Pharmaceutical Regulatory Affairs: Open Access

Shintani, Pharmaceut Reg Affairs 2015, 4:2
DOI: 10.4172/2167-7689.1000137

Review Article Open Access

Sterilization Validation of Gas Plasma Exposure Based on ISO Documents (Mainly ISO TC 198 And 194 Documents)

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Abstract

There are so many ISO documents to understand and utilize for validation studies and routine control. A biological indicator (BI) is essential for conducting sterilization validation and routine control. ISO 11138-1 and ISO 14161 are the major documents to follow for BI manufacturers and BI users, respectively. In ISO 11138-1, BI manufacturers must utilize a BI with 10^6 CFU/carrier as an initial population for validation studies. For routine control, it is approved to use an initial BI population of 10^5 CFU/carrier according to ISO 11138-1. In ISO 14161 BI users need not imitate BI manufacturers and less than 10^6 CFU/carrier BI is approved for use in validation studies and routine control. According to ISO 14161 for BI users, the initial population must be identical for validation studies and routine control. For the BI user, there are four types of procedures for sterilization validation and routine control. These are the half-cycle, over-kill, combined BI/bioburden and the absolute bioburden methods. A BI with An initial population of 10^6 CFU/carrier must be used for validation studies by BI manufacturers according to ISO 11138-1, but BI users can elect to use a commercially available BI with more than 10^3 CFU/carrier as the BI for validation studies and routine control as described in ISO 14161. The approved SAL was defined to be 10^{-6} and this is unchanged for both BI users and BI manufacturers. Therefore, from the initial population of 10^6 CFU (colony forming unit)/carrier to a SAL of 10^{-6}, the survivor curve must be straight and tailing is not allowed as described below (ISO 11138-1). The BI used for gas plasma sterilization, spores of Geobacillus stearothermophilus ATCC 7953, has characteristics that do not result in a tailing phenomenon. A linear survival curve is obtained from an initial population of 10^6 CFU/carrier to a SAL 10^{-6} for all sterilization procedures tested, demonstrating that inactivation kinetics are first order and allowing calculation of the D (decimal reduction) value from the dose or time to decrease 1 log. Chemical indicators (CI) are not approved for use in validation studies; only the use of BI is approved. CIs are only approved for use as a supporting method during routine control according to ISO 11140-1 and 14161. Only a BI is approved for both validation studies and routine control according to ISO 14161.

ISO documents

I am a Japan delegate of the ISO Technical Committee (TC) 198 and 194. TC 198 covers Sterilization of healthcare products. TC 194 covers Biological and clinical evaluation of medical devices. There are currently no ISO documents covering gas plasma sterilization. I have extensive experience in both the gas plasma sterilization field and as an ISO TC delegate. In addition, I have published ISO-relevant books and papers, so based on these experiences I have prepared this article describing the application of ISO requirements to gas plasma sterilization**.** Gas plasma sterilization is a useful procedure that can easily achieve a sterility assurance level (SAL) of 10^{-6} and material/functional compatibility simultaneously [1-5] This is because the gas plasma penetration depth is quite shallow (10-20 nm) [1,2], so material does not deteriorate as easily compared with existing sterilization procedures. Current sterilization validation requires a SAL of 10^{-6} and simultaneous material/functional compatibility. If strict adherence to this requirement were required of the existing sterilization procedures, there would be no sterilization procedures available, so this requirement of simultaneous attainment of SAL of 10^{-6} and material/functional compatibility is in most cases ignored when applied to real sterilization procedures. For example, in our experiments comparing material compatibility among gamma-ray, autoclaving and ethylene oxide gas sterilization, significant deterioration of polyurethane materials was observed [6]. Therefore, if the absolute requirement for sterilization validation were applied [7-20], no available sterilization procedures would suffice; as a result, sterilization validation is currently a compromise. In the ISO 194 document, there are four major degradation documents as follows. Identification and quantification of degradation products from polymeric medical devices is in ISO 10993-13, Identification and quantification of degradation products from ceramics is in ISO 10993-14, Identification and quantification of degradation products from metals and alloys is in ISO 10993-15 and Toxicokinetic study design for degradation products and leachables is in ISO 10993-16. These documents discuss degradation products in the body, not by sterilization, but the mechanism of *in vivo* degradation is quite similar to enzymatic degradation and that of sterilization because bonds with low bonding energy are easily cleaved *in vivo* as well as during sterilization.

In ISO 198, several sterilization procedures are discussed. Sterilization procedures are individually discussed, but for example chemical indicators (CI) or biological indicators (BI) are common topics of discussion for sterilization validation and routine control. CI documents are presented in ISO 11140-1 to 11140-5 and BI document are in ISO 11138-1 to ISO 11138-5. BI is approved for use in both validation studies and routine control, but CI is not approved for use in validation studies and it is only approved for use in routine control in support of BI. This means that BI and CI differ significantly. Unlike CIs, BIs are absolutely essential to attain sterility assurance as described in several ISO documents.

The biological discussion is documented in ISO 11737-1, "Sterilization of medical devices-Microbiological methods-Part 1: Determination of a population of microorganisms on products". CFU (colony forming units) of the biological indicator or bioburden in/

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Received January 19, 2015; **Accepted** March 13, 2015; **Published** March 20, 2015

Citation: Shintani H (2015) Sterilization Validation of Gas Plasma Exposure Based on ISO Documents (Mainly ISO TC 198 And 194 Documents) 4: 137. doi: 10.4172/2167-7689.1000137

on the carrier or products can be determined by using ISO 11737-1. Several methods for the recovery of the bioburden from health care products are discussed in ISO 11737-1. ISO 11737-1 discusses only the retrieval of the bioburden from the products, in contrast to ISO 11138-1 and ISO 14161, which deal with BI retrieval from BI carriers such as paper or SUS. Both ISO documents were discussed at TC 198. Tests of sterility performed in the definition, validation and maintenance of a sterilization process is in ISO 11737-2**.** Aseptic processing**,** BI use and other issues related to sterility assurance are described in the following documents.

- Aseptic processing of health care products -- Part 1: General requirement is in ISO 13408-1
- Aseptic processing of health care products -- Part 2: Filtration is in ISO 13408-2
- Aseptic processing of health care products -- Part 3: Lyophilization is in ISO 13408-3
- Aseptic processing of health care products -- Part 4: Clean-in-place technologies is in ISO 13408-4
- Aseptic processing of health care products -- Part 5: Sterilization in place is in ISO 13408-5
- Aseptic processing of health care products -- Part 6: Isolator systems is in ISO 13408-6
- Aseptic processing of health care products -- Part 7: Alternative processes for medical devices and combination products is in ISO 13408-7
- Sterilization of health care products -- Biological indicators -- Guidance for the selection, use and interpretation of results is in ISO 14161
- Sterilization of health care products -- General requirements for characterization of a sterilizing agent and the development, validation and routine control of a sterilization process for medical devices is in ISO 14937
- Sterilization of health care products -- Chemical indicators -- Guidance for selection, use and interpretation of results is in ISO 15882
- Sterilization of health care products -- Biological and chemical indicators -- Test equipment is in ISO 18472.

I mostly discuss working group 4, which deals with biological indicators (BIs). They are

- Biological indicators -- Part 1: General requirements is in ISO 11138-1
- Biological indicators -- Part 2: Biological indicators for ethylene oxide sterilization processes is in ISO 11138-1
- Biological indicators -- Part 3: Biological indicators for moist heat sterilization processes is in ISO 11138-3
- Biological indicators -- Part 4: Biological indicators for dry heat sterilization processes is in ISO 11138-4
- Biological indicators -- Part 5: Biological indicators for low-temperature steam and formaldehyde sterilization process is in ISO 11138-5

Among these ISO documents from ISO TC 198 and 194, ISO 11138-1, ISO 14161, ISO 14937, ISO 15882 and ISO 11737-1 are the essential ISO documents to read and comprehend for validation study and routine control.

ISO 11138-1 and ISO 14161

For gas plasma sterilization researchers, ISO 11138-1 and ISO 14161 are the essential ISO documents to comprehend. ISO 11138-1 provides general information for BI manufacturing companies and ISO 14161 is specifically for BI users. Therefore, BI manufacturers must obey the ISO 11138 series and BI validation studies and routine control must be carried out following the ISO 11138-1 requirements. According to that document**,** a BI with an initial population of 10^6 CFU/carrier must be utilized and a sterility assurance level (SAL) of 10^{-6} must be attained, indicating that a 12 log reduction is required for BI manufacturers (ISO 11138-1). In routine control, the use of 10^5 CFU/carrier is approved as an initial population, but the SAL requirement of 10^{-6} is unchanged, so a full 11 log reduction must be attained for routine control according to ISO 11138-1.

On the contrary, ISO 14161 provides the requirements for BI users and describes in detail the half cycle method, overkill method, combined BI/bioburden method and absolute bioburden method. In the half cycle method, the initial population (10^6 CFU/carrier) must be reduced down to the half-cycle window (SAL 5 to SAL 10^{-2}), indicating a 6 or 8 log reduction, and these figures double. As a whole, a 12 to 16 log reduction is therefore required. No healthcare product manufacturers conduct the half cycle method due to these very stringent requirements. When using the half-cycle method, it is easy to attain a SAL of 10^{-6}, but material and functional compatibility cannot be attained due to the long exposure time necessary. It is a serious problem if sterilization is successfully completed but the material is no longer useful.

The over-kill method is commonly required for BI manufacturer validation studies, but this method is not always appropriate for BI users (ISO 14161). BI users can conduct other methods besides the over-kill procedure. The BI initial population for the over-kill method is 10^6 CFU/carrier and a SAL of 10^{-6} must be achieved; therefore, a 12 log reduction is required. This is a very difficult requirement for most BI users. Even if a SAL of 10^{-6} can be attained, material and functional compatibility may be impossible to achieve with this method.

According to ISO 11138-1 and ISO 14161, attainment of a SAL of 10^{-6} is absolutely required. However, attainment of material/functional compatibility is required of BI users in ISO 14161, but it is not required for BI manufacturers in ISO 11138-1 because the BI manufacturer has no material being tested. Therefore, the two sets of requirements are clearly different.

The **c**ombined BI/bioburden method in ISO 14161 is specifically for BI users. The initial population is the number of *Geobacillus stearothermophilus* ATCC 7953 spores that is approximately equivalent to the bioburden number, so a few CFU/carrier, and a SAL of 10^{-6} is required. In this case, a six log reduction is required. Unfortunately, BIs with this low concentration are not commercially available, so BI users must prepare the BI themselves. At this point they are temporarily considered to be BI manufacturers, and as a result they must follow ISO 11138-1. BI users who make their own BI must be inspected by the authorities as if they were commercial BI manufacturers, but most BI users do not want to undergo these inspections. Commercial BI preparations with a population of more than 10^3 CFU/carrier are available, and in most cases, to avoid inspections, these will be chosen by BI users for use in the combined BI/bioburden method. In this case, reduction from 10^3 CFU/carrier to a SAL of 10^{-6} represents a 9 log reduction. The combined BI/bioburden method is the most popular,

and is the most realistic method if the authorities understand the BI/bioburden method requirements. The use of 10^3 CFU/carrier in the same area as 10^6 CFU/carrier means that BI clumping will be less, and therefore no tailing phenomenon is observed. Therefore it is easy to obtain a linear survivor curve and a *D* value is easily defined compared with the overkill or half-cycle methods.

The last option is the absolute bioburden method. The initial population is the actual number of bioburden present (determined based on the average from three products) and the microorganism selected is the most sterilization tolerant among the bioburden microorganisms, so it is not always *Geobacillus stearothermophilus* ATCC 7953 or *Bacillus atrophaeus* ATCC 9372. The possibility of *Bacillus subtilis* may be present as a bioburden, but that of *Geobacillus stearothermophilus* is definitely denied because the latter is a definitely thermophilic bacteria growing at 55°C. An initial population of 10^0 CFU carrier level must be reduced to a SAL of 10^{-6}, so at least a 6 log reduction is required. In this case the BI is also self-made, so the ISO 11138-1 requirements must be met and relevant inspections must be conducted. The absolute bioburden method is the most realistic method, but because the BI is self made, and BI users do not want to undergo the required inspections, in most cases the overkill or combined BI/bioburden method with an initial population of 10^6 CFU/carrier or 10^3 CFU/carrier, respectively, are typically used. The absolute bioburden method is not utilized very often since BI users are reluctant to be inspected by the authorities. The required six log reduction of the absolute bioburden method is the most appropriate and realistic and the use of BI organisms (spores) is not always required. The real exposure time for the absolute bioburden method is significantly less than that for the 9 log or 12 log reduction required by the combined BI/bioburden or overkill methods, respectively. A BI with 10^3 CFU/carrier for the BI/bioburden method has less clumping compared with the 10^6 CFU/carrier BI used in the overkill or half-cycle method, so I recommend the use of the combined BI/bioburden method with 10^3 CFU/carrier commercial BI in place of the 10^6 CFU/carrier BI used for the overkill method (12 log reduction) or half-cycle method (12 log to 16 log reduction). For the commercial BI, I recommend the BI from Merck Co. inoculated on modified SUS carrier because the BI from this company has less clumping.

The use of the overkill method is restricted to BI manufacturers although BI users also can be approved to use it, but BI users need not obey the requirements for the BI manufacturer as described in ISO 14161. When the combined BI/bioburden method is applied, BI clumping is low, so it is easy to attain a SAL of 10^{-6} with a linear survival curve from 10^3 to a SAL of 10^{-6} together with material/functional compatibility. For BI manufacturers and for routine control, the use of 10^5 CFU/carrier of BI in ISO 11138-1 is approved. Such a description of a difference of the initial population between validation studies and routine control is not described in ISO 14161 for BI users. According to the requirements of ISO 14161, validation studies and routine control must use an identical BI for evaluation. According to the current ISO documents, less than a 6 log reduction is not allowed because the approved initial population is 10°CFU/carrier and a SAL of 10^{-6} must be obtained; therefore, a at minimum of a 6 log reduction is required, although most engineering researchers are satisfied with 2 log or 3 log reductions, which are not valid.

It is important to mention that chemical indicators (CI) are not approved for use in validation studies. CI manufacturers validate CI characteristics and performance according to the ISO 11140 series and they are also inspected by the authorities. In routine control CI can be utilized as a support for the BI, but use of a CI alone is not approved even for routine control. The BI is the major requirement for sterilization validation and routine control because results using BI and CI do not always coincide.

How to Prepare Survival Curves

Preparation of survival curves is not exactly within the scope of this book, but when considering the cause of tailing, it is an important aspect [14-20]. Methods for the preparation of survival curves can be found in ISO 11138-1 Annex B Survival curve (Normative reference). However, in Annex B, there is no description of tailing, but as mentioned above the user must seriously consider the problems caused by clumping when preparing a BI because the coefficient relationship of the prepared survival curve must be more than 0.8 according to ISO 11138-1 Annex B (**N**ormative reference). This requirement indicates no that tailing is allowed from an initial population of 10^6 CFU/carrier to a SAL of 10^{-6}.

In order to prepare a survivor curve, ten-fold dilutions must be repeated to obtain 30-300 CFU/plate (ISO 14161, 11737-1), and the retrieval procedure to obtain the bioburden or BI from the products or carrier material, respectively, must be carried out according to ISO 11737-1. In ISO 11737-1, several sorts of retrieval procedures are presented. The person who conducts the retrieval must validate which method is most appropriate for their products. The retrieval solution that is commonly used is Tween^R 80 containing phosphate buffered saline solution at pH 7.4. This is not defined in ISO 111737-1, but it is the most popular solution when retrieving microorganisms from health care products or BI. The retrieval procedure must be validated individually by referring to ISO 11737-1.

In ISO 11138 Annex C and D (Normative references), the fraction negative methods for D value calculation are described**;** these include the Sperman-Karber procedure and the Stumbo-Murphy-Cochran procedures. The complete fraction negative method is in ISO 14161 Annex C (Normative reference). On the contrary, no survivor curve method is described in ISO 14161; it is only described in ISO 11138-1 B (Normative reference).

How to Calculate D Values

There are two methods to calculate *D* values. One is by using the survivor curve method, which is in Annex B (Normative reference) of ISO 11138-1 and the others are fraction negative methods in ISO 14161 Annex C (normative reference). In regard to the survivor curve method in ISO 11138-1 Annex B, it is defined that the reduction from an initial population of 10^6 CFU to a SAL of 10^{-6} must be linear with a coefficient correlation greater than 0.8, which is quite a stringent requirement.

Fraction negative methods are the Sperman-Karber procedure and Stumbo-Murphy-Cochran procedures. They are described in Annex C (Normative reference) of ISO 14161 and are for BI users. A description of methods for the calculation of *D* values is outside of scope of this book, but BI users need to keep in mind that less than 10^6 CFU can be used as an initial population because BI users must obey ISO 14161, not ISO 11138-1.

Conclusion

Many ISO documents must be read and understood in order to conduct sterilization validation and routine control. If published papers and books do not follow ISO requirements, these books and papers are considered invalid. Most of the engineering researchers' papers and books are invalid because their survivor curves do not attain a SAL of 10^{-6}, primarily because of the use of BI with clumping. The SALs presented by the engineering researchers' are 10^2 to 10^3 at most, which

differs significantly from 10^{-6}, which is definitely required by the ISO documents. Therefore, the data in their books and papers are invalid and unreliable.

In order to avoid the tailing phenomenon from clumping, less than 10^6 CFU/carrier BI (such as 10^3 CFU/carrier BI) can be used to attain a SAL of 10^{-6}. It should be relatively easy to attain a SAL of 10^{-6} using the combined BI/bioburden method in ISO 14161 and BI users should obey this ISO document, not ISO 11138-1, which is only for BI manufacturers. If BI users, including researchers do not follow the requirements of ISO 14161, their papers and books are useless and invalid.

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