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Editorial Open Access

# Considering an Important Point When Handling Gas Plasma Sterilization

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Gas plasma sterilization is currently of interest mainly to engineering researchers; however, in many cases their publications contain significant errors because they do not have a background/understanding of microbiology and sterilization. As a result, several misconceptions about the use and efficacy of the gas plasma sterilization process have been published. As a result relatively few microbiological and chemical researchers are involved in gas plasma sterilization research. In 2011, Sakudo and Shintani published a book in which they noted that many current publications on similar topics contain several mistakes in their discussion and interpretation of microbiology and sterilization. For that reason Shintani included a chapter entitled "Several Points to Consider When Conducting Plasma Experiments" for their benefit. However no revisions or corrections have been published in the literature since then. In this editorial, it discusses about new up-to-date information and critically evaluates the current status of the technology.

For example, one misinterpretation of the engineering studies is in regard to the meaning of the required "6 log reduction" for sterility assurance. A 6 log reduction does not mean zero, as $10^0 = 1$, and the possibility of survival at a sterility assurance level (SAL) of $10^0$ is 63%. A 6 log reduction is the requirement for the absolute bioburden method in ISO 14161, which addresses biological indicator (BI) users only. The bioburden is the type and number of viable microorganisms in or on the product, and in sterilization validation the real target of sterilization is not the BI, but rather the bioburden. Bioburdens of $10^6$ CFU (colony forming units)/carrier do not exist in real situations. For example, according to the absolute bioburden method in ISO 14161, an initial population of $10^0$ CFU/carrier level (i.e., a few CFU) that undergoes a 6 log reduction attains a SAL of $10^{-6}$. $10^{-6}$ is the closest number to zero, which was defined from the stochastics in ISO 11137-1.

To achieve a 6 log or 12 log reductions, the survivor curve must be a straight line. BI manufacturers are required to use an initial population of $10^6$ CFU/carrier, so reduction by 6 logs to a SAL of $10^{-6}$ represents a 12 log reduction: this is required in ISO 11138-1 as an overkill method for sterilization validation. An initial population of $10^6$ CFU/carrier to a SAL of $10^0$ is not recognized as a 6 log reduction in ISO 14161. Since ISO 11138-1 is for BI manufacturers and ISO 14161 is for BI users, researchers, as BI users, must obey the ISO 14161 requirements for sterilization validation. The main difference between ISO 11138-1 (BI manufacturer) and ISO 14161 (BI user) is that in ISO 14161, the overkill method is described together with another method, but in ISO 11138-1, only the overkill method is described. Detailed information about ISO requirements will be found out in ISO 14161 and ISO 11138-1 and all researchers associating with gas plasma sterilization procedures must read and understand well these ISO documents.

Stacking is often mistaken as clumping in the BI by the engineering researchers, and the presence of clumping in the BI (biological indicator) is a serious problem. In order to attain a straight survivor curve from an initial population of $10^6$ CFU/carrier to a SAL of $10^{-6}$ (a 12 log reduction), the BI should be free from any clumping. A 12 log reduction is required for BI manufacturers in ISO 11138-1, but it is not always required of the BI user in ISO 14161. To attain a 12 log reduction, it is necessary to avoid clumping in the BI; otherwise a curved (tailing) survivor curve is obtained. In this case even a SAL of $10^0$ cannot be attained, indicating that sterilization validation failed. Official documentation of sterilization validation is required, and relevant authorities conduct inspections to confirm sterilization validation. The straight line survivor curves were obtained using the BI free from clumping. These curves were obtained with $10^6$ CFU/carrier and a SAL of $10^{-6}$, which represents a full 12 log reduction. ISO 11138-1 requires that the coefficient correlation of the survivor curve must be greater than 0.8 (ISO 11138-1, Normative Annex B).

It is quite important to note that the D value (decimal reduction value, i.e., the time or dose required to decrease by 1 log) is only one per one microorganism. Often the tailing phenomenon can be explained by a difference in the kinetics of killing. Therefore, D values are calculated for each kinetic curve, indicating that more than one D value was present per microorganism, which a serious mistake is done by the engineering researchers. Why does clumping cause tailing? The penetration depth of gas plasma is quite shallow (~10-20 nm) except for oxygen gas plasma, so when spores are in multiple layers (clumping), only those at the surface of the first layer are immediately killed. As a result, killing of the second or third layer is delayed by the interference of the first layer, and this delay causes the observed tailing of the curve. Tailed survivor curves are not the true survivor curves. The BI *Geobacillus stearothermophilus* ATCC 7953 is the standard endospore former used in gas plasma sterilization. The average size of *G. stearothermophilus* spores is 1 μm X 3 μm (rod), so if the BI has clumps, those spores below the surface layer will not be killed because the penetration depth of gas plasma sterilization is ~ 10-20 nm.

Because gas plasma penetration is so shallow (~10-20 nm), the bioburden is killed but the product itself is not damaged, which is the best benefit of the gas plasma sterilization procedures. To kill the bioburden without deterioration of the material being sterilized is called simultaneous attainment of material/functional compatibility and a SAL of $10^{-6}$. The bioburden is scattered on the surface of the product and i.e., a few to 10-20 CFU/product surface without clumping is the real estimated bioburden. This level of bioburden is scattered over a larger area of the product than the BI, so no clumping is observed, and thus no tailing phenomenon is observed for the bioburden following ISO 14161 (absolute bioburden method).

In order to avoid further misinterpretation by the engineering researchers, we, as microbiologists and chemists, need to contribute by conveying appropriate information and correct mechanisms to the engineers to work together well. For that purpose understanding of ISO descriptions is quite useful for cooperation to obtain validation studies in success.

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