Standard Operating Procedure for the Validation of an Autoclave by a Bio-indicator

1. Purpose

The purpose of this SOP is to guide users on the proper procedure to validate the efficiency of an autoclave-induced sterilization by means of a commercially available Bio-indicator.

2. Scope

It applies to all lab staff.

3. Responsibility

3.1. Lab staff shall ensure that the biological waste is fully sterilized before disposal as general waste.

4. Safety Precautions

4.1. Always make sure there is enough water in the autoclave to cover the bottom plate.

4.2. Do NOT put in the hand or finger between the lid and the autoclave when closing the lid to avoid injury.

4.3. The lid, chamber, gasket and panel are extremely hot after completion of the operation. Do not touch the equipment.

4.4. Put on heat gloves when unloading.

4.5. Ensure that the drain bottle is not full before the start of autoclave.

5. Procedure

Sterikon® plus Bio-indicator, an ampoule containing a nutrient broth, sugar, a pH indicator and spores of a non-pathogenic organism, *Geobacillus stearothermophilus* ATCC 79531 (sporulation optimized) shall be utilized for the purpose of the said validation.

5.1. **At least 2** ampoules should be used for autoclaves with a capacity of up to 250 litres.

5.2. Shake each Sterikon® plus Bio-indicator vigorously to disperse spore before placing in the autoclave.

5.3. Place each of the 2ml ampoule in either a 25 ml beaker or un-capped bottle. Ensure that the beakers are tagged with autoclave tape.

5.4. The beaker/ un-capped bottle containing the ampoule(s) are to be placed in the metal baskets at the bottom and in the middle of the autoclave cavity. These positions are usually considered to be the most unfavorable for autoclaving.

5.5. Autoclave the ampoules according to the regular procedures using appropriate cycle/settings. An appropriate sterilization time will be 15 minutes at 121°C.

5.6. Remove the ampoules and incubate them in the incubator for 48 hours at 60 ± 2°C. A non-sterilized ampoule should also be incubated to serve as a control.

5.7. After the incubation process, the ampoules are visually inspected to view the results and the follow-up actions detailed in the following table implemented.

	Red-Violet	Yellow-Orange
Colour of Ampoule		
Results	PASS	FAIL
Remarks	Adequate sterilization; *Geobacillus stearothermophilus* spores are killed off	Inadequate sterilization; ampoule becomes turbid due to microbial growth
Follow-up Actions	-	**Service engineer for the autoclave to be contacted to rectify the problem**

5.8. The incubated ampoules MUST be autoclaved first before disposing it as a general waste. Record the date and result of test in the logbook.

6. Frequency of Bio-indicator validation

6.1. The biological indicator validation should be carried out every 6 months.

7. Notes

7.1. The ampoules should be stored at temperature between 2 °C to 8 °C. Room temperature (up to approximately 25°C) storage is possible but for a limited period of not exceeding 10 days. Storage at temperature above 30 °C adversely affects product stability.

7.2. Inadequate sterilization indicates that the autoclave may not be functioning optimally and efficiency is likely compromised. It is mandatory to carry out regular maintenance and annual inspection of the autoclaves to ensure smooth operation.

8. Reference

Refer to Merck Microbiology Manual 12th Edition, pp. 449-450

http://www.mibius.de/out/oxbaseshop/html/0/images/wysiwigpro/Sterikon_plus_110274_engl.pdf