Simultaneous Achievement of Sterility Assurance Level (SAL) of $10^{-6}$ and Material and Functional Compatibility in Gas Plasma Sterilization

**Running Title: Simultaneous SAL and Compatibility**

Hideharu Shintani*
Faculty of Science and Engineering, Chuo University, Tokyo, Japan

**Abstract**

In the existing sterilization procedures, it is quite hard or impossible to achieve sterility assurance level (SAL) of $10^8$ and material/functional compatibility simultaneously. Simultaneous achievement of both is required in ISO 14161 and sterilization validation. As gas plasma sterilization penetration was quite shallow at around 10-20 nm level from the surface, it can kill only one layer of bioburden and can maintain material and functional compatibility in success without any difficulties. Bioburden means sort and number of viable microorganisms in/or the products. It is so-called contaminant. Sterilization was finished in success but material was damaged and useless, such a phenomenon must be avoided. In the current sterilizations, gamma-ray irradiation, electron-beam irradiation, autoclaving, dry heating, hydrogen peroxide gas or ethylene oxide gas sterilization has inferiority not to obtain material and functional compatibility. If gas plasma sterilization will be applicable to the real healthcare products, simultaneous achievement of SAL of $10^{-6}$ and material/functional compatibility cannot maintain without any difficulties, at this time simultaneous achievement is addressed to the existing sterilization procedures and sterilization validation. In that means gas plasma sterilization is the future promise sterilization procedure because only gas plasma sterilization can achieve both in success.

**Keywords:** Plasma; Sterilization; Microorganisms; Irradiation

**Introduction**

Gas plasma sterilization is popular among sterilization researchers and small number of commercial base gas plasma equipment is available from for example AST Products Inc (http://www.astp.com/plasma-equipment). Sterad® from J & J is not exact H$_2$O$_2$ gas plasma. Plasma is used for aeration of residual H$_2$O$_2$. However, gas plasma sterilization is not popular due to narrower space of sterilization chamber. Sterilization is the toughest concept against microorganisms. Sterilization can kill all types of microorganisms including spores and vegetative cells [1-3]. Spores are the most tolerable organisms among microorganisms (Table 1). In addition according to ISO 14161 and sterilization validation, exact sterilization must maintain sterility assurance level (SAL) of $10^8$ and initial population of $10^6$ CFU (Colony Forming Unit). From initial population down to SAL $10^{-6}$ is required 12 log reduction. It's a mistake that from initial population of $10^6$ CFU/crrier to $10^0$ CFU/crrier, which is not correct requirement of 6 log reduction. The correct 6 log reduction is from $10^0$ CFU/crrier to SAL of $10^{-6}$ in ISO 14161 and sterilization validation. For that purpose straight survivor curve must be indispensable. Initial population of $10^6$ CFU/crrier is the same population to bioburden and SAL of $10^{-6}$ is definitely required in ISO 11138-1 and ISO 14161 as well as sterilization validation by the authority. The six log reduction required to BI user is the absolute bioburden method in ISO 14161. This requirement is not addressed to BI manufacturer in ISO 11138-1.

**Requirement of Sterilization Procedure**

This requirement exists in sterilization validation and ISO 14161 and 11138-1. To attain SAL of $10^{-6}$, survivor curve must be straight from the initial population (No) to half-cycle window (SAL 5 to SAL $10^{-1}$) must be straight (Figure 1) and from SAL $10^{-1}$ to $10^0$ can be confirmed experimentally straight and from SAL of $10^0$ to SAL of $10^{-6}$ speculated to be straight because from SAL of $10^{-1}$ to SAL of $10^0$ cannot be confirmed experimentally and only speculated from stochastics in ISO 11137-1. Up to SAL of $10^{-1}$ ($1/100$) it can be confirmed experimentally, but less than $10^{-1}$ ($1/1000$), it has more possibility to be contaminated, thus exact SAL is uncertain below SAL of $10^{-1}$. Therefore, SAL of $10^{-6}$ is the speculatio and this amount is defined as the closest amount to zero from stochastics and this concept is explained in ISO 11137-1. In this means that during 6 log reduction, any tailing

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>More resistant</td>
<td></td>
</tr>
<tr>
<td>Prions</td>
<td>Scapie, Creutzfeldt-Jakob disease, chronic wasting disease</td>
</tr>
<tr>
<td>Bacterial spores</td>
<td>Bacillus, Geobacillus, Clostridium</td>
</tr>
<tr>
<td>Protozoal oocysts</td>
<td>Cryptosporidium</td>
</tr>
<tr>
<td>Helminth egg</td>
<td>Ascaris, Enterobius</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>M. tuberculosis, M terrae, M chelonea</td>
</tr>
<tr>
<td>Small, Nonenveloped virus</td>
<td>Poliovirus, Parvoviruses, papillomaviruses</td>
</tr>
<tr>
<td>Protozoal cysts</td>
<td>Giardia, Acanthamoeba</td>
</tr>
<tr>
<td>Fungal spores</td>
<td>Aspergillus, penicillium</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>Pseudomonas, Providencia, Escherichia</td>
</tr>
<tr>
<td>Vegetative fungi and algae</td>
<td>Aspergillus, Trichophyton, Candida, Chlamydomonas</td>
</tr>
<tr>
<td>Vegetative Helminth and Protozoa</td>
<td>Ascaris, Cryptosporidium, Giardia</td>
</tr>
<tr>
<td>Lagre, Nonenveloped virus</td>
<td>Adenovirus, Rotavirus</td>
</tr>
<tr>
<td>Gram–positive bacteria</td>
<td>Staphylococcus, Streptococcus, Enterococcus</td>
</tr>
<tr>
<td>Enveloped virus</td>
<td>Human immunodeficiency virus, hepatitis B virus, Herpes simplex virus</td>
</tr>
</tbody>
</table>

Table 1: Tolerance order to sterilants among microorganisms.

*Corresponding author: Hideharu Shintani, Faculty of Science and Engineering, Chuo University, 1-13-27, Kasuga, Bunkyo, 112-8551, Tokyo, Japan, Tel: +81425922336, Fax: +81425922336, E-mail: shintani@mail.hinocatv.ne.jp

Received December 22, 2014; Accepted January 23, 2015; Published January 27, 2015


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curve due to clumping (Figure 4) (Curved survivor line, Figure 5A) is not approved. The reason why tailing curve can be observed and how to avoid this is also explained in the NOVA book [1-3], but curved survivor line less than 6 log can be observed in the papers and books from engineering researchers. All of them are invalid data.

Initial population of $10^6$ CFU/carrier down to SAL of $10^{-6}$ is quite difficult to attain by gas plasma sterilization because the penetration depth of gas plasma is quite shallow at around 10 to 20 nm [4] (Figure 2). Figure 2 was the polystyrene surface after (upper) and before (lower) gas plasma exposure and observed by Atomic Force Microscopy (AFM). From the upper figure, it can observe to be etched around the depth of 10 to 20 nm. From the presented scale the deepest etched depth can speculate to be around 20 nm. From this, the average etching scale is around 10-20 nm. The *Geobacillus stearothermophilus* ATCC 7953 spore has 1 m width and 3 µm length (Figure 3), indicating gas plasma cannot pass through even one spore. This indicates gas plasma can kill only one layer of spore and multi layers cannot kill sufficiently from the shallow penetration depth. Multi-layers (clumping, Figure 4) are the reason why survival curve presents curved line before SAL of $10^6$ [1-3]. Multi-layers are called clumping among microbiologists (Figure 4) and same phenomenon as stacking among engineering researchers, must be avoided to obtain straight survival curve up to SAL of $10^{-6}$, not SAL of $10^6$ (Figure 5).

As gas plasma sterilization was quite shallow penetration depth, so products themselves are quite safe from damage, indicating simultaneous achievement of SAL of $10^{-6}$ and material/functional compatibility can easily obtain compared with the existing sterilization procedures such as gamma-ray irradiation, autoclaving, dry heating, hydrogen peroxide gas sterilization, ethylene oxide gas sterilization and so on [5-78].

We have a data to indicate gas plasma sterilization does not cause serious damage to the material. Polystyrene (PS) was sterilized by
because penetration depth of the sterilization factors can be only 10-20 nm from the surface, which can kill only scattering bioburden in one layer on the products. Both achievement of SAL of 10^-6 and material/functional compatibility can be completed in success required in ISO 14161 and sterilization validation to the BI user by the authority. The existing sterilization procedures are all failed to attain material and functional compatibility in the exact status, therefore compatibility is not strictly applied to the existing sterilization procedures, and if strictly applied to the existing sterilization procedures, no sterilization procedures are available in the current status, so the use of the existing procedures was connived. But this kind of status is not correct and the real procedures to attain SAL of 10^-6 and material/functional compatibility must be realized.

**Table 2: Analysis of exhaust gas from polystyrene (PS) treated with nitrogen gas plasma**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Before and After Treatment to PS</th>
<th>Detected Gases, ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Pressure Gas plasma</td>
<td>Sort of gases</td>
<td>CO^2</td>
</tr>
<tr>
<td>Before</td>
<td>&lt;2</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>After</td>
<td>3.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>

The data is cited from Shintani et al, Biocontrol Science (2007), 12, 131-143. 1 UV-absorbance method 2 chemical luminescence method 3 pyrazolone light absorption method 4 ozone detector 5 GC-MS N.D. not detected, indicating less than limit of detection (LOD)

In addition, we have a tensile and elongation strength test of Latex rubber before and after nitrogen gas plasma exposure (Table 3) [4,80-88] and leaching test of latex rubber before and after nitrogen gas plasma exposure (Table 4) [4,89-91]. Using Table 3 data, it is statistically tested with Student t test (paired t test using Statt View) and no significant difference was observed. In Table 4, statistical analysis cannot be done, but it can speculate that the significant difference may not exist.

**Conclusion**

As mentioned in the above, gas plasma sterilization can attain SAL of 10^-6 and material/functional compatibility without any difficulties...
H2O2 sterilizer because chamber is too large when considering as H2O2 possible. SteradR from J & J is not real H2O2 plasma sterilizer, but simply meaning commercial base gas plasma sterilizer is anticipated as soon as functional compatibility to the existing sterilization procedures. In that healthcare materials, the present connivance to the existing sterilization procedures cannot be approved by the authority in future. It may be before and after nitrogen gas plasma exposure

<table>
<thead>
<tr>
<th>Sample</th>
<th>No</th>
<th>Max Tensile test (N)</th>
<th>Max elongation test (%)</th>
<th>300% Elongation tensile test (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non treatment</td>
<td>1</td>
<td>3.58</td>
<td>656.0</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.83</td>
<td>684.5</td>
<td>1.09</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.73</td>
<td>781.5</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3.73</td>
<td>695.0</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.70</td>
<td>678.0</td>
<td>1.30</td>
</tr>
<tr>
<td>Ave</td>
<td></td>
<td>3.71</td>
<td>699.0</td>
<td>1.28</td>
</tr>
<tr>
<td>Plasma 40 min treatment</td>
<td>1</td>
<td>3.30</td>
<td>685.5</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.45</td>
<td>694.5</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.98</td>
<td>788.0</td>
<td>1.15</td>
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<tr>
<td></td>
<td>4</td>
<td>3.80</td>
<td>626.2</td>
<td>1.43</td>
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<td></td>
<td>5</td>
<td>4.73</td>
<td>812.5</td>
<td>1.32</td>
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<tr>
<td>Ave</td>
<td></td>
<td>3.85</td>
<td>721.4</td>
<td>1.08</td>
</tr>
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</table>

If gas plasma sterilization procedures can be applicable to the real healthcare materials, the present connivance to the existing sterilization procedures cannot be approved by the authority in future. It may be required to improve in order to attain SAL of 10\(^{-6}\) and material and functional compatibility to the existing sterilization procedures. In that meaning commercial base gas plasma sterilizer is anticipated as soon as possible. Sterad\(^{4}\) from J & J is not real H\(_2\)O\(_2\) plasma sterilizer, but simply H\(_2\)O\(_2\) sterilizer because chamber is too large when considering as H\(_2\)O\(_2\) plasma sterilizer because sterilization factors are short-lived and small flight distance. Plasma in Sterad\(^{4}\) is used for H\(_2\)O\(_2\) residue removal, not for sterilization.

References

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