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Current Mistaken Interpretation of Microbiological Data on Gas Plasma Sterilization

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Abstract

Even when using vegetative cells, a tailing phenomenon can be observed in survivor curves in engineering researcher's papers on gas plasma sterilization. This indicates that even disinfection was not even achieved. By definition, sterilization is a process that kills all types of microorganisms including bacterial spores and vegetative cells. In contrast, disinfection kills only vegetative cells and does not kill bacterial spores. Tailing of a survivor curve is often caused by clumping of the biological indicator (BI), and engineering researchers who make their own BI without critical knowledge, appropriate techniques and confidential know-how to avoid such clumping frequently publish nonlinear survivor curves. Preparation of a monolayer of BI is quite a difficult task even for BI manufacturers and it requires proprietary information. Therefore, engineering researchers should purchase commercially available BI. Among the BIs on the market, I recommend purchasing BI from Merck Co., as clumping was found to be minimal based on results of scanning electron microscopy (SEM) observation. When such a tailing phenomenon is observed, a SAL of 10^{-6} cannot be attained, and therefore no D value (decimal reduction value) can be determined and the exposure time for a 9 or 12 log reduction remains undefined. The D value must be determined from the straight line of a 9 log or 12 log reduction survivor curve, and there can only be one D value per microorganism; there is never more than one D value per one microorganism. The BI is defined as the most tolerant microorganism (typically bacterial spores) to the sterilization procedure being used, so if the BI is killed, then other contaminants (microorganisms) can be speculated to also be killed. Therefore, the use of a BI is essential in sterilization validation studies and routine control. Strategies to avoid clumping, tailing phenomena and to attain a SAL of 10^{-6} will be considered in this article.

Introduction

Several papers and books on gas plasma sterilization have been published, mostly by engineering researchers. Due to their insufficient knowledge of sterilization and microbiology, their papers and books contain many mistaken interpretations of data in the Figures and Tables and descriptions in the text [1]. A typical example is the curved survival curve. In this article, I would like to convey the correct information on sterilization, and how to conduct sterilization validation studies and routine control for BI users and BI manufacturers.

In contrast, microbiologists have misunderstood that Sterad^{R} from J & J Co. is a hydrogen peroxide gas plasma sterilizer [2,3], but this is not the case; the engineering researchers know that Sterad^{R} is not a gas plasma sterilizer. This is because microbiologists lack appropriate engineering knowledge; therefore there are misunderstandings on both sides. Gas plasma sterilization chambers cannot have such a large capacity as that of Sterad^{R} 100 (152 L), because gas plasma sterilization factors are short-lived and have very short flight distances. For example, the OH radical has a life period of a few μs and a flight distance of 0.003 cm/μs.

The requirements for sterilization validation studies and routine control for BI manufacturers and BI users are different. BI manufacturers should use 10^{6} CFU/carrier BI and must attain a SAL of 10^{-6} in validation studies (ISO 11138-1). In routine control, it is approved to use 10^{5} CFU as an initial population, but a SAL of 10^{-6} must be attained according to ISO 11138-1. For BI users, the use of various initial populations is approved according to ISO 14161, although the initial population used in validation studies and routine control should be the same.

Sterilization

Sterilization kills all types of bacterial spores and vegetative cells, which means the material is totally free of bioburden. The bioburden is the number and types of viable microorganisms in/on the products, or the so-called "viable contaminants". In contrast, disinfection only kills vegetative cells, but not bacterial spores. Decontamination is the removal of spores and vegetative cells by a process that does not kill them. The definitions of sterilization, disinfection and decontamination are described in the book edited by Sakudo and Shintani [4].

Sterilization is defined by the ISO 11138 series and ISO 14161. However, no ISO documents on gas plasma sterilization are currently available. Recent Pub Med searches with the key words ISO and gas plasma sterilization did not result in any matches. An ISO 11138 series is addressed to BI manufacturers and ISO 14161 is for BI users. According to ISO 11138-1, a BI with an initial population of 10^{6} CFU/carrier must be used and a sterility assurance level (SAL) of 10^{-6} must be attained, indicating that a 12 log reduction is required in validation studies. This requirement is only for BI manufacturers, not for BI users, so BI users need not follow the 12 log reduction procedure. For example, 10^{3} CFU/carrier BI has been approved for use by BI users (Combined BI/bioburden method) and a SAL of 10^{-6} is required, which is a 9 log reduction. Because of the lower population for a BI with 10^{3} CFU/carrier, clumping is less of a problem compared with a 10^{6} CFU/carrier BI; this can be experimentally confirmed by using scanning electron microscopy (SEM). If there is less clumping, the survivor curve is more likely to be a straight line and any tailing phenomenon will be avoided [1,5-8]. If the survivor curve shows tailing, a SAL of 10^{-6} cannot be attained, the *D* (decimal reduction) value, time or dose to decrease one log reduction, cannot be determined, and the sterilization exposure time to achieve a 9 log or 12 log reduction cannot be obtained without any information regarding a *D* value. In other words, no valid

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results can be attained when there is clumping of the BI and tailing of the survivor curve. The *D* value must be determined from the straight slope of a 12 log reduction survivor curve, and there is only one *D* value per one microorganism. The inactivation kinetics follows a first order equation. Even if tailing occurs, there cannot be more than one *D* value per microorganism and inactivation kinetics cannot be second or third order when restricted to BIs such as *Geobacillus stearothermophilus* ATCC 7953 or *Bacillus atrophaeus* ATCC 9372 because they are spore forming bacteria with straight survivor curves against all sorts of sterilization processes tested.

Most engineering researcher's data show curved survivor curves and report that inactivation kinetics is first or second order, indicating more than two *D* values per each microorganism. Their data are invalid according to the requirements of ISO 14161, because the *D* value must be determined from the straight slope from the initial population of 10^6 CFU/carrier to a SAL of 10^{-6}, indicating a 12 log reduction survivor curve. This indicates that the inactivation kinetics follows a first order equation because the use of *Geobacillus stearothermophilus* ATCC 7953 spores as the BI should not result in a tailing phenomenon. In sterilization validation, a 6 log reduction is not approved except in the absolute bioburden method in ISO 14161 for BI users.

D values can be obtained by the fraction-negative method using the Stumbo-Murphy-Cochran procedure and Spermann-Kerber procedure (ISO 14161). These methods require that from an initial population of 10^6 CFU/carrier to a SAL of $5\text{-}10^{-2}$, the survivor curve must be a straight line, and clumping should not be present. As a description of the methods for calculation of *D* values, please refer to ISO 14161 and ISO 11138-1. Procedures for the calculation of *D* values are more clearly described in ISO 14161.

A BI for gas plasma sterilization is not currently defined in any ISO TC 198 documents. The BI is defined the most sterilization-tolerant non-pathogenic microorganism, and is generally a bacterial spore former. From the available experimental data, *Geobacillus stearothermophilus* ATCC 7953 is the most appropriate candidate. In some cases *B. atrophaeus* ATCC 9372 has been used, but its bacterial spore are less tolerant than that of *Geobacillus stearothermophilus* ATCC 7953 [9,10]. Most engineering researchers typically use bacteria that do not form endospores, such as *Escherichia coli*, *Legionella* spp., and so on, but these microorganisms are very susceptible to sterilization compared with bacterial spores, so their use is invalid. Even if sterilization studies confirm that *E. coli* or *Legionella* spp. can be disinfected, what about *Bacillus cereus*, which is a pathogenic spore forming microorganism? This is a major concern because there is a reasonable chance that bacterial spore formers may be present as a bioburden. How can such studies address whether contaminants such as *B. cereus* can be sterilized or not? In order to sterilize pathogenic bacterial spores it is necessary to use an appropriate BI for confirmation, and the BI must be the most tolerant bacterial spore to the sterilization procedure because pathogenic bacterial spores present as a bioburden can also be speculated to be killed. Therefore, sterilization, not disinfection or decontamination must be carried out. If sterilization is attained using an appropriate BI, it is reasonable to consider that other pathogenic microorganisms of vegetative cell type or even bacterial spores would also be killed by the same sterilization process, and further experimentation is unnecessary. This means that for gas plasma sterilization the use of *Geobacillus stearothermophilus* ATCC 7953 as the BI is required and use of *Bacillus atrophaeus* ATCC 9372 is inappropriate.

Gas plasma sterilization has a quite shallow penetration depth of approximately 10-20 nm [10]. Because penetration is so shallow, only one layer of bioburden can be sterilized, and the healthcare products being sterilized remain undamaged. In validation studies, a SAL of 10^{-6} and material/functional compatibility must be simultaneously attained. Among the existing sterilization procedures, no sterilization methods can currently achieve simultaneous achievement of a SAL of 10^{-6} and material/functional compatibility. If this requirement is strictly enforced for the existing sterilization procedures, there are no compliant sterilization procedures available, and therefore the use of the existing sterilization procedures is the result of a compromise. As gas plasma sterilization can successfully comply with both sterilization requirements, the current compromise will be problematic, as other sterilization methods will then be strictly required to simultaneously attain both a SAL of 10^{-6} and material/functional compatibility, which is an impossible requirement for existing sterilization procedures.

Engineering researchers have insufficient knowledge of sterilization and microbiology; therefore, these should cooperate with microbiologists and chemists to correctly evaluate their experimental results. Currently, many engineering researchers misinterpret the meaning of a six log reduction and ignore the need to attain a SAL of 10^{-6}, as seen by the tailing phenomenon in their survival curves due to clumping [11,12]. These researchers need to read ISO 14161 and comprehend the usefulness and importance of using an appropriate BI to conduct sterilization validation.

Engineering researchers' understanding of six log reduction is from an initial population of 10^6 CFU/carrier to SAL of 10^*, but this is wrong. The correct requirement is the reduction of an initial population of 10^* CFU/carrier to a SAL of 10^{-6}. As already mentioned, a SAL of 10^* CFU is the bioburden level. For this purpose a linear survivor curve is required at least from a SAL of 10^* to an initial population of 10^6 CFU/carrier. If a tailed survivor curve is obtained, the experiment is not valid, sterilization has not been attained, and hno useful information such as the *D* value can be determined. A curved line at around a SAL of 10^3, which is commonly seen in engineering researchers' papers, provides no useful information. They obtain one *D* value from the initial straight line and another *D* value from the next curved line, which is incorrect. Only one *D* value exists per one microorganism.

Conclusion

Obtaining a SAL of 10^{-6} and material and functional compatibility is a difficult task for existing sterilization procedures. However, in gas plasma sterilization, this requirement can be easily attained because of the shallow penetration depth of the sterilization factors, which include radicals and metastables; therefore, the BI must be completely free from clumping to avoid any tailing phenomenon due to clumping.

If the survivor curve shows tailing, a SAL of 10^{-6} cannot be reached and therefore the *D* value cannot be defined and the exposure time to attain a 9 or 12 log reduction cannot be determined. Under these conditions sterilization cannot be attained. As engineering researchers have an insufficient understanding of sterilization and microbiology, they should cooperate with microbiologists and chemists to evaluate and interpret their experimental results.

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