Standard Operating Procedure

Title: Selection and Use of Biological Indicators during Validation Studies

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Purpose

The use of Biological Indicators (BI's) for the validation and in-process monitoring of sterilisation cycles is becoming increasingly complex and highly regulated. Therefore, a defined approach to the use and control of biological indicator validation exercises is required. The purpose of this Standard Operating Procedure is to define the selection and usage of Biological Indicator Spore Strips and ampoules when validating thermal processes or equipment.

Scope

The biological indicators defined in this SOP are applicable for the validation and monitoring of sterilisation cycles utilised at a GMP site, in the production of biopharmaceutical products. The usage procedures defined in this SOP shall apply to all validation activities carried out at a GMP site using biological indicator spore strips for process and equipment validation or testing work.

Definitions

D value: The time taken, in minutes, to reduce a population by 90% (one Log10). This term is usually followed by a reference to the sterilisation modality concerned, in sub-script; thus for steam it is D121, for dry heat D160 and so on.

Z value: The change in temperature required to change the D-value by one Log10. i.e. A Z value of 10°C will change a D value of 5 minutes at 121°C to a D value of 0.5 minutes at 131°C

F_0: The equivalent time, in minutes, at 121°C, calculated from time(s) at different temperatures, assuming a Z value of 10 °C. Used to include heat up and cool down times in cycle lethality calculations.

Time/Temperature Sterilisation: A sterilisation cycle based on a load being held at a set temperature for a set amount of time. For example, an autoclave cycle of fifteen consecutive minutes at a minimum temperature of 121°C.

Bioburden: The term used to encompass the total biological challenge to a process. Usually, but not exclusively, referring to those organisms recovered from the product matrix prior to sterilisation.

Lethality Input (LI): An estimation of the process challenge calculated from the D value and the population of the BI.

Example:

Lethality input = Log10 population of BI x D Value of BI
Example = Log10 1.7 x 106 x 1.95 minutes = 6.23 x 1.95
LI = 12.15

The Lethality Input is to be used to record the challenge to a sterilisation process and to ensure that future validation activities use an equivalent challenge to the process.
Lethality Input is also known as $F_{biological}$ or F_{bio}.

Sterility Assurance level (SAL): Probability of a single viable micro-organism occurring on an item after sterilization.
Note: The term SAL takes a quantitative value, generally 10^{-3} or 10^{-6}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} takes a lower value but provides a greater assurance of sterility than an SAL 10^{-3}.

Probability of a Non-Sterile Unit (PNSU): The probability of a unit (product container) being non-sterile after the application of a lethal agent. The distinction between SAL and PNSU is that non-sterile units may have more than a single surviving micro-organism.

D-values can only truly be made if all test conditions are the same. D-values reported in literature are therefore specific to the test systems in which the studies were performed. However, what can be taken from literature is that where comparisons are made, G. stearothermophilus is routinely identified as the most resistant and typically a D-value between 0.5 – 2.5 minutes is reported.

For instance, a site can choose to use two types of biological indicators for dry goods sterilisation (direct steam) paper spore strips (figure 1) and EZ-Test ampoules (figure 2). A third sealed ampoule (figure 3) is used for liquid cycles.

Paper Spore Strips have a filter paper carrier enumerated with a spore concentration of *Geobacillus Stearothermophilus* packaged inside a steam penetrable glassine envelope. The spore strips inside the glassine envelope turn turbid if spores grow or remain clear if no spores have grown. A typical biological indicator spore strip is illustrated in figure 1.

Figure 1: Typical Geobacillus Stearothermophilus spore strip

These ampoules contain a known quantity of bacterial spores inoculated onto filter paper and placed inside a plastic culture tube with a crushable glass ampoule containing the culture medium. The appearance of a yellow colour indicates growth while no colour change indicates adequate sterilisation. Typical biological indicator EZ-test ampoules are illustrated in figure 2.

Figure 2: typical Geobacillus Stearothermophilus EZ-test ampoules

Glass Ampoules are self-contained biological indicators used for monitoring the steam sterilization of liquids. The glass ampoule biological indicator contains Geobacillus stearothermophilus spores and a specially-formulated culture medium that turns a dramatic yellow if spores grow or remain pink/purple if adequate sterilisation occurred.

2.2.5 Use of the Paper Spores Strips during testing in which the BI may be subjected to physical hardship requires a customized approach. The delicate nature of the glassine protective layer must be protected from the extreme conditions. This will be achieved by placing the spore strips in a short piece of silicone tubing. The tubing is closed but not sealed at the ends by tie-wraps. This will protect the spore strips while providing adequate steam penetration to kill the spores. Figure 5 illustrates how this system is put together.

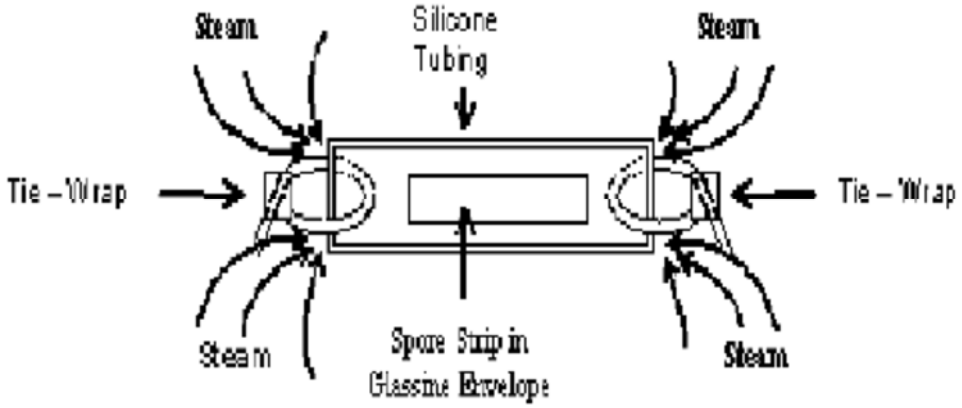

Figure 5: Protected wrapping method used during validation studies

2.2.6 The BI strips should be examined upon completion of a run and any damage to the envelope or wetness of the strips should be documented the relevant protocol comments section.

3. Storage Conditions of Biological Indicators

3.1 **Geobacillus Stearothermophilus spore strips –** spore strips must be stored at room temperature (20 – 25^{o}C) until the expiry date (24 months from the manufacture date). Do not dessicate the spore strips.

3.2 **Geobacillus Stearothermophilus ampoules –** ampoules must be stored at room temperature (20 – 25^{o}C) until the expiry date (24 months from the manufacture date). Do not dessicate the spore strips.

3.3 **Geobacillus Stearothermophilus glass ampoules –** glass ampoules must be refrigerated between +2^{o}C and +8^{o}C until the expiry date. Storage at room temperature (up to 25^{o}C) is possible for a limited period of 1 to 2 weeks. Storage at temperatures exceeding +30^{o}C affects the products stability.

4. Transport of Biological Indicators

All three types of biological indicators must be transported in a safe manner and in a container that is correctly labelled, break resistant and suitably sealed.

Any spills or breakages must be reported immediately to the Environment, Health & Safety Specialist and contained by trained staff utilising the spill kit.

5. Disposal of Biological Indicators

Biological indicators must be disposed of once they pass the expiry date or are unusable for a particular reason.

All three types of biological indicators (spore strips, EZ Test ampoules and glass ampoules) must be autoclaved for no less than 30 minutes for 121^{o}C. Selected Autoclave and Cycle run must be used for the disposal of all biological indicators. Once the biological indicators have been autoclaved they must be disposed in a biological waste bin.

Appendix 1: Biological Indicator Test Results Sheet – Glass Ampoules

Procedure

Fill out table and transfer sheet to Quality Control with all samples.

Cycle Number:		Run #:		Initials/Date:	
QC Serial Number:					
Commenced Incubation at	^{o}C	Time:		Initials/Date:	

1. Place the ampoules in the incubator rack and incubate immediately for 48h at (60 ± 2)^{o}C.
2. Record the examining time and results in the table below.

Acceptance Criteria - Geobacillus stearothermophilus

- **Vibrant Yellow colour** indicates bacterial growth.
- **Clear Purple, Violet, Brown or Bourbon colour** indicates adequate sterilisation.

Vibrant Yellow

Purple

Violet

Brown or Bourbon Colour

Table 1. Test Results for Indicators				
Position	**Op. Init.**	**Date**	**Biological Indicator Result** **Purple, Violet, Brown or Bourbon = –** **Vibrant Yellow = +**	**Sign/Date**
Control				
Test Result: ☐ **Pass** ☐ **Fail**				
Comment:				
Tested by/Date		**Verified by/Date**		
Review by/Date				

Appendix 2: Biological Indicator Test Results Sheet

Project Number	Equipment ID

Procedure

Fill out table and transfer sheet to Quality Control with all samples.

Cycle Number:		Run #:		Initials/Date:	
QC Serial Number:					
Commenced Incubation at	^{o}C	Time:		Initials/Date:	

3. To activate the media, place the indicator in an upright position in a plastic crusher. Gently squeeze the crusher to break the glass ampoule. Place the activated indicator in the incubator rack and incubate immediately.
4. Examine the indicator for any colour change at 24h. Record the examining time and results in the table.

Acceptance Criteria - *Geobacillus stearothermophilus*

- Yellow colour indicates bacterial growth.
- No colour change indicates adequate sterilisation.

Position	Op. Init.	Date/Time	Biological Indicator Purple = - Yellow = + 24h	Initial/Date
Control				
Operator Initial/Date				
Test Result: ☐ Pass ☐ Fail				
Comment:				
Tested By/Date				
Verified By/Date				

