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A theoretical basis for choosing the dose in radiation sterilization of medical supplies

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Summary

A method for the choice of the dose of treatment in radiation sterilization is proposed. It appears possible to validate doses lower than the usual value of 25 kGy by using a substerilizing dose to determine an intermediate level of sterility assurance level (SAL), after elimination of the most radiation-sensitive contaminants, and by extrapolating for this intermediate SAL to the required level, on the basis of an assumed maximum value of the D_{10} for the remaining contaminants. This can be done by sterility testings on 3 sets of 50 samples, among which two sets are irradiated at two suitably chosen substerilizing doses. Such a procedure will allow, in most cases, the use of a smaller sterilization dose. It will then significantly reduce the cost of the process by a limited amount of experimental testings, and will be useful for limited series of production.

Introduction

The choice of the dose of treatment in radiation sterilization is an important matter because of its practical and economical consequences (Ley and Tallentire, 1964). It is difficult, because it involves a statistical analysis which is never obvious or intuitive.

In most of the countries of the world, a minimum dose of 25 kGy (2,5 Mrad) is used. This dose is considered, and it was proven by a long experience, as efficient for any product made according to good manufacturing practices (Fitch et al., 1985); but it is clearly higher than necessary in many cases. The Association for the Advancement of Medical Instrumentation (AAMI of the U.S.A.) published a "Process Control Guide Lines" (Anon., 1984) where several procedures are proposed to determine the specific dose necessary to reach a given sterility assurance level (SAL) for a production. In many cases, and possibly most cases, this dose will be less than 25 kGy.

However, the procedures given in the AAMI document need many experimental tests, which are costly. They are not well fitted to the limited productions of many small companies. It would then be very interesting if it were possible to use procedures that allow one to estimate levels of sterilization doses lower than 25 kGy by a smaller number of tests than in the AAMI procedures, even if those doses are not the lowest achievable ones. Such procedures could bring the best profit–cost ratio for limited series of production.

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Here we shall discuss the theoretical basis for such a procedure.

Sterility assurance level (SAL)

When a population of micro-organisms of a given species is irradiated, the number of survivors decreases with the dose. Schematically, the curve of decrease is a straight line in a semi-logarithmic scale (Fig. 1), characterized by the slope, which is the dose allowing to reduce the number of survivors by a factor 10 (D_{10}). Beyond the point where only one micro-organism is surviving, and for the continuity, the proportion of contaminated items containing one survivor has to be substituted to the number of micro-organisms.

This defines the SAL which is, for a batch or a population of radiation-sterilized products the *proportion* $-n$ (estimated directly or by statistical extrapolation) of non-steriles equal to or less than 10^{-n}. The proportion is not exactly equivalent to a probability of finding a given number of contaminated items in a sample, which must be calculated from the Poisson distribution. If, for example, we consider a sampling of 10^6 items from a population having a proportion of contaminated ones of 10^{-6}, the probability of finding 0 or 1 contaminated item is $P(0) \# P(1) = 0.368$. They are $P(3) = 0.061$ and $P(4) = 0.015$ for 3 and 4 contaminated items (see Table 3). It means that there are probabilities to find more than one contaminated item in the sample, which are balanced by a probability to find no contaminated item, to make an average probability of one.

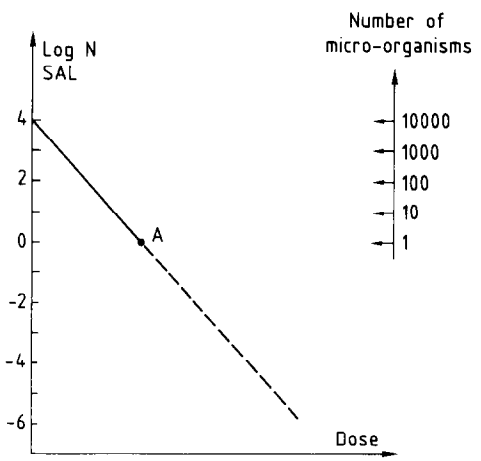

Fig. 1. Dose survival curve ($N/No. = 10^{-(D/D_{10})}$).

In practice, the real contaminations of medical devices are characterized by the presence of a range of contaminating species, with various D_{10} values and various initial contamination, and by an uneven contamination, i.e., the products are not bearing the same number of contaminants.

Every method of determination of the sterilization dose for getting a given level of SAL must take those practical facts into account. However, it can be understood that there is no method which could cover every case of incidental heavy contaminations by highly radio-resistant contaminants under acceptable economical conditions. The analysis has then to consider the contaminations which can be reasonably expected under conditions of good manufacturing practice.

Principles of the practicable methods for the determination of the sterilization doses (D.S.)

The levels of SAL which are considered as achieving an acceptable sterility may depend on the use of the sterile item, but are always high: generally -6, and at least -4. It follows that they can never be measured experimentally, because the necessary number of sterility testings would be far too high. The sterilizing dose (D.S.) must always be determined either by statistical extrapolation of experimentally obtainable data, or by assuming the worst characteristics of the contamination, or by a combination of both (Davis et al., 1984; Doolan et al., 1985): a maximum level of initial contamination can be specified, and a maximum value of D_{10} can be taken into account to estimate the D.S. by the formula:

$$\text{D.S.} = D_{10}\ (\text{Log No.} - \log \text{SAL}) = 25 \text{ kGy},$$

with No. $\leqslant$ 100 micro-organisms per item,

SAL $= -6$ and $D_{10} \leqslant 3.12$ kGy (Darbord et al., 1985)

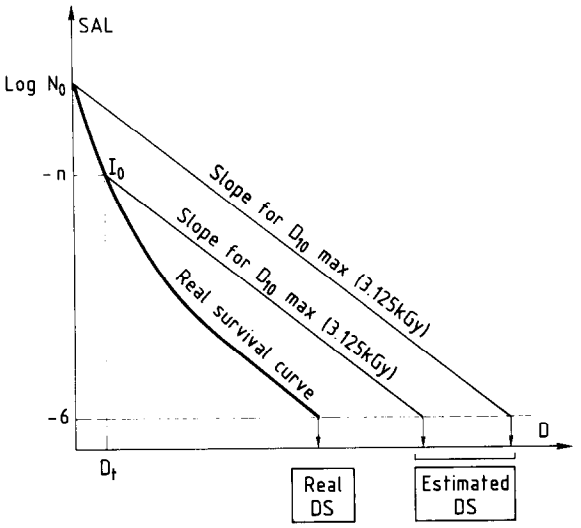

Fig. 2. Principle of the determination of a D.S.

This method will obviously in many cases lead to an overestimation of the D.S.: the initial contamination (maximum or mean value) is often much lower than the upper limit specified, whereas most of the micro-organisms will have a D_{10} smaller than the maximum assumed value.

If a substerilizing dose (D_t), leading to a SAL between 0 and -1, is delivered (Fig. 2), an order of magnitude of the actual level of SAL ($-n$) can be experimentally determined (point I_0) by sterility testings. At that level of SAL, a large number of the microorganisms of low D_{10} have already been eliminated. From this point I_0, it is then possible to take into account a maximum value of D_{10}, as in the conventional way to determine the D.S. by extrapolation. In other words, this procedure is purely an improvement of the present method, by eliminating the overdose resulting of a too pessimistic estimation of the initial part of the survival curve.

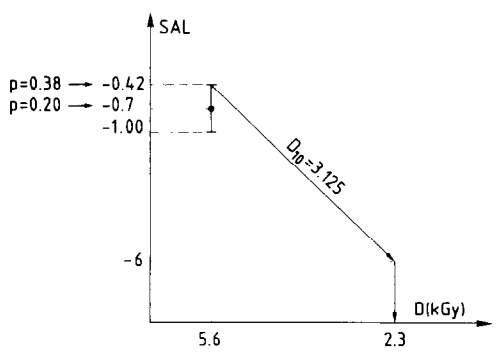

Fig. 3. Application of the determination of a D.S. (example).

An example will illustrate the method (Fig. 3): a sterility test is carried out on 50 items irradiated at a testing dose $D_t = 5.6$ kGy. The number of positives found by the sterility test is 10. The number of positives in the sampling allows to estimate, by using of the binomial law (Snedecor and Cochran, 1967), the maximum range of the proportion of positives in the population. In the present case, at a 99% confidence level, the proportion can go from 0.08 to 0.38. It means that the worst probable SAL of the population, corresponding to 38% of positive, is -0.42. To reach a SAL of -6, we need to add 5.58 unit of SAL, or 5.58 D_{10} kGy. We shall take into account, for computing the equivalent dose, the conventional maximum D_{10} (3.125 kGy): $\Delta D = 5.58 \times 3.125 =$ 17.4 kGy, and the D.S. $= D_t + \Delta D = 5.6 + 17.4 =$ 23.0 kGy.

Remark. The experimental conditions in the sterility testing may lead to false positives. It is interesting to point out that those false positives lead to an increase of the estimated D.S., which brings only an increase in the safety of the procedure. On the other hand, the incidence is very small. In the given example, let us assume the presence of 3 false positives. It would mean that the true number of positives is only 7, the maximum percentage 31% (SAL $= -0.51$), and the ΔD would be $(6 - 0.51) \times 3.125 = 17.2$ kGy, instead of 17.4 kGy. In this approach, it can be easily understood why an accurate knowledge of the number of positives is not really very critical. The important point is to be able to estimate the SAL of the sample and the worst probable SAL of the population.

Verification of the proposed method by simulation

Need of a verification

The basic methodology of the approach adopted here is very simple. This is not enough to prove its efficiency in the real conditions of the radiation

sterilization. In reality, the bioburden consists of a range of micro-organisms of various D_{10}; it is not evenly distributed on items; the sterility testings (whatever may be the dose) will give various numbers of positives, which must be interpreted; the applied doses must be suitably chosen, etc. The best way of investigating the efficiency of the method (and, practically, the only way) is simulation by computation. It avoids every bias related to the problems coming from the statistical aspects of the determination of the D.S.: the answer to that kind of problems is far from being obvious or intuitive, except by simulation, which is a method already used by previous authors (Davis et al., 1984; Doolan et al., 1985; Dwyer et al., 1985; Fitch et al., 1985).

Principles of the simulation

The considered populations were those used by Davis et al. (1984) for developing the AAMI protocols. They cover a large range of radiosensitivity, from populations expected to occur in the real conditions of production to assumed populations of very high resistance. By combining the choice of the population and that of the level of initial contamination (Fig. 4), a wide range of cases can be considered, corresponding to D.S. between 8.5 and 45 kGy. For a given initial contamination and a given population, the test dose (D_t) corresponds

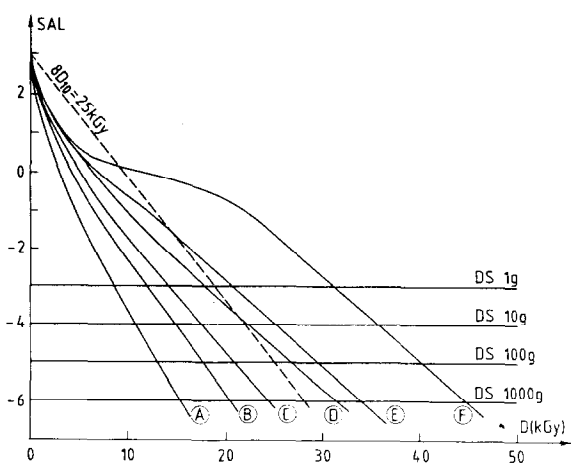

Fig. 4. Variation of the SAL with the dose for the various populations.

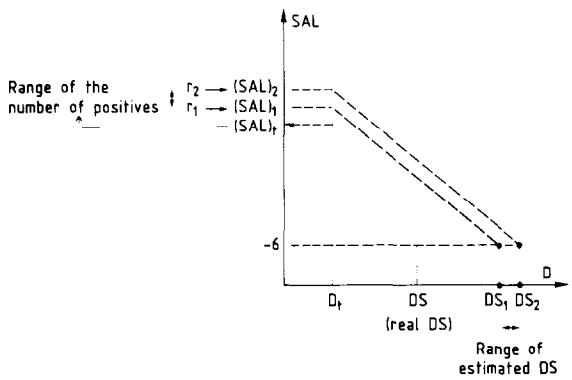

Fig. 5. Principle of the simulation.

to a value SAL_t (Fig. 5). This value allows to compute the lower and upper limits, r_1 and r_2, of the numbers of positives which may be found at 99% confidence level in a sterility testing of a given number of samples. From those values, and following the method previously described, the lower and upper limits of the estimation of the D.S. are computed, and can be compared to the real value.

A given protocol will be considered as satisfactory if the lower limit of the estimated D.S. is equal or higher than the real D.S.

Remark. Recent results (Doolan et al., 1985; Dwyer et al., 1985) show the possible existence of spikes of contamination. The effect of such spikes is equivalent to an increase of the mean con-

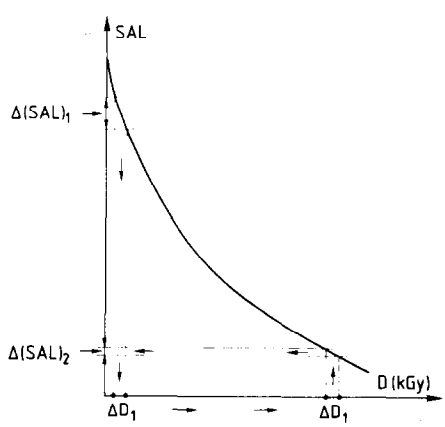

Fig. 6. Impact of a variation of the initial SAL on the level of SAL at the sterilization dose.

tamination and of the dose required to reach a given SAL. It seems, however, that the order of magnitude of this variation of SAL is only 0.3 unit. It is located in the initial part of the curves of Fig. 4, and thus will not much modify the final SAL at the D.S., as shown in Fig. 6, due to the difference of the slopes between the initial and the final parts of the curves.

Validation by simulation

We shall hereafter analyse the main questions for checking the validity of the method. The contaminations are characterized by the radioresistance (populations A–F of Fig. 4) and the initial number of contaminants (1–1000 micro-organisms per item). A contamination B 100 is a contamination of 100 micro-organisms per item, those organisms belonging to population B.

Limits of validity

To be acceptable, the method must validate a dose equal to or higher than the true D.S., for the every contaminations covered by the present usual specification (i.e. a D.S. $\leqslant$ 25 kGy, an initial contamination $\leqslant$ 100 organisms/item, and an average maximum D_{10} of 3.125 kGy). This can be checked by simulating the use of the method for a range of populations and initial numbers of contaminants inside those limits or in the border-line cases. The results are shown on Table 1. In every case belonging to the specification, the estimated D.S. is equal to or higher than the real D.S.. Even in the cases having one characteristic (initial number of contaminants or radioresistance) outside the specification, the minimum D.S. which are determined are not really far from the real value. This validates the method from the point of view of the safety of the sterilization process.

On the other hand, the minimum D.S. which can be estimated will be found when all the samples of the test will be sterile at a D_t of 0 kGy. It varies slightly with the number of samples, but it is in the range 15–17 kGy as shown in Table 2. For productions having a low number or radiosensitive contaminants (e.g. A_1 or B_1, where D.S. are 8.5 and 12 kGy) this is a relatively large overestimation, but anyway far smaller than the conventional 25 kGy.

TABLE 1

Limits of validity of the method

Population	Initial contamination (micro-organisms/item)			
	1000	100	10	1
A	+	+	+	+
B	+	+	+	+
C	−1	+	+	+
D	–	–	−1/−3	+
E	–	–	−5/−6	−2/−3

+ = validated DS higher or equal to true DS; negative values = difference between validated DS and true DS (kGy). Conditions of simulation: 50 samples; homogenous contamination. (On the border of the limit of validity, the difference in kGy between the estimated DS and the real DS is indicated in parentheses). – = $>10^{-6}$ (not validated).

TABLE 2

Lowest estimated D.S.

Number of samples tested	20	50	100
P_{max}	0.21	0.10	0.05
$DS = (6 - \log P_{max}) \times 3.125$ (kGy)	17	16	15

TABLE 3

Probabilities of finding 0, 1, 2... n non-sterile items in a sample of 10^6 items with a proportion of 10^{-6} non-steriles

Number of non-sterile	Probabilities (%)	
	Homogenous contamination	Heterogeneous contamination *
0	36.8	40.9
1	36.8	34.5
2	18.4	15.6
3	6.1	5.4
4	1.5	2
5	0.3	1
6	0.1	0.6
7	–	0.4
8	–	0.3
9	–	0.2
10	–	0.1

* Dispersion of the number of contaminants is that considered in AAMI document

TABLE 4

Effect of the dispersion of the number of contaminants per item on the determination of D.S.

Population	D_t (kGy)	Minimum and maximum number of non-sterile		Range of P_{max} used for estimating D.S.		Range of estimated D.S.	
		Ho	He	Ho	He	Ho	He
C 100	5	2–13	1–34	0.17–0.45	0.14–0.85	21.3–22.6	21.1–23.5
	7	0–14	0– 5	0.10–0.23	0.10–0.26	22.6–23.8	22.6–23.8
C 1000	7	5–19	2–48	0.26–0.57	0.17–1.00	23.9–25.0	23.3–25.7
	8	1–10	0–16	0.14–0.38	0.05–0.51	24.1–25.4	24.1–25.4

Ho = homogenous, He = heterogenous number; 50 samples tested.

Influence of the dispersion of the contamination

The previous analysis was carried out in the case of an evenly distributed contamination. In practice, the bioburden is distributed heterogeneously over the items. What is the influence of this dispersion on the result? Let us first consider the case of two sets of 10^6 items, respectively homogeneously and heterogeneously contaminated. Table 3 gives the probabilities of finding 0, 1, 2... contaminated items in both samples. Those probabilities differ to a very limited extent. In a more direct way, Table 4 gives an example of the applications of the method and compares the results obtained with an homogeneous contamination and in the case of the dispersion of AAMI protocols. It can be seen that such a dispersion does not modify significantly the values of D.S.

Size of the sample

The maximum proportion of contaminated items in the production is statistically estimated to a given level of probability from the number of positives in the sample. From this point of view, a decrease in the size of the sample leads to a somewhat larger range of the estimates of this proportion. In fact, the increase of the maximum proportion of positives of the population at the testing dose D_t does not change much the final estimated value of D.S., as shown in Table 5 through two examples. From the point of view of

TABLE 5

Influence of the size of the sample

Population *	D_t (kGy)	Number of samles submitted to a sterility test	Range of the determined sterilizing dose DS **	Real DS (kGy)
B–100	3	50	20.6–21.3	18
		30	20.6–21.4	18
		20	20.7–21.4	18
		15	20.7–21.6	18
		10	20.7–21.6	18
C 100	4	50	21.3–22.1	21.3
		30	21.3–22.2	21.3
		20	21.3–22.4	21.3
		15	21.3–22.5	21.3
		10	21.3–22.5	21.3

* Homogenous contamination

** Maximum proportion of non-sterile in the population estimated at 99% confidence level.

mathematical statistics, it would then be possible to estimate the proportion of contaminated samples of a production series, with an accuracy sufficient for the further estimation of D.S. from a very low number of testings. On the other hand, the sampling has to be sufficient to take into account the possible variations of the number of contaminants from one item to another. For this reason it would not seem reasonable to reduce the number of testings to less than 50 samples.

Influence of the dose of testing on the estimated value of D.S.

Let us assume that samples are submitted to successive increasing doses, and that the procedure described above is applied for determining the D.S. at each of those doses. Qualitatively, it is clear that, for the lowest doses, all the tested samples will be positive, allowing no estimation of the SAL. Then the probability of finding positive samples will increase. If the first few samples give steriles, this will allow an estimation of a maximum proportion of positives in the population, of the corresponding level of SAL, and the extrapolation to the D.S. For still higher doses eventually, all the samples will be sterile. They will still allow the estimation of D.S., but with a larger excess. If higher testing doses, above 7–8 kGy, are used the validated D.S. become too high to be of interest.

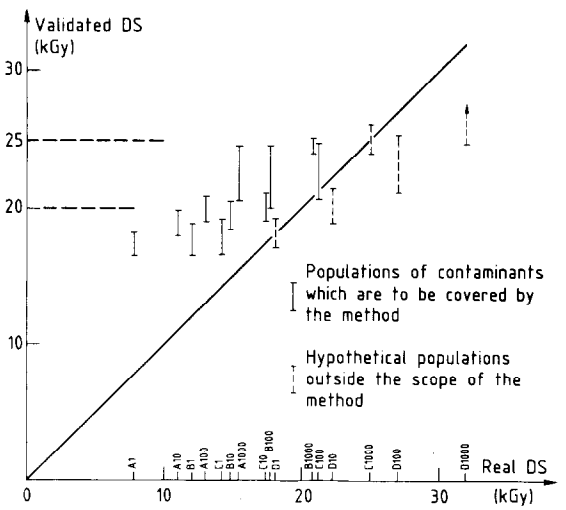

Fig. 7. Comparison of the validated D.S. and the real D.S. for 16 AAMI populations, after application of 3 experimental doses ($D_t = 1 - 3.6 - 8.6$ kGy, sets of 50 samples).

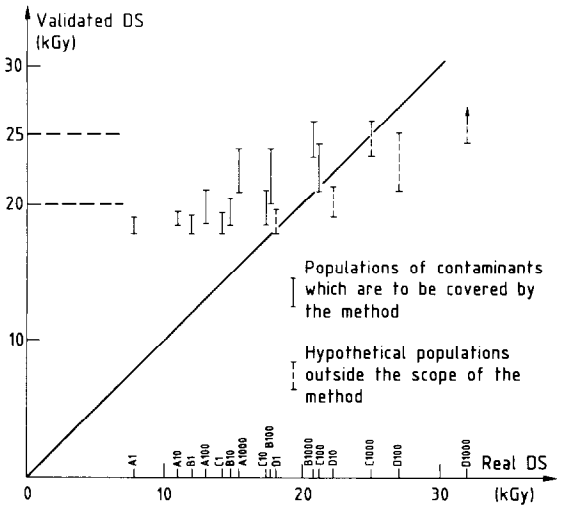

Fig. 8. Comparison of the validated D.S. and the real D.S. for 16 AAMI populations, after application of two experimental doses ($D_t = 0 - 3 - 7$ kGy, sets of 50 samples).

Practical use of the method

Qualification of a D.S.

It is practically not very convenient to apply a range of successive increasing doses to sets of samples of a production. This multiplies the number of sterility testings and the number of different irradiations. But we just saw that the D.S. can be determined from testings in a range of testing doses, provided that one accepts that the D.S. so determined is not exactly the minimum dose which can be estimated. Fig. 7 shows the range of the D.S. which will be validated by applying the 3 D_t 1, 3.6, and 8.6 kGy. The use of such a "3 testing doses procedure" does lead to D.S. achieving a SAL of -6 for all the cases which are to be considered. The D.S. which are so determined are in many cases appreciably lower than 25 kGy. The D.S. determined from slightly different D_t (e.g. 3 and 3.6, or 8 and 8.6 kGy) are not much different. This means that the experimental D_t can be somewhat different from the intended doses. This fact is important, as it is often experimentally difficult

TABLE 6

Determination of D.S. from various samplings of production

Item	D_t testing dose (kGy)	Facility	Positives samples	Positive fraction	Maximum positive fraction	D.S. * (kGy)
Operative fields 1 × 1	0	–	26/50	0.52	0.68	18.2
	3.4	CARIC	3/50	0.06	(0.20)	(20.0)
Occlusive dressings	0	–	25/50	0.5	0.66	18.2
	3.4	CARIC	2/50	0.04	(0.17)	(19.8)
Air inlets	0	–	19/50	0.38	0.54	17.9
	3.4	CARIC	1/50	0.02	(0.14)	(19.5)
	3.5	CSVT	0/50	0	(0.10)	(19.2)
Biological needles	0	–	37/50	0.74	0.87	18.6
	3.9	CARIC	0/50	0	(0.10)	(19.6)
Compress	0	–	50/50	–	–	–
	1.1	CARIC	43/50	0.86	0.97	19.8
	3.4	CARIC	21/50	0.42	(0.58)	(21.4)
Lockable blood plasma sets	0	–	50/50	–	–	–
	1.1	CARIC	46/50	0.92	(1.02)	(19.9)
	3.4	CARIC	33/50	0.66	0.80	21.8
One connector blood plasma set	0	–	49/50	0.98	1	–
	1.1	CARIC	35/50	0.70	0.83	19.6
	3.4	CARIC	24/50	0.48	(0.64)	(21.5)
Latex gloves	0	–	50/50	–	–	–
	3.4	CARIC	38/50	0.76	0.88	22.0
Drains	0	–	50/50	–	–	–
	1.1	CARIC	40/50	0.80	0.92	19.7
	3.4	CARIC	6/50	0.12	(0.27)	(20.4)
	3.5	CSVT	3/50	0.06	(0.20)	(20.0)
1.2 m blood plasma sets	0	–	50/50	–	–	–
	1.1	CARIC	44/50	0.88	0.99	19.8
	3.4	CARIC	7/50	0.14	(0.30)	(20.5)
"Cicagraph"	0		10/50	0.20	0.36	17.4
	3.6	CARIC	1/50	0.02	(0.14)	(19.7)
	8.6	CARIC	4/50	0.08	(0.22)	(25.3)
	3.6	CSVT	2/50	0.04	(0.17)	(20.0)
	8.6	CSVT	0/50	0	(0.10)	(24.3)
"Cicaplaie"	0	–	18/50	0.36	0.53	17.8
	3.6	CARIC	3/50	0.06	(0.20)	(20.2)
	8.6	CARIC	2/50	0.04	(0.17)	(25.0)
	3.6	CSVT	3/50	0.06	(0.20)	(20.2)
	8.6	CSVT	0/50	0	(0.10)	(24.3)
Finger-stalls Package of 25	0	–	50/50	–	–	–
	1.1	CARIC	31/50	0.62	0.76	19.5
	3.6	CARIC	1/50	0.02	(0.14)	(19.7)

TABLE 6 (continued)

Item	D_t testing dose (kGy)	Facility	Positives samples	Positive fraction	Maximum positive fraction	D.S. * (kGy)
Finger-stalls package of 100	0	–	50/50	–	–	–
	1.1	CARIC	26/50	0.52	0.68	19.3
	3.6	CARIC	1/50	0.02	(0.14)	(19.7)
Obturators IVS	1.2	CARIC	21/250	0.084	0.14	17.3
	3.9	CARIC	4/248	0.016	(0.05)	(18.6)
Spatula Dow Corning	0	IRE	0/50	0	0.10	15.7
	3.6	IRE	0/100	0	(0.06)	(18.5)
Catalyst 1.2 ml	0	IRE	0/50	0	0.10	15.7
	3.6	IRE	0/100	0	(0.06)	(18.5)
Base SFD 20 g	0	IRE	0/50	0	0.10	15.7
	3.6	IRE	0/100	0	(0.06)	(18.5)

CARIC = electron beam irradiation; CSVT (France) and IRE (Belgium) = γ-irradiation. Values in brackets = a lower DS has already been validated.

to reach exactly an intended dose. The use of the set 0 – 3 – 8 kGy (Fig. 8) gives satisfactory results with only two irradiations at two doses. That makes the procedure very economical.

Control procedure

Once the D.S. is validated, it will be necessary to periodically check that it remains valid. For this, 50 samples will be irradiated at a suitably chosen D_t, as shown in the following example:

- D.S. to be audited: 20 kGy
- maximum number of positives accepted: 3 (this number is chosen to take into account the possible false positives)
- for 3 positives over 50 samples: $P_{max} = 0.2$, or SAL $\leqslant -0.70$
- $D = \text{D.S.} - D_t = (6 - 0.70) \times 3.125 = 16.6$ kGy
- then testing dose $D_t = 20 - 16.6 = 3.4$ kGy. If the number of positives is less than 3, the D.S. is validated.

Experimental study of various products

Several samples of various products were studied by applying the procedure described above as a conclusion of this work. The results are shown in Table 6. They show that in those cases, D.S. significantly lower than 25 kGy are sufficient for reaching a SAL equal to -6.

It is shown that the irradiation of a sample to a substerilizing dose allows to estimate an intermediate level of SAL, from which the sterilizing dose can be extrapolated by using a maximum value of the D_{10} of the remaining micro-organisms. The analysis is carried out by stimulation, using various initial contaminations and profiles of radiation resistances. The use of at most 3 testing doses will be sufficient to validate reduced sterilization doses if they are suitable, which leads to a very economical method, even if it does not allow to validate the lowest achievable sterilizing doses.

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