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by

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Abstract

This report describes the basis of radiation sterilization of medical utensils in Denmark and of the choice of dose for sterilization at Risö of disposable equipment of dry plastic.

The possibilities of establishing general criteria for efficient sterilization by comparison with autoclaving are dealt with.

A description is given of the selection of test strains and test pieces for use in fixing the dose for sterilization of a particular category of utensils and in controlling the microbiological effect of the irradiation.

The possibilities of continuous control of disposable equipment based on counting of the micro-organisms present on the equipment prior to irradiation are discussed. Such day-to-day control must be assumed to warrant a reduction of the routine dose below the minimum of 4.5 Mrad used in Denmark for dry plastic equipment.

Contents

1. Introduction

Radiation sterilization of medical equipment in Denmark began in 1958, when a company manufacturing oxygenators for lung and heart operations inquired at the Danish A. E. C. about the possibilities of having their products sterilized by irradiation. The first oxygenators were irradiated the same year in the Co^{60} facility of Risö, but from 1961 sterilization has taken place in the linear accelerator. Since then a steadily increasing amount of various products has been treated: in 1961 400, in 1962 5,200, in 1963 14,300, in 1964 20,400, and in 1965 39,100 boxes, each containing 10 pounds. The products comprise

hypodermic syringes
infusion and transfusion sets
urine bags
Petri dishes
catheters

and a large variety of surgical appliances. All the products are dry plastic equipment.

From the very beginning a great amount of bacteriological research was carried out in co-operation with Statens Seruminstitut, which is in a consultative position to the National Health Service. Until recently there have been no legislation or regulations applying to the manufacture and sterilization of medical utensils, and our procedures as described below have therefore been shaped on the patterns found in well-established methods such as autoclaving. An advisory committee on sterilization problems was established a year ago. This committee is working out a set of recommendations which, when followed, allow manufacturers to label their products as being manufactured according to health authority recommendations. The essential point in these is the definition of sterility as less than 10^{-6} viable units per item. The recommendations will specify demands on factories with regard to sanitary precautions and supervision of the procedures by a competent bacteriologist approved by the National Health Service; further they will give some guidelines for handling and packaging of the products.

The basis on which the routine dose for radiation sterilization of dry plastic equipment at Risö has been fixed is in fact a postulate.

This postulate is: It is desirable that industrially sterilized disposable equipment is of as good a bacteriological standard as equipment steri-

lized by hospitals.

This fundamental point of view has been approved by the Danish health authorities.

If the hospital standard of sterilization is optimal - that is, provides adequate safety without superfluous costs - the radiation sterilization at Risö may thus have been adjusted to the optimum standard; on the other hand, if the sterilization procedures of the hospitals incorporate a requirement of an exaggerated margin of safety, the same margin may be expected to be found in the Risö standard of radiation sterilization.

However, the advantages of adopting the same standard for industrial radiation sterilization as for hospital sterilization are considerable. The consumers may count on industrially sterilized equipment affording the same safety as that to which they are accustomed, and the radiation dose may be fixed by analogy with traditional methods for bacteriological control of autoclaving.

The common autoclaving procedures rest on a solid foundation of experience, and the recommendations of the authorities concerning these procedures vary little from one country to another. Moreover it is agreed that autoclaving performed lege artis offers a very good guarantee of sterility.

2. The Microbicidal Effect of Autoclaving Procedures

In Denmark the control of autoclaved hospital equipment is based on spore test pieces being placed, at suitable intervals of time, in that part of the piece of equipment which is supposed to be most resistant to sterilization. An autoclaving procedure is accepted if the test pieces are always sterilized by it. Of course the day-to-day operation is controlled by physical and chemical measuring methods, but the spore test is the decisive control measure.

The test organisms are spores of a strain of Bacillus subtilis. The spores, in a number of about 10^6 per test piece, are diffusely distributed in sand, which is wrapped up in two layers of paper. It is recommended to use at least six test pieces for each check of an autoclaving procedure.

With a view to comparing different sterilization methods, the inactivation factor of the test strain was investigated by placing such spore test pieces in various hospital equipment and autoclaving it at 120°C. In all cases the recommended sterilization times yielded inactivation factors larger than 10^{12} [3].

From the way in which the spore test is used in routine control in Danish hospital departments it appears that autoclaving procedures have hitherto been accepted if the inactivation factor was greater than 10^8 [3]. It should be emphasized, however, that such acceptance has been conditional on the equipment having been cleaned, assembled and packaged by the hospital staff under good hygienic conditions.

3. Basic Demands on Routine Sterilization by Ionizing Radiation

On the basis of the foregoing, the following requirements were laid down for routine sterilization by means of ionizing radiation:

(a) In the part of the object to be sterilized in which irradiation has the lowest microbicidal effect, this effect must not be lower than that corresponding to the inactivation factor 10^8 for a bacterial strain fulfilling the radiation-resistance criteria for a test strain in sterilization;

(b) The test pieces used for measurement of the inactivation factor must be prepared in such a way that the radiation resistance of the test organisms contained in them may be supposed to be at least equal to the greatest resistance that may occur in the equipment to be sterilized;

(c) The equipment must be manufactured under hygienic conditions.

Health authorities in some countries may find the inactivation factor 10^8 too small as compared with the widely recommended factor 10^{12} for sterilization of foodstuffs. The reasons for choosing an inactivation factor larger than 10^8 instead of larger than 10^{12} were:

1. Alone on account of the risk of contamination with pyrogenes and toxins, industrially produced hospital equipment must be manufactured under good hygienic conditions. In the case of rather uncomplicated plastic equipment, the fulfilment of this requirement ensures that the number of germs prior to sterilization is small;

2. In radiation sterilization the most unfavourable possibility of microbicidal effect may be determined with greater accuracy than that obtainable in similar determinations connected with sterilization methods such as autoclaving and ethylene-oxide sterilization[3,4]. This must justify a narrower safety margin in radiation sterilization than in autoclaving.

3. Streptococcus faecium, which, as mentioned in the following, was employed as test strain for the calculation of the radiation-sterilization dose used at Risö, occurs more sparsely - i. e. in a smaller number of viable units per weight unit - in dust and dirt than the aerobic spore-formers.

By the experimental technique mentioned in the following, the most radiation-resistant of the bacterial spores examined so far have been shown to have an inactivation factor of about 10^{12} on irradiation with 4.5 Mrad[5, 6].

4. Preparation of Test Pieces

It is well known that the environment of micro-organisms before, during and after irradiation may greatly influence the inactivation factor at a given dose. It is also well known that in their natural environment micro-organisms are often considerably more resistant to sterilization by traditional methods than they are when prepared under laboratory conditions[10]. To the authors' knowledge there is no basis for assuming that the above does not apply to radiation sterilization as well.

In working out the method for investigation of the radiation resistance of different micro-organisms it therefore had to be taken into account that the purpose was to sterilize dry plastic equipment, which might be contaminated with dust.

Dust contains droplet nuclei and other particles such as skin scales, bits of hair, plant fibres, manure particles, etc. Therefore the contaminating flora might be enclosed in organic matter and dried salts under conditions impeding the free oxygen exchange. Drying-up, the presence of organic matter and reduced oxygen tension are factors known to be capable of increasing the radiation resistance of micro-organisms in laboratory experiments, and it must be supposed that at worst all these factors might contribute to increasing the radiation resistance of micro-organisms on dry plastic equipment. The test pieces required therefore had to be different from the cleansed preparations which are in general a very suitable material for the study of inactivation curves under varying experimental conditions.

The inactivation curves on which the fixing of the Risö dose for sterilization of dry plastic equipment rests were all worked out on the basis of irradiations of bacteria and bacterial spores in test pieces prepared by the following uniform technique, which was chosen in order to make the curves suitable for this particular purpose: the micro-organisms were scraped-off plate cultures, which, uncleansed, were suspended in serum broth and dried as uniform drops in atmospheric air at room temperature (22-26°C). The drops were dried on polyethylene foil and wrapped up in the same kind of foil[6].

Figures 1 and 2 show the results of a great number of such investigations (see also the next section).

5. Selection of Test or Reference Strain

The test organisms used in connection with autoclaving, dry-heat sterilization and ethylene-oxide sterilization were chosen because they are more resistant to sterilization by the respective methods than all known pathogenic strains and at least as resistant as all contaminating bacteria of common occurrence in the environment concerned. The same criteria might be applied to the selection of a strain to be used in radiation sterilization as a parallel to the test strains approved for other sterilization methods.

That a test organism should also be innocuous and easy to work with is a point of minor interest in this connection. In radiation sterilization a test organism would hardly be used for routine tests as the dose control required in the day-to-day operation is carried out better and cheaper by physical methods. The test organism would only be applied for comparison with other test organisms used in connection with other sterilization methods, and possibly as a reference strain in comparisons between the microbicidal effects of different irradiation procedures.

Figures 1 and 2 show that, according to the technique of investigation used here, the commonly occurring bacterial spores are not the most radiation-resistant organisms in the microflora present in dust.

Further the figures show that some bacterial strains, such as Micrococcus radiodurans R_1[1] and the most resistant mutant of Streptococcus faecium Y_9[7], may have a very great radiation resistance as compared with the majority of the organisms studied. However, the existence of these extremely resistant strains need not influence the choice of test or reference organism if they can be assumed to be apathogenic and are not common in the relevant environment. Also in heat sterilization one must resign oneself to the existence of apathogenic micro-organisms far more resistant to heat than the test strains.

The conclusion of the investigation was that the most resistant of the faecium strains isolated from dust samples fulfilled the criteria for a test strain for radiation sterilization of dry plastic equipment.

It is well known that Str. faecium is ubiquitous; strains of this species have in fact been isolated from all dust samples examined hitherto. Str. faecium has been found in human infections and may thus be pathogenic, although this is the exception. It occurs in a great number in the human intestinal canal and, for a vegetative bacterium, shows a considerable resistance to drying and heat.

6. Choice of Dose

The most resistant of the faecium strains isolated from dust samples had an inactivation factor of 10^8 after irradiation with 4.5 Mrad by means of a linear electron accelerator[5].

The consequence of the basic demands on radiation sterilization and the choice of test pieces and test strain must therefore be that a minimum dose of 4.5 Mrad was required for radiation sterilization of dry plastic equipment in the electron accelerator plant at Risö.

If the number of germs on the unsterilized equipment is under efficient control, the dose may probably be fixed on the basis of the initial germ number as determined through the control; to the authors' knowledge, however, such control, based on a reasonable number of random samples taken from the unsterilized equipment, has not been established as a routine measure anywhere.

Our task was to evaluate the microbicidal effect of a particular dose in irradiation of dry plastic equipment in an accelerator plant. We have had no opportunity to compare the microbicidal effect in the accelerator plant with that in a cobalt plant at doses exceeding 2.5 Mrad, but to the extent to which the experimental conditions have made a comparison reasonable, good agreement between the microbicidal effects of the doses in the two plants has been found up to this dose in numerous experiments with dried spores as well as with dried vegetative bacteria.

Of course it is possible that continued investigations, carried out by the technique used so far or by altered growing and preparation methods, may show that other micro-organisms, either pathogenic or commonly occurring apathogenic ones, are more radiation resistant than the most resistant among the faecium strains found. No applicable investigation technique can offer all micro-organisms optimal possibilities of development, and the bacteriological methods used have inevitably led to a selection, also where it was not intended.

7. Germ Counts on Unsterilized Plastic Equipment

In the foregoing statement of the reasons for fixing a routine dose of not less than 4.5 Mrad for radiation sterilization in Denmark, the initial number of germs on disposable plastic equipment has only been mentioned in passing. A review of Danish observations of the numerical size of the contaminating flora may perhaps be of interest.

In general, hypodermic syringes appear to be little contaminated. The number of germs per syringe demonstrable by the technique used, which is based on membrane filtering of rinsing water from the equipment, is considerably less than 100 in all cases examined (fig. 3). However, Dr. Kelsey, Colindale, has reported counts of the order of 10^5-10^6 spores per syringe in about one third of a hundred units taken from a single batch[8]. When so large numbers may occur where the syringes are produced under bad hygienic conditions, it is necessary always to manufacture disposable equipment under good hygienic conditions, which must be ensured by control.

As would be expected, more complicated equipment such as donor sets shows larger counts. Figures 4a and b indicate the number of germs per donor set from a Danish factory before a qualified bacteriologist was attached to it. Figures 5a and b give the count per set after the bacteriologist had organized the manufacturing procedure.

On the whole the investigations have shown that the average number of germs prior to sterilization is small, but that the germs may be very irregularly distributed as regards number as well as species. However, a sterilization method must also afford a reasonable guarantee against rare accumulations of resistant germs, and in fixing a radiation dose for the sterilization of a specific group of articles, the most unfavourable possibilities must be taken into account.

By efficient supervision of the manufacturing process it must be possible to make the occurrence of badly contaminated units a rare phenomenon. If the initial number of germs on disposable equipment were controlled by examination of a suitable number of random samples, the sterilization dose for such equipment might probably be fixed on the basis of the germ counts. After the control has been carried on for some time, it must be possible to estimate also the frequency of occurrence of badly contaminated units.

There is reason to believe that donor sets manufactured by a well-organized procedure - subject to control based on germ counts on unsterilized random samples - may be sterilized with a dose of 3.5 Mrad to the same standard as by autoclaving.

8. Sterility Testing and Safety Margin

A short time ago Ley and Tallentire[9] suggested that the basis for the choice of a radiation-sterilization dose for disposable plastic equipment used hitherto be taken up for revision.

They pointed out that sterility testing is an unsatisfactory way of evaluating the efficiency of sterilization methods. This is the prevalent point of view in Denmark, too, although sterility testing is still used because it may be the only means of control available to the authorities. In the deliberations leading to the fixing of a radiation dose at Risö, results of sterility testing were not taken into account, but such results may necessitate a revision of the basis of dose choice presented here. As far as we understand, Brewer[2] has observed some radiation-sterilized equipment to fall through at a sterility test, even when the dose was between 4 and 5 Mrad. The reason might be the presence of a very great number of germs on the equipment prior to irradiation. If the initial germ number was of the same magnitude as that on the syringes examined by Kelsey[8], the positive finding at the sterility test would confirm the realistic nature of the evaluation presented here of the resistance of the microflora on plastic equipment.

It should be emphasized once more that if radiation sterilization is to be applicable on a par with heat sterilization, it must be subject to the requirement of a reasonable margin of safety. A few fatal accidents due to surviving radiation-resistant bacteria on, for instance, donor sets for use in blood banks may easily cause radiation sterilization as such to fall into discredit. There may be good reasons for permitting a narrower safety margin for equipment such as hypodermic syringes and urethral catheters than for blood bank equipment; however, the Danish health authorities do not at present fall in with this view, but want all sterile equipment judged by the same standard.

As a matter of fact, the authors of the present work consider it possible that a dose of 2.5 Mrad suffices to ensure that the risk of infections after the use of thus irradiated hypodermic syringes is so small as to be insignificant in practice; even if this should be so, however, it would not necessarily mean that the syringes were sterile.

For long-established reasons, a very high degree of safety is wanted for the patients, who are the ultimate 'consumers' of the disposable equipment; if any doubt is entertained as to the acceptability of the basis on which the dose is fixed, the dose and thus the safety margin must be increased until a new basis has been established through experiments.

We consider it possible that autoclaving and dry-heat sterilization as well as radiation sterilization fulfilling the basic requirements drawn up here provide an unnecessarily wide safety margin for hypodermic syringes, but we think that 2.5 Mrad is too small a dose to justify the designation 'sterile'. We are sure that a dose of 2.5 Mrad is too small to sterilize

donor sets and similar equipment, where a few surviving germs may mean
a risk of fatal accidents because the micro-organisms may have a possibil-
ity of undisturbed growth after the 'sterilization'.

The present report is based on work carried out by a group consisting
of staff members of Statens Seruminstitut and Risö.

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10)   G. Sykes, Disinfection and Sterilization. Second edition. (Ed. by E. and F. N. Spon Ltd., London, 1965) pp. 112-120.
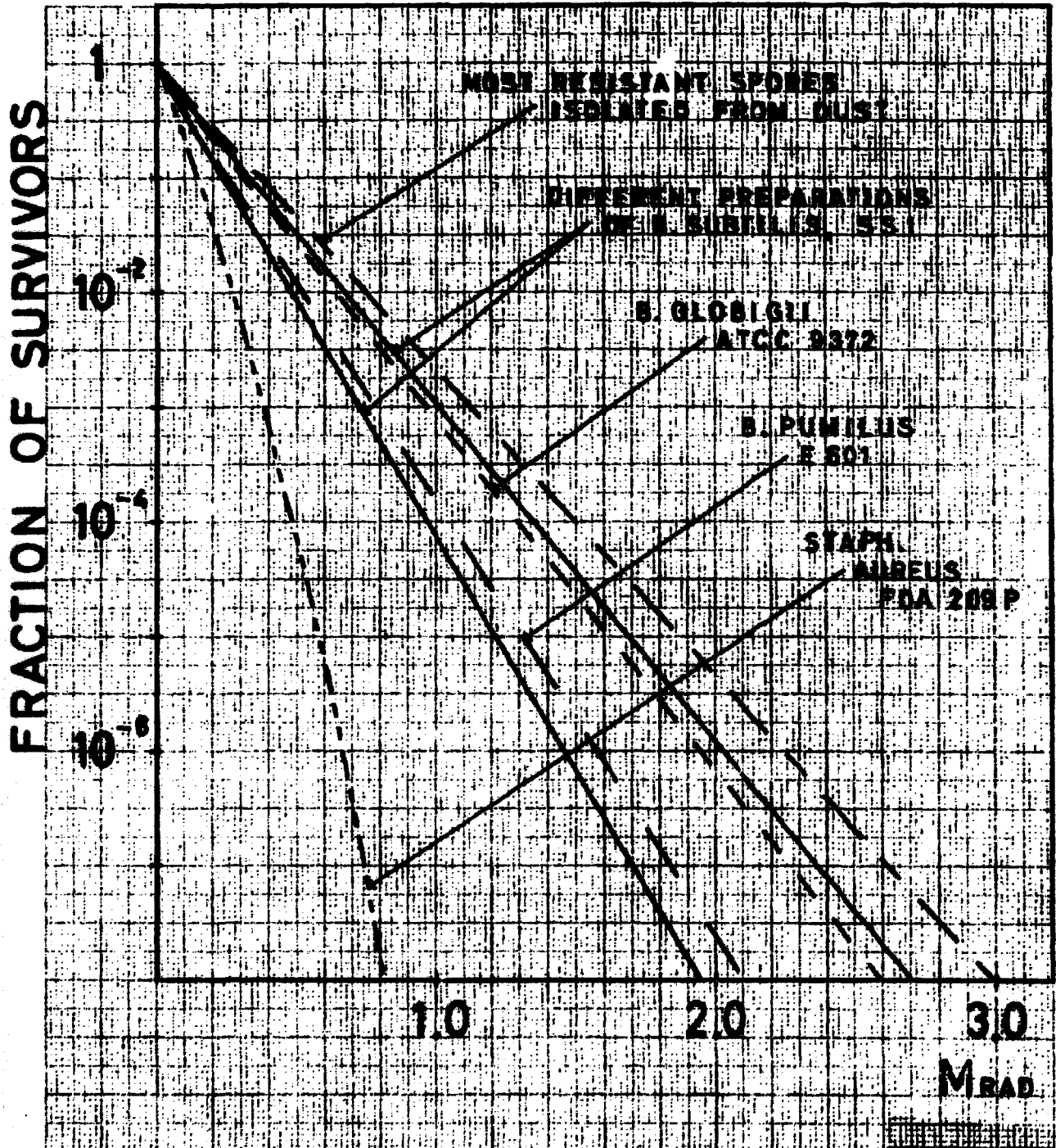
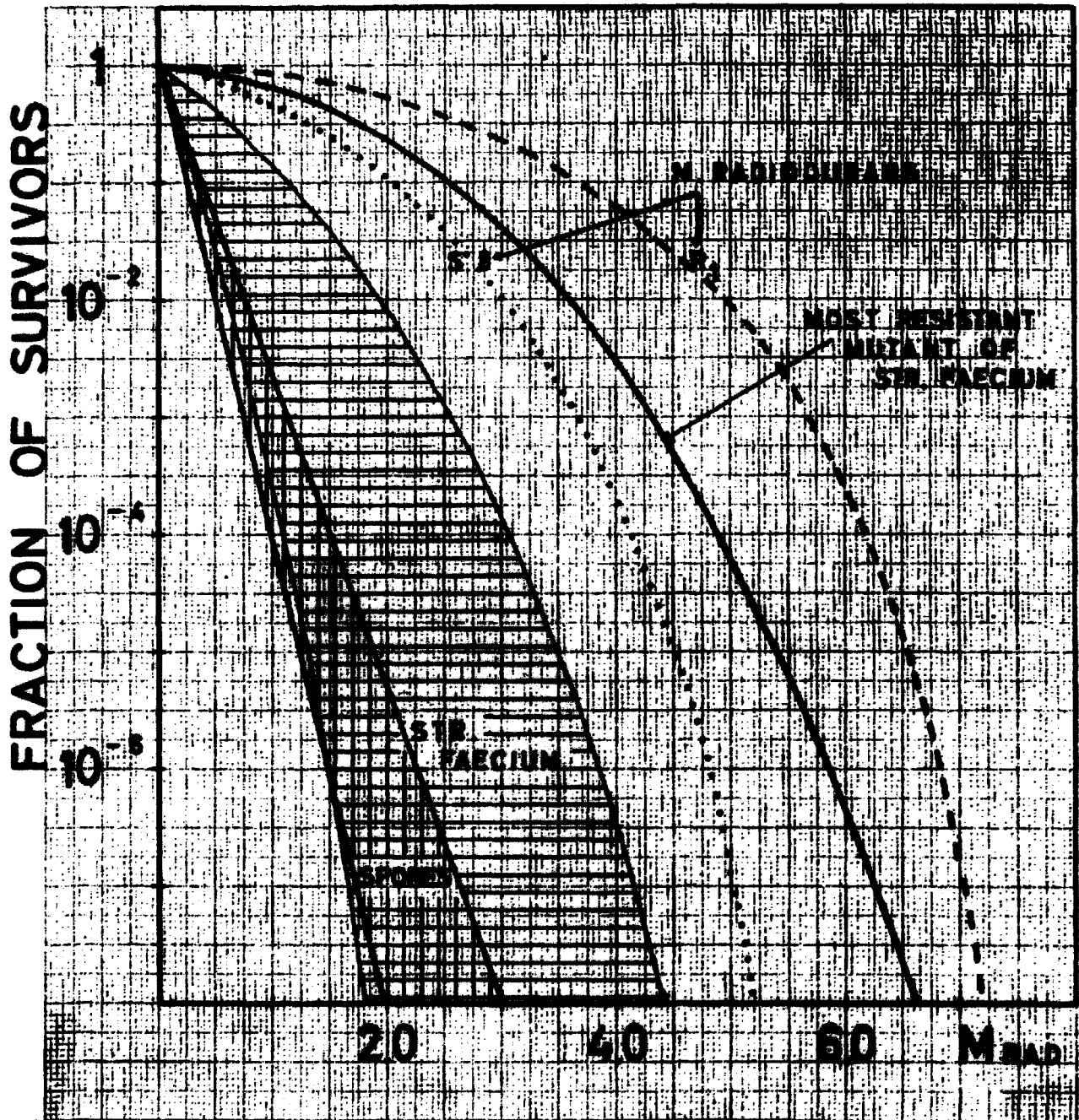Fig. 1. Inactivation curves for various dried bacteria and spores.

Fig. 2. Horizontal hatching indicates the range in which inactivation curves have been obtained for laboratory strains as well as for strains isolated from dust of Str. faecium.

Vertical hatching indicates the corresponding range for the spores studied. Inactivation curves for two strains of M. radiodurans and the most resistant mutant of Str. faecium are given for comparison.
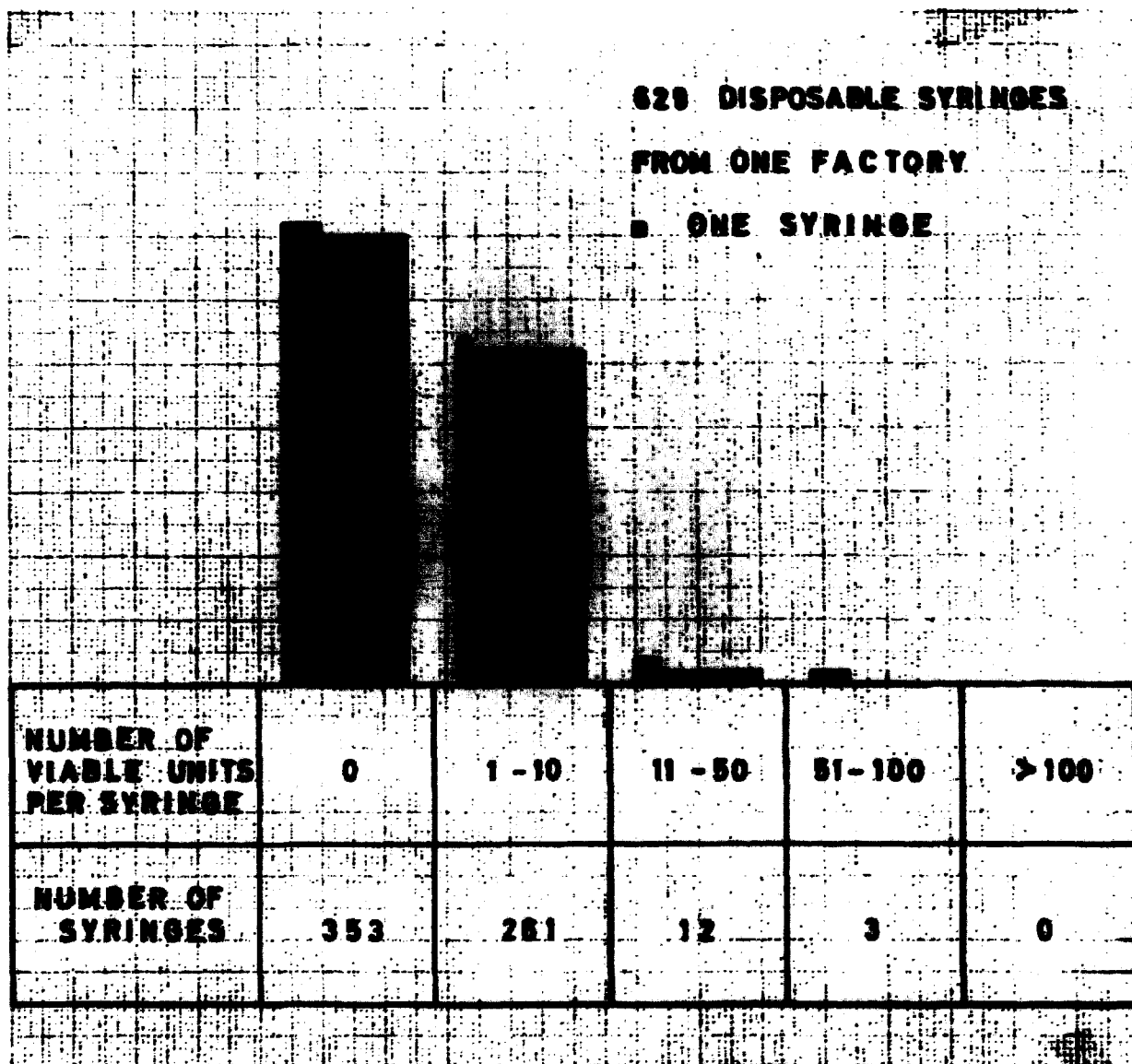
Fig. 3. Viable counts in rinsing water from the inside of the syringes.
The rinsing water was membrane filtered, whereupon the filters were
cultured on blood-agar plates at 37°C.

OUTER SURFACES

TOTAL: 181 SETS

■ ONE DONOR SET

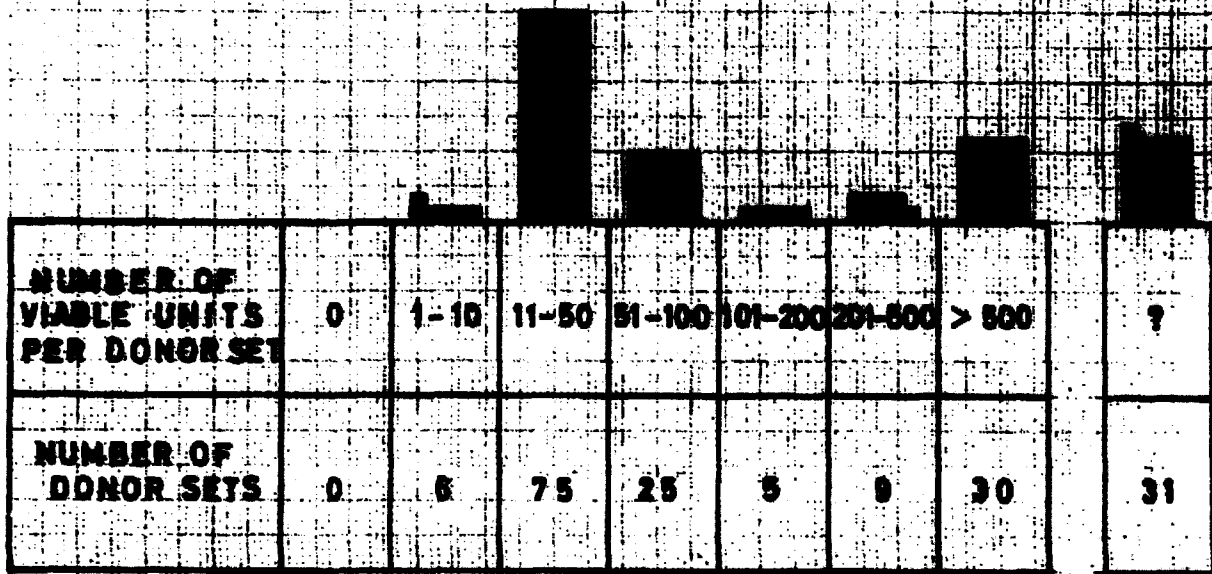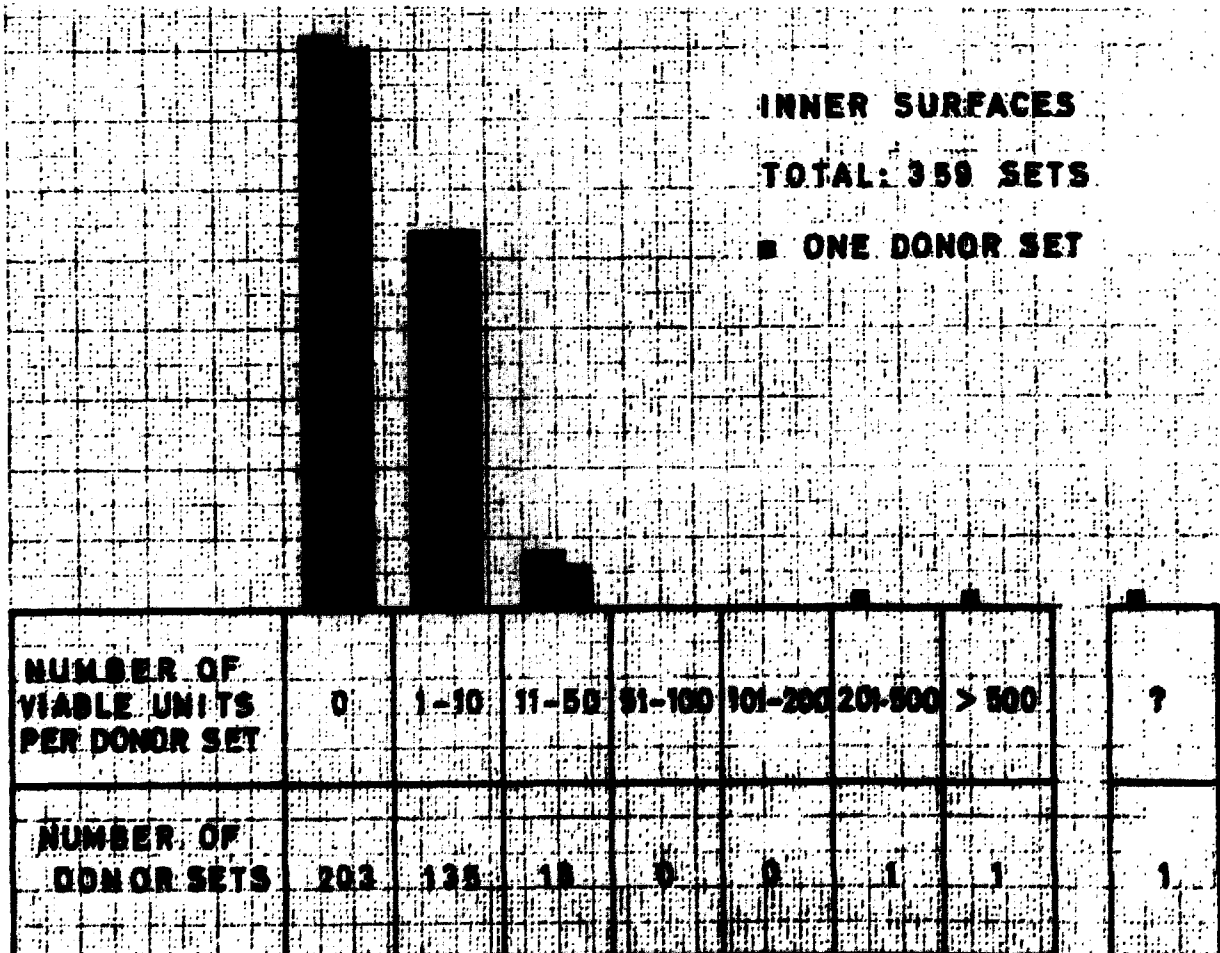| NUMBER OF VIABLE UNITS PER DONOR SET	0	1-10	11-50	51-100	101-200	201-500	> 500		?
NUMBER OF DONOR SETS	0	6	75	25	5	9	30		31

Fig. 4a and b. Viable counts from donor sets produced without supervision by a qualified bacteriologist.

INNER SURFACES

TOTAL: 359 SETS

■ ONE DONOR SET

NUMBER OF VIABLE UNITS PER DONOR SET	0	1-10	11-50	51-100	101-200	201-500	> 500		?
NUMBER OF DONOR SETS	203	135	18	0	0	1	1		1

OUTER SURFACES

TOTAL: 54 SETS

■ **ONE DONOR SET**

NUMBER OF VIABLE UNITS PER DONOR SET	0	1 - 10	11 - 50	>50	?
NUMBER OF DONOR SETS	2	54	8	0	0

Fig. 5 a and b. Viable counts from donor sets produced under the supervision of a qualified bacteriologist.

INNER SURFACES

TOTAL: 126 SETS

■ **ONE DONOR SET**

NUMBER OF VIABLE UNITS PER DONOR SET	0	1 - 10	11 - 50	>50	?
NUMBER OF DONOR SETS	98	28	0	0	0