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Research Article

The limits of sterility assurance

Die Grenzen der Sterilisationssicherheit

Abstract

Sterility means the absence of all viable microorganisms including viruses. At present, a sterility assurance level (SAL) of 10^{-6} is generally accepted for pharmacopoeial sterilization procedures, i.e., a probability of not more than one viable microorganism in an amount of one million sterilised items of the final product. By extrapolating the reduction rates following extreme artificial initial contamination, a theoretical overall performance of the procedure of at least 12 lg increments (overkill conditions) is demanded to verify an SAL of 10^{-6}. By comparison, other recommendations for thermal sterilization procedures demand only evidence that the difference between the initial contamination and the number of test organisms at the end of the process amount to more than six orders of magnitude. However, a practical proof of the required level of sterility assurance of 10^{-6} is not possible. Moreover, the attainability of this condition is fundamentally dubious, at least in non-thermal procedures. Thus, the question is discussed whether the undifferentiated adherence to the concept of sterility assurance on the basis of a single SAL of 10^{-6} corresponds with the safety requirements in terms of patient or user safety, costs and energy efficiency. Therefore, in terms of practical considerations, a concept of tiered SALs is recommended, analogous to the comparable and well-established categorization into “High-level disinfection”, “Intermediate-level disinfection” and “Low-level disinfection”. The determination of such tiered SALs is geared both to the intended application of the sterilized goods, as well as to the characteristics of the products and the corresponding treatment options.
In the case of aseptic preparation, filling and production procedures, a mean contamination probability of 10^{-3} is assumed. In automated processes, lower contamination rates can be realized. In the case of the production of re-usable medical devices, a reduction of at least 2 lg increments can be achieved through prior cleaning in validated cleaning and disinfecting devices. By chemical disinfection, a further reduction of ≥5 lg increments is achieved. In the case of sterilized surgical instruments, an additional concern is that they lay opened in contaminated air for the duration of the operation, at least in conventionally ventilated operating theaters. Finally, the amount of pathogens necessary to cause an infection must be considered. By logical consideration of all aspects, it seems possible to partially reduce sterility assurance levels without any loss of safety. Proceeding from this, we would like to make the following suggestions for tiered SAL values, adjusted according to the respective sterilization task:

- SAL 10^{-6} for heat-resistant pharmaceutical preparations (parenterals), suggested term: “Pharmaceutical sterilization”,
- SAL 10^{-4} for heat-resistant medical devices, suggested term: “High-level sterilization”,
- SAL 10^{-3} for heat-sensitive re-usable medical devices, under the precondition of a validated cleaning efficacy of >4 lg increments, suggested term: “Low-level sterilization”.

Keywords: sterility, sterility assurance level (SAL), draft of tiered SAL values

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Zusammenfassung

Sterilität bedeutet die Abwesenheit aller vermehrungsfähigen Mikroorganismen einschließlich Viren. Derzeit wird für Sterilisationsverfahren ein Sterilitätssicherheits-Wert (SAL-Wert) von 10^{-6} gefordert, d.h. in einer Menge von einer Million sterilisierten Gütern darf höchstens ein lebensfähiger Mikroorganismus zu erwarten sein. Durch Extrapolation der Mikroorganismen-Reduktionsraten nach artifizieller extremer Ausgangskontamination (=10^{6} Test-Mikrorganismen pro Prüfobjekt) wird zum Nachweis eines SAL-Werts von 10^{-6} eine theoretische Gesamtreduktionsleistung des Verfahrens um mindestens 12 lg-Stufen abgeleitet („Overkill"). Demgegenüber verlangen andere Empfehlungen lediglich den Nachweis, dass die Differenz zwischen Ausgangszahl und Zahl der Testorganismen nach Ende des Prozesses mehr als sechs Zehnerpotenzen beträgt. Da der praktische Nachweis des geforderten Sterilisationssicherheitsniveaus von 10^{-6} unmöglich ist und zumindest bei nichtthermischen Verfahren die Erreichbarkeit dieses Zustands grundsätzlich zweifelhaft ist, wird die Fragestellung diskutiert, ob ein undifferenziertes Festhalten an dem gegenwärtigen praktizierten Konzept der Sterilisationssicherheit auf der Basis eines SAL-Wertes von 10^{-6} unter dem Aspekt der Patienten- bzw. Anwendersicherheit sowie der Kosten und des Energieverbrauchs den tatsächlichen Sicherheitsanforderungen entspricht.

Unter praktischen Gesichtspunkten wäre daher ein Konzept von abgestuften SAL-Werten analog der Differenzierung in „High-level", „Intermediate-level" bzw. „Low-level disinfection" sinnvoll, deren Festlegung sich sowohl an der vorgesehenen Anwendung des Sterilisierguts als auch an dessen Eigenschaften und den damit verbundenen Behandlungsmöglichkeiten orientiert.

Bei aseptischen Zubereitungs-, Abfüllungs- und Herstellungsverfahren wird von einer mittleren Kontaminationswahrscheinlichkeit von 10^{-3} ausgegangen, bei automatisierten Prozessen können auch geringere Kontaminationsraten realisiert werden. Im Fall der Aufbereitung wieder verwendbarer Medizinprodukte ist durch die vorausgehende Reinigung in Reinigungs-Desinfektions-Geräten eine Reduktion um mindestens 2 lg-Stufen erreichbar. Durch die chemische Desinfektion wird nach der Reinigung eine weitere Reduktion um ≥5 lg-Stufen erreicht. Bei sterilisiertem chirurgischem Instrumentarium kommt hinzu, dass das Sterilgut im konventionell belüfteten Operationssaal für die Dauer der Operation geöffnet auf dem Instrumentiertisch lagert. Schließlich muss auch die Erregermenge berücksichtigt werden, die eine Infektion auszulösen vermag. Bei konsequenter Berücksichtigung aller Teilaspekte erscheint es möglich, die terminale Sterilisationsbehandlung ohne Sicherheitsverlust deutlich zu reduzieren. Hierfür wird ein Vorschlag für an die jeweilige Sterilisationsaufgabe angepasste abgestufte SAL-Werte unterbreitet:

- SAL 10^{-6} für thermostabile Arzneizubereitungen, vorgeschlagener Terminus: „Pharmazeutische Sterilisation",
- SAL 10^{-4} für thermostabile Medizinprodukte, vorgeschlagener Terminus: „High-level-Sterilisation",
- SAL 10^{-3} für thermolabile Medizinprodukte unter der Voraussetzung einer validierten Reinigungseffektivität >4 lg-Stufen, vorgeschlagener Terminus: „Low-level-Sterilisation".

Schlüsselwörter: Sterilität, Sterilitätssicherheits-Wert (SAL), Konzept abgestufter SAL-Werte

The concept of sterility assurance – a compromise between overkill, viability and safety requirements

Sterility of a product or object means the complete absence of viable microorganisms, including viruses, which could pose a risk during administration [67], [81], [61], [22]. A sterility assurance level (SAL) of 10^{-6} is currently required for sterilization procedures, i.e., a probability of not more than one viable microorganism in one million sterilized items of the final product [18].

The inherent problem with these requirements is that evaluating the success of such sterilization by means of a final inspection is all but impossible, since contamination rates on the order of an SAL of 10^{-6} cannot be recorded in experiments [59], [67], [46], [26].

Thus, model situations have to be created, with the help of which conclusions can be drawn regarding the treatment conditions necessary to attain sterility meeting the SAL. Therefore, representative test organisms with a maximum resistance to the procedure to be examined are used for the purposes of auditing and qualifying sterilization procedures. Consequently, the inactivation of such highly resistant microorganisms encompasses all less resistant organisms, including most pathogens. Furthermore, the test organisms should be cultivable under simple and most easily reproducible conditions. In general, innocuous bacterial endospores fulfill these requirements [41], [50], [31], [4]. In order to verify the efficacy of sterilization procedures, and to determine treatment parameters, extremely large volumes of these test organisms are used, typically $\geq 10^6$ bacterial spores per test object, e.g., in the form of bio-indicators [18].

When using the "Half-cycle method", the action of half of the intended sterilization cycle – usually half of the treatment period – is examined. If, following this action, a certain number of bio-indicators contaminated with 10^6 resistant bacterial spores are inactivated, it can be concluded that when applying the full cycle, an SAL of 10^{-6} is guaranteed at a theoretical spore-inactivation rate of ≥12 lg increments. This corresponds to "Overkill conditions" [37], [41], [30], [26].

However, in a course of action of this kind, which is based on the complete inactivation of a limited number of test objects, it must be taken into account that there is a statistical connection between the mean number of contaminated test objects after treatment and the total number of identically treated test objects. Accordingly, the more test objects are included in the test, the greater the duration of treatment, concentration or dosage of antimicrobially active agents must be to completely inactivate a limited number of bio-indicators [66], [64], [87], [65].

In contrast, other directions and recommendations – also for heat sterilization procedures – only demand evidence that the difference between the initial number of test microorganisms and the number of test organisms at the end of the process amount to more than six orders of magnitude, i.e., an inactivation rate ≥6 lg increments in order to consider the SAL of 10^{-6} as attained and indicate the product treated in this way as sterile [14], [3].

What all methods used to prove the efficacy of sterilization procedures have in common is that the conditions necessary to attain an extremely low probability of contamination of 10^{-6} are inferred from the treatment conditions required to reduce extremely high artificial test contaminations. This procedural method is based on the general assumption of exponential inactivation kinetics for microorganisms under the influence of antimicrobially effective parameters, from which a linear mortality curve results, given a semi-logarithmic diagram.

There have been detailed trials and discussions, in particular concerning heat inactivation kinetics. As early as 1921, Bigelow put forward logarithmic inactivation kinetics for microorganisms under the influence of heat. The monograph by Konrich and Stutz [35], which was considered to be a standard work for many years, as well as works by Machmerth [39], Pflug and Holcomb [48], Russell [51], Gould [23] and Knöller [34] provide detailed analyses and discussions of the relevant literature, including experiments on this issue. The inactivation kinetics for microorganisms initially postulated for heat treatments, which corresponded to first-order reaction kinetics, was also applied in principle to the conditions of the action of ionizing irradiation as well as chemical agents with antimicrobial effect [28], [83], [63], [42], [53].

Divergence from a strict semi-logarithmic course of the mortality curve is usually explained by inhomogeneity of the test organism populations used. The actual complexity of microbiological inactivation kinetics cannot be clearly and comprehensively described using simple mathematical models, so that there is no uniform theory which takes into account all possible courses of mortality curves for the inactivation of microorganisms under the influence of noxa with antimicrobial effect [83]. Consequently, while in many cases the assumption of the linearity of inactivation kinetics in the semi-logarithmic standard simplifies the actual circumstances, it offers the only practicable possibility for interpretation and utilization of data gained through experiments [39], [48], [37], [81], [34]. The current European Pharmacopoeia also puts forward the view that "the inactivation of microorganisms by physical or chemical means follows an exponential law..." [18].

The fact that the objective of sterilization is to ensure a rate of microbial contamination of $\geq 10^{-6}$ surviving microorganisms per test object results in the necessity of extrapolating mortality curves from the area that can be recorded in experiments to determine SAL-compatible sterilization parameters. As the description of the inactivation kinetics by means of a simple mathematical model, such as first-order kinetics, already shows an approximation within the area ascertainable in experiments, the associated uncertainties must considerably increase in the case of extrapolation outside this area [83]. Nevertheless, procedural parameters intended to guarantee the attainment of sterility that meets the SAL are usually defined on the basis of extrapolation of this kind [41], [48], [4].

Thus, both the determination of procedural parameters for sterilization processes and the proof of a SAL $\leq 10^{-6}$ as the quantitative end-point which has to be guaranteed by a sterilization process are not based on scientifically proven data, but are only rules of thumb and approximate values [34].

Conclusion: Using the SAL concept as a basis for the evaluation of the performance of sterilization procedures constitutes a situation (which is probably unique) in which the certain attainment of a condition is required by law, but there is no way of proving the attainment of this condition in practical terms [22], [25], [24], [2]).

Concept for proving sterilization assurance for heat-sensitive medical devices

In our own tests to examine new concepts for the gentle sterilization of irritable goods, applying non-thermal physical and/or chemical treatment procedures and using *Bacillus subtilis* spores as test organisms, test conditions were selected that, after treatment, either viable test organisms were still in evidence on the test objects or both sterile and unsterile test objects were present simultaneously. By combining direct cell counting methods using classical microorganism recovery and counting techniques [65], [48], [1] with the "fraction negative method" [64], [65], [48], [62], mortality curves on the basis of experimental data were maintained in a range of about 8 orders of magnitude ($\geq 10^6$ to around 10^{-2} test organisms per test object). With reference to the known initial contamination of the test objects, exact reduction factors could be stated for the treatment parameters applied in each case, to derive from these the treatment conditions necessary to attain sterility assurance in accordance with the SAL [73], [74], [75].

Under this condition, the attainable degree of reduction of the test organisms can be exactly quantified on the one hand, and the actual inactivation kinetics can be depicted on the other, at least in the range that can be recorded using microbiological methods of proof. Additionally, possible inhomogenities of the mortality curves can be taken into account for extrapolation into the SAL area.

Conclusion: Exact quantitative statements as to the inactivation kinetics and, accordingly, the antimicrobial efficacy can be made only if not all test objects are fully inactivated, starting from a known initial contamination following sub-effective treatment. Thus, for the quantitative characterization of the efficacy of antimicrobial procedures, the experimental conditions are to be chosen so that in the result of treatment, surviving test organisms are detectable for various individual treatments.

Various experiments by the current authors demonstrated that inactivation kinetics within the experimentally accessible range between 10^6 and 10^{-2} bacterial spores per test object never exhibited a linear course [33], [77], [76], [74], [75]. In part, there were very pronounced concave curves, consisting of an initial steep section which then levelled off. At least with the non-thermal antimicrobially active treatments examined, it was apparently possible to reduce the high starting incidence in the test objects to a low level using relatively short action duration, low substance concentrations or low irradiation. However, the efficacy against the residual contamination was considerably lower. Consequently, an extrapolation of the steep section of the respective inactivation curve into the SAL range of 10^{-6} would result in treatment durations, irradiation doses or substance concentrations which are actually much too brief to guarantee an adequate sterility assurance. On the other hand, an extrapolation of the second, flat part of the mortality curve would result in all cases in extreme sterilization conditions, which could not be applied practically.

Analogous mortality curves can be found again and again primarily in older publications. Seidl et al., for example, reported on experiments on radio sterilization of medicinal products, in which it was possible to destroy 99.9% of a test organism population with an irradiation dose of 0,1 Mrad (1 kGy); for the remaining 0.1%, however, a dosage at least five times stronger was necessary [57]. Pronounced concave mortality curves for *Bacillus subtilis* spores dependent on varying gamma irradiation doses can also be found in Wallhäußer [80]. Pfeiffer also reports non-linear inactivation kinetics when applying ionizing irradiation, because of disproportionate survival of radiation-resistant microorganisms in the range of higher irradiation doses [45], [47]. Furthermore, various works on sporocidal efficacy of hydrogen peroxide show concave, flattening-out mortality curves [72], [11], [5]. Van Ooteghem describes non-linear survival curves for microorganisms under the influence of preservatives [71].

Such inactivation curves, also called "tailing" curves, are explained predominantly (e.g., by Hermann [28] as well as Wickramanayake and Sproul [83]) by the existence of microorganism populations on the test object with inconsistent resistance to the antimicrobially active treatments examined. Consequently, the less resistant fraction is killed first (steep curve section); the predominance of the surviving, more resistant fraction then results in a flatter course of the mortality curve. Spicher explains this phenomenon in detail, coming to the conclusion that the determination of the parameters of a sterilization procedure must be oriented to such extreme values represented by highly resistant test organism fractions, since that reflects the actual circumstances [67].

The range between experimentally detectable contamination rates up to ca. 10^{-2} test organisms per test object and the SAL of 10^{-6}, still encompasses 4 lg increments, which cannot be proven by experimental data. Due to the inhomogeneity of the inactivation kinetics already present in the experimentally accessible range, it is not possible to make any certain statement regarding the continuing course of such mortality curves, which may increasingly flatten out. Spicher already in 1993 expressly pointed out that, in unfavorable cases, the highly resistant test

organism fractions, and thus the flat part of the mortality curve, may no longer be determined using the usual microbiological testing procedures. As a result, the customary extrapolation of such curves into the SAL range involves considerable risks [67].

Thus, it is clear that the general assumption of first-order exponential inactivation kinetics, which was used originally to describe heat inactivation of bacteria, cannot be applied without restrictions to non-thermal processes. One approach for the interpretation of these experimental results is the hypothesis that there is a basic difference between thermal and non-thermal antimicrobial modes of action, from which follows the assumption that such non-linear mortality curves are not attributable solely to the inhomogeneity of the test populations. Possibly, they display a characteristic that can be generalized for all inactivation procedures based on non-thermal actions [77], [76]. From those treatment parameters that effect elimination of the conventionally high test organism numbers present on bioindicators for sterilization control, it is therefore not permissible – for sterilization procedures in which efficacy relies on irradiation and/or chemical effects – to directly derive treatment conditions which are intended to guarantee a reduction into the experimentally no longer detectable but for sterilization assurance essential range. Consequently, treatment conditions that ensure an SAL-compliant reduction up to a contamination rate of at least 10^{-6} cannot be clearly determined for such procedures. The conclusion to be drawn from this is that when non-thermal treatment procedures are applied, the condition of sterility that would conform with a contamination probability of 10^{-6} cannot in fact be guaranteed. Thus, the SAL concept is not a procedure suitable for showing the efficacy of non-thermal sterilization processes.

Consequently, only steam sterilization and sterilization using dry heat should be described as sterilization procedures in the proper or traditional sense.

Sterility according to a SAL of 10^{-6} should, logically, only still be required for medical devices and preparations that can be subjected to steam or hot air sterilization using the required standard and equivalent procedures. This is because it is possible that a homogeneous linear mortality curve, and thus the sufficiently certain determination of the treatment conditions necessary to guarantee an SAL of 10^{-6}, can be presumed only in thermal procedures.

Other authors who carried out a detailed mathematical analysis of the intrinsic uncertainties of the exponential model of mortality of test organisms reach the same conclusions in principle (while also taking into account thermal inactivation kinetics). They explain that for decades, this evidently inadequate theoretical basis has been adhered to without question, using the argument that, due to extreme safety premiums, the safety of sterilized products in practice is secured by assuming higher contamination rates with extremely resistant test organisms when examining sterilization procedures [8], [9].

In order to ensure the highest possible level of safety in the application of non-thermal procedures, a proof of “Antimicrobial efficacy on the highest experimentally accessible level” should be required.

This proof should show that the inactivation kinetics dependent on the number of test organisms in the entire range ascertainable in experiments can be evidenced with performance data. As a rule, the performance characterization for non-thermal antimicrobial procedures should be carried out using test bodies contaminated with low levels of highly resistant test organisms. This is in order to reflect the fact that, apparently in contrast to the relatively simple option of reducing high numbers, the inactivation of low levels of residual contamination is disproportionately more difficult to achieve. Test microorganisms with a high level of resistance to the procedure to be examined should be used as test organisms, e.g., bacterial spores. It must be proven that a reduction of the number of test organisms by at least five lg increments up to a contamination rate of 10^{-2}, which can only just be proven in experiments, has been achieved. In general, only the strict concentration on data that can be recorded in experiments affords the possibility of being able to directly compare various procedures and procedural steps using inactivation kinetics and, consequently, to make available differing, but equivalent inactivation procedures for various products. The extrapolation of such inactivation kinetics recorded in experiments by merely one additional lg increment to a contamination level of 10^{-3} would guarantee a sufficient "Safety premium” for the determination of the necessary treatment parameters. Here, a “tiered” SAL of 10^{-3} could be introduced for non-thermal sterilization procedures. This value is also referred to repeatedly in the literature on this subject [22], [24], [86].

In order to differentiate it from actual sterilization with an SAL of 10^{-6}, which should be restricted to thermal procedures, such a gentle sterilization procedure targeted at a contamination probability of 10^{-3} could be called ”Low-level sterilization”. The efficacy of ”Low-level sterilization” concentrates primarily on the range of low levels of residual contamination with highly resistant microorganisms on goods to be sterilized, following effective preparation (aseptic processing and/or cleaning and subsequent disinfection), which is very important in practice.

Do the current theoretical sterility assurance requirements reflect the actual safety requirements?

It was ascertained that the practical proof of the required level of sterility assurance of 10^{-6} is not possible. Moreover, the attainability of this condition is fundamentally questionable, at least in non-thermal procedures. Furthermore, it is questionable whether the undifferentiated adherence to the currently practiced concept of

sterility assurance on the basis of an SAL of 10^{-6} complies with the actual safety requirements in terms of patient or user safety [2]. The practical relevance of an SAL of 10^{-6} is not conclusively proven, i.e., it is practically impossible to find a difference in the rate of infection resulting from the application of products that were sterilized with a (theoretical) SAL of 10^{-6} on the one hand, or on the other hand were treated using a procedure in which only a contamination likelihood of 10^{-3} is ensured [85].

By extrapolating the reduction rates following extreme artificial initial contamination to an SAL of 10^{-6}, a theoretical overall performance of the procedure can be established by at least 12 lg increments (overkill conditions). Such an SAL, however, is neither evidence based nor can it be accurately ascertained in experiments, or attained practically.

Furthermore, it must be taken into consideration that, by adhering to an SAL of 10^{-6} as a "gold standard" for sterilization, unnecessarily high costs are incurred, the introduction of new sterilization procedures is made considerably more difficult [2], and no allowance is made for the requirement of sustainable development.

Therefore, for practical reasons, a concept of tiered SALs would make sense, the establishment of which is oriented towards the intended application of the sterilized goods, the characteristics of such goods, and the corresponding treatment options. In disinfection procedures, an analogous categorization of "High-level disinfection", "Intermediate-level disinfection", and "Low-level disinfection", has been customary for some time already [69], [10], [52], [55], [60].

Since the mid-1990s, there have been repeated suggestions for the definition of tiered sterility assurance values, such as a "Concept of tiered SALs", the suggestion of the declaration of "Asepsis assurance values" [22], [86], or a declaration as "Aseptic: Safe for its designated use", which is oriented more towards the intended application of the product [24], [2].

The determination of the respective safety level should be oriented towards both the features and the quality of the products (e.g., new products being used for the first time and single-use products, as opposed to re-usable and re-processed goods etc.), as well as the intended application.

In practice, in the course of modern quality-controlled production and preparation procedures for pharmaceuticals and medical devices, only a very low level of contamination is to be expected. In the case of aseptic preparation, filling and production procedures, a contamination probability of 10^{-3} is assumed; lower contamination rates can also be realized in automated processes [18], [45], [41], [85], [81], [17], [24], [13], [44]. For the use of gamma radiation for sterilization, possibilities of determining dosage by ascertaining the number, type and radiation resistance of microbiological contaminants on the goods to be sterilized have been discussed and applied in practice for several years [45], [49], [27], [47].

The possible inclusion of the real contamination risk for the products to be sterilized in the course of the quality control of production processes could become general practice to determine the necessary sterilization treatment conditions and the resulting level of safety. This would also correspond to the suggested concentration of the proof of efficacy of a sterilization procedure on the low level of residual contamination with more highly resistant microorganisms.

In addition to the degree of contamination prior to sterilization, the period between sterilization and intended use as well as the risk of recontamination and/or proliferation of microorganisms during the storage period must also be taken into account. Thus, e.g., for aqueous parenterals, it is by all means reasonable to demand an SAL of 10^{-6}.

In the case of the production of re-usable medical devices, a reduction of at least 2 lg increments can be achieved through prior cleaning in validated cleaning and disinfecting devices (CDD). In CDDs for containers for human egesta, the reduction rate was 3 to >5 lg increments, depending on the procedure [82]. Due to the requirements for chemical disinfection, a further reduction by ≥5 lg increments is achieved through disinfection following cleansing in the reprocessing procedure [21]. This means that because of the preceding reprocessing procedure for medical devices, at least 7 lg increments are usually added to the actual sterilization performance. Based on a consistent consideration of the reduction performance of all partial steps of reprocessing and sterilization procedures, it would be possible to markedly reduce the final sterilization treatment without a reduction in safety. The prerequisite for this is the validation of all partial process steps, which is required in any case.

In the case of sterilized operating instruments, an additional factor must be taken into account in the risk assessment. In conventionally ventilated operating theaters, sterile goods are removed from their sterile packaging in the theater and are stored opened on the instrument table for the duration of the operation. During this time, they are exposed to the risk of contamination from sedimentation of airborne or particle-borne pathogens, released mainly by the operating team. Even in the case of low-turbulence displacement flow (LDF) and subject to the premise that the sterile goods are opened within the LDF and the table of instruments is also situated completely within the LDF, according to DIN 1946-2 and -4, up to 10 CFU/m^3 (CFU – colony forming units) may be contained in the airflow following the filter emissions of the ventilation equipment (around 10 cm apart) in an empty, unused, and previously cleaned and disinfected operating theater [15], [16]. The pathogens released by the operating team, which cannot be fully removed by the LDF, are added to this. Thus, the overkill sterilization safety level currently required is disproportionately high compared to the actual probability of contamination of the operating instruments following sterilization [58].

The infection dose must also be taken into account as a further factor in the risk assessment. With the exception of a possible divergent infectivity specific to a certain strain, one can assume that, as a rule, fewer than 10^5 bacteria per 1 g of tissue are not sufficient for the creation

of a manifest wound infection. In the case of special infectivity, however, even small numbers of bacteria (10^3–10^4) can potentially cause infections. Other aspects, such as the location of the infection, intensity of the wound infection, irritation caused by foreign bodies, circulation, and immunity also influence the rate of infection. Thus, in the presence of suturing material, just 10^2 staphylococci per gram of tissue can be sufficient to cause a wound infection [20], [56], [25], [2]. However, even in the most unfavorable case of an infection dose $\geq 10^1$ (for which there is no evidence), this as a rule refers to one species, e.g., *S. aureus*. Since an initial contamination of operating instruments with $>10^9$ of the same species is more than unlikely (1 g of excrement contains up to 10^{11} bacteria per gram in relation to the total number, but not to one species [6]) and at least 10^7 lg increments are eliminated by the reprocessing procedure preceding sterilization; even the inactivation of 6 lg increments only in thermal procedures includes a sufficient security premium for all contamination eventualities.

Based on these considerations, we would like to make the following suggestions for discussion of tiered SAL values adjusted to correspond to the respective sterilization task [78]:

- SAL 10^{-6} for heat-resistant pharmaceutical preparations, suggested term: “Pharmaceutical sterilization”
- SAL 10^{-4} for heat-resistant medical devices, suggested term: “High-level sterilization”
- SAL 10^{-3} for heat-sensitive re-usable medical devices on the condition of prior validated cleaning efficacy >4 lg increments, suggested term: “Low-level sterilization”
- Proof of antimicrobial efficacy on the highest experimentally accessible level for all other products which, according to understanding to date, are to be applied sterile, suggested term: “Microbiologically safe for the designated use”

In addition to product damage, which is well known and which has been examined above all in connection with radiation sterilization [43], [32], [40], [84], more recent tests show that the interaction between antimicrobial treatment processes and the material characteristics or the functional features of the products treated obviously has to be assessed in a considerably more differentiated fashion than was previously believed in the case of sensitive goods that are to be sterilized [12], [36], [74], [79]. Consequently, even if the microbiological validation of gentle sterilization procedures is optimized, its use especially for sensitive goods with special areas of application may continue to be subject to considerable and possibly even more stringent restrictions. Ultimately, the extent to which an increase in the intensity of the effect of an antimicrobial noxa actually offers such an increase in microbiological safety that the possible consequences for the functionality and/or bio-compatibility can be justified, must be analyzed in detail in each individual case.

Due to the multitude of interactions between antimicrobially effective components in a procedure and the products treated, a conclusive decision about the suitable antimicrobial treatment for very specific goods can be made only following a specific risk assessment.

Thus, the sterilization procedure in each individual case of application must be optimized anew.

Generally, the result of this is that uniform and universally applicable recommendations for gentle sterilization, above all of thermolabile products, cannot be made.

Because sterility is currently demanded for products sensitive to the established sterilization methods, the use of tiered SAL values can avoid the situation in which this demand practically cannot even be met at the outset, and, consequently, these products must currently be implemented on an assurance level that is inadequately microbiologically defined in terms of its ability to prevent infection.

This sophisticated concept of sterility assurance levels would also result in the entitlement for both bacteria-retentive filtration and aseptic preparation to be classified as methods of preparation of sterile products. Although this is the case in the current version of the European Pharmacopoeia [19], to date this has been neither theoretically nor practically validated.

Furthermore, the introduction of tiered SAL levels could also make the introduction of alternative sterilization methods considerably easier.

In general, in order to guarantee a high level of microbiological safety, the entire production or preparation process, but above all the steps preceding the actual sterilization treatment, would have to be taken into account more than they have been up to now to ensure the microbiological safety of the final product. This approach, which builds on concepts already introduced in the 1980s [39], [38], [29], takes into account the effects attainable on all levels within an overall outcome to ensure the microbiological quality of a final product. With regard to the growing epidemiological importance of viruses and especially prions, which are extremely difficult to inactivate, this is the only way to ensure sufficient levels of infection-prevention safety [54], [70], [68].

In the future, it will be imperative that aspects of sterilization or sterilizability are taken into account as early as possible in the design of both products and the production and application processes involving these products, so that ultimately, an optimized strategy is adjusted to each specific product to ensure the highest possible level of infection-prevention safety. This will enable the manufacturer and the user “to build sterility into a product as opposed to building a product and testing it for sterility” [41].

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