14.11 Evaluation of Sterilization Dose for Disposable Hypodermic Needles Manufactured under Ultra-clean Condition

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ABSTRACT

Relationship between characteristics of bioburden and sterilization dose setting for disposable hypodermic needles, produced on same manufacturing lines, is presented in this study. The number of bioburden for 512 needles (18 lots) was changed from 0 to 6 and its distribution was extremely shifted to 0. Most of isolates (401 strains) were Gram positive cocci (60.3%) and spore forming bacteria (31.7 %). The distribution of gamma radiation resistances (D_{10}) showed two peaks at about 1.1 kGy and 0.4 kGy. The sterilization doses estimated by Japan log formula method on the basis of above data became higher than those by AAMI B2 (UDS) and B3 methods.

KEYWORDS

disposable hypodermic needle, radiation sterilization, sterilization dose, bioburden, radiation resistance

INTRODUCTION

Generally speaking, the lack of informations for bioburden brings about undesirable results in sterilization dose setting. Recently, following with GMP control, medical products with a very low bioburden have been mass-produced, but such products essentially have difficulties to obtain sufficient informations for bioburden. In AAMI methods (AAMI,1984; AAMI,1991), known as a flexible dose setting method, a low bioburden may influence the estimation of the extrapolation factor or the results of auditing experiments.
Therefore, to establish a resonable sterilization dose with a large safety margin for such products, the dose setting method should be carefully selected or developed. From above reasons, the investigation on characteristics of bioburden in such products have increasingly become important.
We have examined a relationship between characteristics of bioburden in a product manufactured by ultra-clean condition and differences of sterilization dose estimated with several dose setting methods.

MATERIALS AND METHODS

Needles
About 9500 samples (20 lots) of disposable hypodermic needles (18G×$1^{1}/_{2}$ - 27G ×23 mm, Misawa Medical Industry), produced on the same manufacturing lines for about 3 years , was used.

Measurements of Bioburden
One needle and 10 ml of washing solution (0.1% Tween 80 + 0.85% NaCl in distilled water) in a screw-cocked test

tube (30 ml) were agitated by a shaker for 5 min. (about 360 strokes / min). Moreover, efficient collecting microorganisms, 10 samples and 30 ml of washing solution in a Duran bottle (100 ml) were agitated by the same condition as above. After agitating, washing solution was filtrated with membrane filter (MF: Toyo Advantech, pore size: 0.45 μm). MF was incubated on a paper filter pad immersed with 2 ml of Trypticase Soy Broth (TSB, BBL) at 35 °C for 7 days, and these bioburden were calculated from viable counts of colony on MF. Sterility tests of pre-sterililized needles were carried out with TSB at 32°C for 14 days and the probability of positive culture was examined.

Simple classification of microorganisms

After Gram's and spore staining, microorganisms were classified with regard to morphology by microscopic observation.

Samples for irradiations

After simple classification of bioburden by microscopic observation, spore forming microorganisms were incubated on Trypticase Soy Agar medium (TSA, BBL) contained 10 ppm of Mn^{2+}, and non-spore forming microorganisms were incubated on TSA at 35 °C for 2-10 days. Fungi and yeasts were incubated on Potato Dextrose Agar medium (PDA: Difco) at 25 °C for 14 days. Microorganisms grown on the culture media were scratched with a glass spreader. Microorganisms were filtrated with glass wool, and then washed by centrifugation (6000 rpm, 20 min., 4 °C , 3 times) with 0.85 % saline water.

20 μl of harvested washed microorganism suspensions were dropped on a glass fiber paper (Toyo Advantech, GA-100, about 1×1 cm) or a piece of polypropylene (PP) cap of needle (about 1×1 cm) with a micropipette. After drying in a clean bench, these test pieces were enclosed in sterilized bags (Hogi Medical, HM-301) one by one.

Irradiation

Gamma ray was irradiated by Co-60 source (185 TBq). Dose range of 1-15 kGy and dose rates of 0.36-2.2 kGy/hr were used. Electron beam (EB) was irradiated by a 5 MV Dynamitron accelerator. Beam currents were 0.8-1.0 mA, cart speed under the beam were 9.5-17.5 m/min and dose range were 1-16 kGy. Dosimetry for gamma ray and EB radiation were done using Fricke dosimeter and radiochromic film dosimeter (FWT-60), respectively.

Radioresistance of microorganisms

Irradiated test pieces were agitated for 5 min. with 3 ml of washing solution and about 15 glass beads (Ø: 2-3 mm) in test tube by an automatic mixer. Homogenized suspensions of glass fiber paper and agitated suspensions of PP cap were adequately diluted with 0.85 % saline water. An adequate amount of diluted suspension was mixed in a petri dish with TSA at about 50 °C and shaked ,and then incubated at 35°C for over 40 hours. Fungi and yeasts were incubated at 25°C for 5 days after spreading on PDA.

Survival curves were made from viable colony counts on the agar culture, and were approximated to linear or quadratic expression with least squares method. D_{10} values were calculated from survival curves as the average values of 6D/6.

RESULTS AND DISCUSSION

Bioburden

On the bioburden experiments of 512 needles (18 lots) by MF method, the number of bioburden was from 0 to 6 and its distribution extremely shifted to 0 (total average bioburden: 0.123). Furthermore, the average probability of detecting microorganisms (8.0%) of all lots was larger than that of positive cultures in sterility tests (3.8%), and both were independently changed from lot to lot (Fig.1). Following AAMI B2 (UDS) method (AAMI, 1984), needles randomly sampled from 3 lots, which probability of positive sterility test in non-irradiated needle was 2 %, were separately irradiated in the incremental dose experiments by EB and gamma ray. All samples were negative except the sample irradiated at a dose of 1 kGy by EB (one positive). The results of sterility test experiments for non-irradiated 1800 needles (18 lots × 100 samples), which were carried out for the same lots as the bioburden experiments, and some of AAMI auditing experiments were shown in Table 1. The number of positive culture was changed from 0 to 11 of 100 samples. In AAMI auditing experiments, 5 of 17 auditing experiments showed more than 3 positive, that original sterilization dose was not accepted. Usually, we observed microscopically the existence of limited types of microorganisms. 401 contaminants isolated from MF and sterility test experiments were classified

into four groups with regard to morphology: Gram positive cocci (60.3 %), spore forming bacteria ,*Bacillus*, (31.7 %), yeasts and fungi (3.5 %) and unclassified microorganisms (4.5 %).

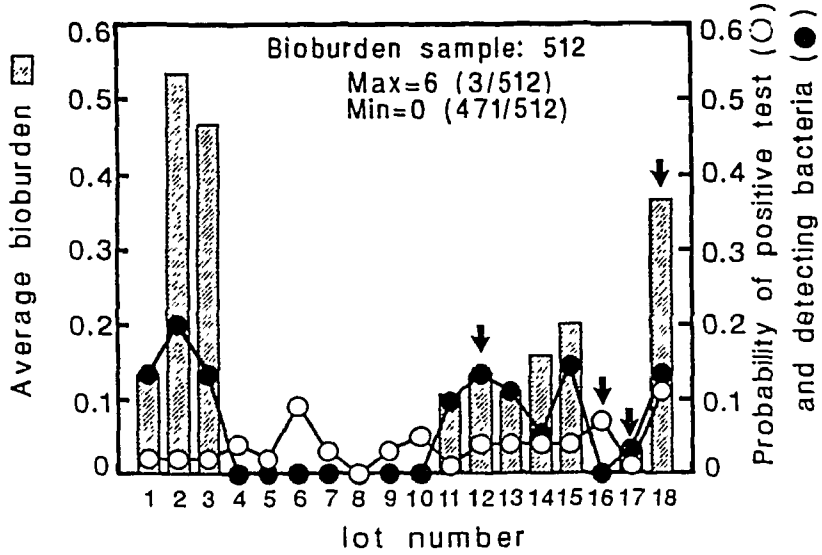

Fig. 1. Relationship between bioburden and sterility test
↓ : failed lot in AAMI audit experiments

Table 1. Results in AAMI audit experiments

	Positive number of 100 samples											
	0	1	2	3	4	5	6	7	8	9	10	11
Non-irradiated	1	2	4	1	5	1	0	1	0	1	0	1
Gamma ray (1 kGy)	4	2	1	1	2	0	0	0	0	0	0	0
Electron beam (1 kGy)	4	1	0	1	1	0	0	0	0	0	0	0

* The number in column show frequencies of audit experiments

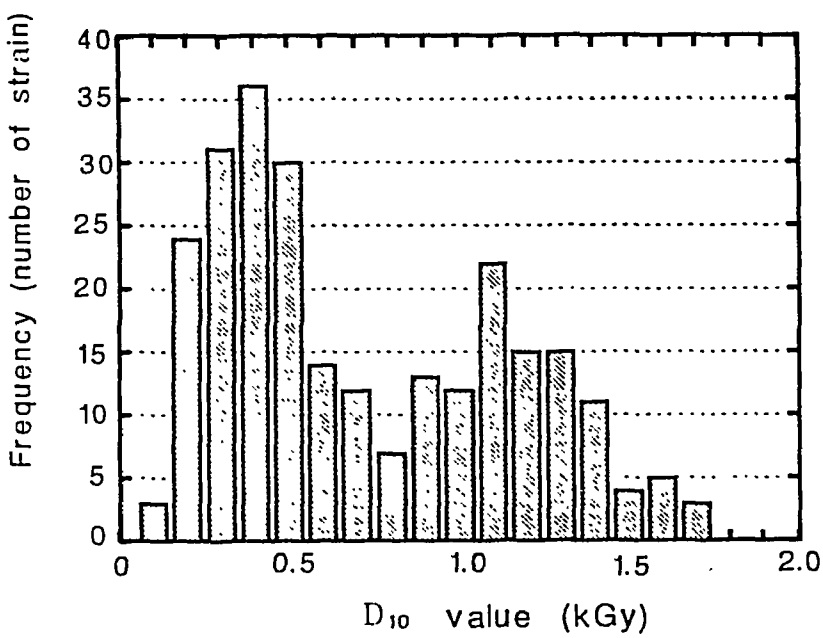

Fig. 2. D_{10} value distribution of natural bioburden (257 strains) on the hypodermic needles for gamma ray irradiation. (carrier material : glass fiber paper)

Table 2. Radioresistances of microorganisms isolated from hypodermic needles

	D_{10} value for electron beam (kGy)		D_{10} value for gamma ray (kGy)	
	glass fiber paper	PP test piece	glass fiber paper	PP test piece
Bacillus pumilus ATCC 27142	1.55	1.52	1.51	1.55
No.1 (*Bacillus*)	1.38	1.55	1.40	1.51
No.10 (Gram positive cocci)	1.67	1.97	1.61	1.93
No.21 (*Bacillus*)	1.78	1.88	1.68	1.68
No.24 (*Bacillus*)	1.50	1.73	1.59	1.98

Radioresistance of isolated microorganisms

Distribution of radioresistances (D_{10}: 6D/6, carrier: glass fiber paper) for isolated microorganisms showed two peaks at about 1.1 kGy and 0.4 kGy. The maximum and minimum D_{10} values were 1.76 kGy and 0.15 kGy, respectively (Fig.2). Spore forming bacteria and a part of Gram positive cocci were contained in the former peak, and fungi, yeasts, and the rest of Gram positive cocci were contained in the later peak.

And then four strains of high radioresistant bacteria that were selected from a series of experiments and reference bacteria (*Bacillus pumilus* ATCC 27142) were used to the estimation of radioresistances by EB and gamma ray irradiation: Three strains (No.10, 21 and 24) were isolated from bioburden experiments, and one strain (No.1) was isolated from the experiment followed AAMI-B3 method. Radioresistances of those bacteria were influenced by carrier material (Table 2). Radioresistance of bacteria inoculated on PP cap of needle were higher than that on glass fiber paper. The maximum D_{10} value was less than 2 kGy.

Estimations of sterilization doses

Sterilization doses of needles were estimated based on above data. When the sterilization dose was calculated by Japan log formula method (Ministry of Health and Welfare, Japan, 1993), 1.97 kGy (EB) and 1.98 kGy (gamma ray) as the maximum D_{10} values, and 10 organisms per needle as the safety estimation value instead of the maximum of bioburden (6 organisms per needle) were used. As the results, sterilization doses were 13.79 kGy (EB) and 13.86 kGy (gamma ray), respectively.

The maximum D_{10} values obtained by AAMI B3 method were 1.55 kGy (EB) and 1.51 kGy (gamma ray), respectively. The sterilization doses calculated from those data were 8.95 kGy (EB) and 9.00 kGy (gamma ray).

On AAMI B2 (UDS) method, D^{**} were 0.95 kGy (EB) and 1.00 kGy (gamma ray) , and UDS of both were 1.8 kGy. And then, sterilization doses were 8.15 kGy (EB) and 8.2 kGy (gamma ray). When AAMI method 1 (AAMI, 1991) was used to these samples, sterilization dose correspondent to the average bioburden (0.53) was 13.5 kGy, provided that the verification test was passed. The sterilization doses estimated by Japan log formula method were higher than those by AAMI B2 (UDS) and AAMI B3 method, and almost equal to those by AAMI method 1 by chance.

As shown in Fig.1 and Table 1, the auditing of sterilization dose in AAMI B2 and B3 method was sometimes failed by fluctuation of bioburden. If AAMI method 1 is applied to low bioburden products, the sterilization and auditing dose may be changed remarkably with fluctuation of bioburden. The increase of the difference between average and maximum bioburden may bring about the decrease of the efficacy of the auditing as shown in Table 1. The radioresistant distribution of AAMI population C was obtained with sub-lethal screening and expressed as populations with D_{10} values of more than 1 kGy (Whitby *et al.*, 1979). Therefore, if populations with D_{10} values of less than 1 kGy are omitted from the radioresistant distribution of the hyperdermic needle bioburden (Fig.2), the survival curve of the residual populations (average bioburden=1) is plotted as shown in Fig.3.

The survival curve for PP cap carrier (solid line) was estimated by multiplying radioresistances of the residual populations by 1.245, which value was the ratio of the D_{10} value of 1.98 kGy to that of 1.59 kGy for No.24 strain in Table 2 and also the muximum ratio of the D_{10} values for PP cap to those for glass fiber paper in Table 2. The auditing dose obtained from the survival curve for PP cap carrier was greater than that of AAMI population C.

If dried samples of bacteria suspended in TSB, as Whitby's experiments, have great radioresistances compared with those in distilled water, the auditing dose obtained by the survival curve for PP cap carrier may be further increased. Therefore, those methods are undesired to apply to medical products with a very low bioburden. On the other hand, Japan log formula method, using a maximum D_{10} and safety value instead of maximum bioburden, is thought to be relatively stable against fluctuation of bioburden and providing a reasonable sterilization dose with a satisfactory safety margin.

From above reasons, microbiological informations by routine control following with GMP, are very important to estimate a sterilization dose for ultra-clean medical products.

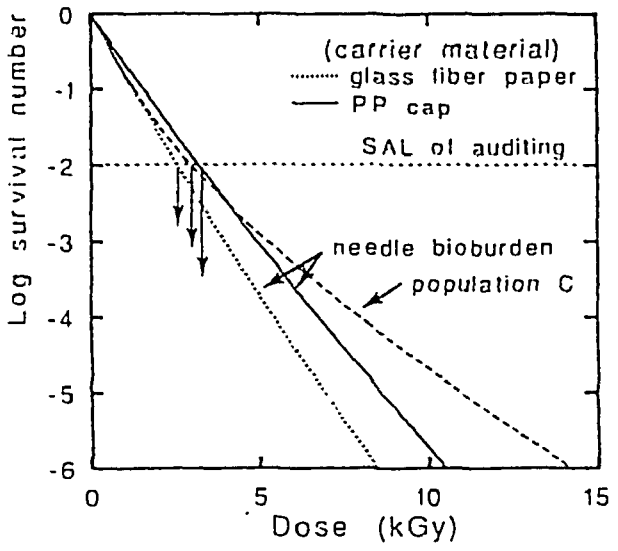

Fig. 3 Survival cureves for the hypdermic needle bioburden and AAMI population C, and auditing doses (average bioburden = 1)

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