syringes which had been exposed to the radiation-sterilization process ("known sterile" syringes) to establish the level of "false-positive" results to be expected, due to accidental contamination occurring in the microbiological laboratory itself. These data are shown in Table 1. The procedures employed in this study were sufficiently more complex than those used in routine sterility testing that the relatively high level of "false-positive" results was not unexpected, even in "good" industrial laboratories. When the incidence of syringes found to be uncontaminated was corrected for the level of "false-positive" results in each of the laboratories, clear differences among the three manufacturers became evident in the percentage of syringes produced "sterile" prior to irradiation. These differences were verified by the cross-assessment of syringes of one manufacturer in the laboratory of another (Table 2).

Figure 4 shows the differences in numbers of aerobic organisms per syringe obtained by the laboratories of each of the three manufacturers examining their own syringes. These data are not corrected for "false positives." It was orginally hoped that these data represented real differences between the individual manufacturers, but the data in Fig. 5 show that, on cross-assessment of

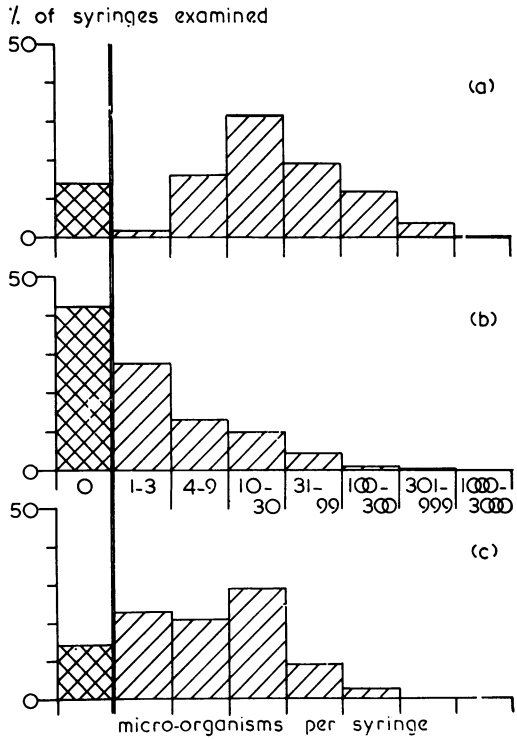

FIG. 4. *Number of aerobic organisms per syringe based on examination in the laboratories of their manufacturers.*

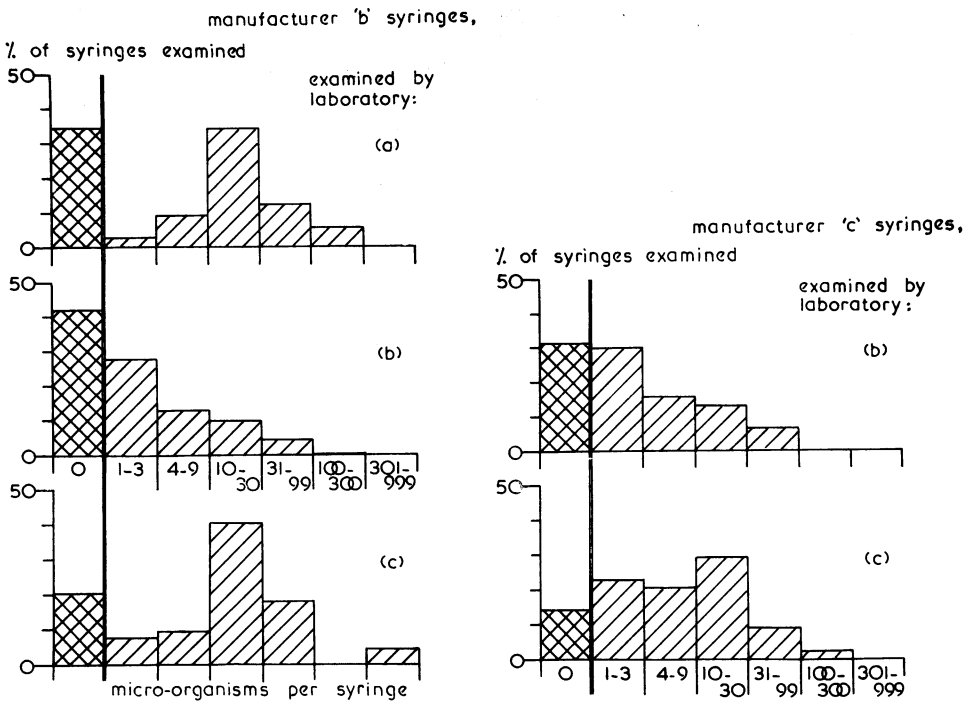

FIG. 5. *Number of aerobic organisms per syringe based on examination of one manufacturer's syringes in the laboratories of another manufacturer.*

one manufacturer's syringes by the laboratories of another manufacturer, the distribution of aerobic microorganisms per contaminated syringe differed depending upon the laboratory in which the microbiological examination was carried out. Each laboratory tended to produce a distribution pattern similar to that observed in syringes produced by its own parent manufacturer, rather than one similar to the distribution which another manufacturer's laboratories had found in assessment of their own syringes. Because of these inter-laboratory differences, it was not considered useful to attempt to characterize the cumulative distribution of numbers of organisms per syringe as to its mathematical form.

Characterization of contaminating organisms. The results of the examination of morphology, Gram-staining, and presence of coagulase in coccal organisms did not permit species differentiation. However, the characteristics examined did allow a classification which is outlined in Table 3. The results from the two laboratories which participated in this portion of the study are in good agreement, with the exception of the occurrence of coagulase-positive organisms and anaerobes, for which the results indicate differences in laboratory technique.

The general pattern of distribution among the contaminants was similar for syringes from both manufacturers, although, despite laboratory variations, the syringes produced by manufacturer b showed a higher incidence of gram-positive cocci and a lower incidence of rod-shaped organisms than those of manufacturer c. Fungal contamination of syringes appeared to be seasonal, with the highest incidence occurring in April and May. The accidental contamination occurring during laboratory examination, as assessed by the results with "known sterile" syringes, showed a similar pattern in the case of laboratory b; in the case of laboratory c, however, accidental contamination was predominantly with a bacillary organism producing pink or orange colonies.

Deliberate contamination. Exposure to dust did not significantly increase the number of microorganisms found on contaminated syringes unless the syringes were placed in the extractor fans which exhausted air from the syringe assembly premises until they were black with accumulated dirt. The maximal number of aerobic colonies found on syringes exposed for 1 week in the assembly area of two manufacturers was only 100.

Data obtained by two manufacturers for the syringes exposed in lavatories are shown in Fig. 6. It is significant that, even after 7 days of exposure to deliberate handling in the lavatory, a small

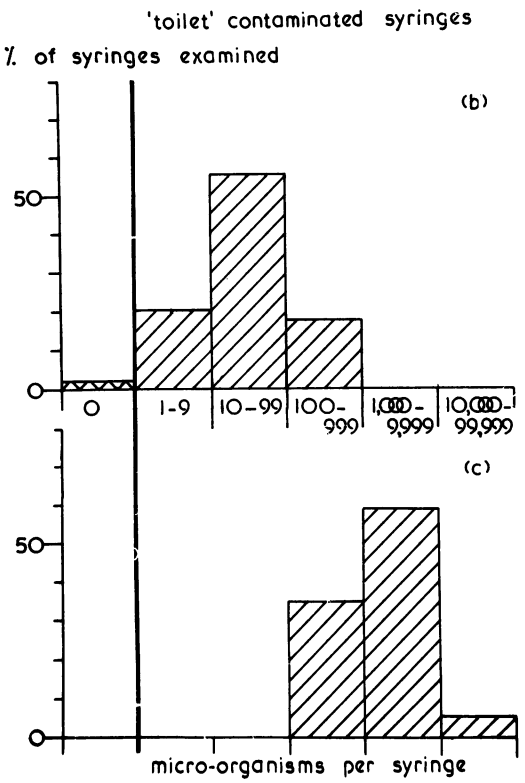

FIG. 6. *Microbial contamination of syringes after exposure for 1 week in the lavatories of two manufacturers.*

proportion of syringes remained sterile. The maximal contamination with aerobic organisms (manufacturer c) on any syringe was less than 13,000 organisms. Many syringes exposed in the lavatory were also contaminated with anaerobic organisms, but all of these yielded fewer than 1,000 colonies per syringe.

The most common organisms found on the deliberately heavily contaminated "lavatory" syringes were gram-positive, coagulase-negative cocci. A remarkably small number of coliform organisms was detected, even on the syringes exposed in the lavatory. This may be due to the inability of these organisms to survive on a plastic surface, but might also be due to the insensitivity of the media used in recovering coliforms.

DISCUSSION

The present results show that the disposable hypodermic syringe, manufactured under hygienic conditions, is often sterile prior to exposure to any sterilizing process. Accidental contamination in the present series of 964 syringes resulted in only one bearing as many as 1,133 aerobic organisms, and only one bearing 4,275 anaerobic organisms. Even after protracted exposure to deliberate handling in the lavatory, no more than 13,000 organisms were recovered from any syringe. This maximal figure, around 10^4 organisms per syringe, is a far more realistic estimate of the contamination likely to be found under the worst possible conditions within a well-run manufacturing plant than the earlier estimates of 10^5 organisms and above (4).

The radiosensitivity of microorganisms found on disposable plastic syringes when irradiated in the micro-environment of the syringe is not known, but inactivation curves produced under laboratory conditions, for even the most resistant bacterial spores isolated from dust in the UK, yield approximately one survivor in 10^7 organisms irradiated with 2.5 Mrad. Even the radioresistant *Streptococcus faecium*, isolated from dust in a Danish factory producing medical devices, when irradiated under laboratory conditions which maximize its radioresistance, has only about one survivor in 1,000 organisms irradiated with 2.5 Mrad (3). If these data are freely translated to the present series of plastic syringes in which only about 1 syringe in 1,000 has a chance of contamination with as many as 1,000 organisms, unless all organisms are of a uniformly resistant type and are in a resistant condition on the syringe, the chance of a single syringe contaminated with only one surviving organism being dispatched for use after radiation sterilization with a dose of 2.5 Mrad will be very small indeed (ca. 10^{-6}). However, further experiments are in progress in an attempt to examine the radiosensitivity of the microorganisms isolated from these plastic disposable syringes under environmental conditions which simulate their micro-environment on the production-line syringe.

The discrepancies between the results obtained by large, well-equipped, and conscientious industrial microbiological laboratories suggest that any attempt to exercise national control of the presterilization cleanliness of any type of medical product which is to be sterilized by radiation, gas, or heat (10) will have as a corrollary the requirement that central microbiological facilities are available in which all such examinations can be undertaken by one laboratory for which the incidence of "false-positive" results is known, and any apparent differences between distributions of numbers of contaminating organisms on similar items produced by different manufacturers can be meaningfully established.

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