PROTOCOL: BIOLOGICAL INDICATORS
Monitoring Effectiveness of Sterilization of Common Biology Autoclaves

IMPORTANT: USERS PLEASE READ CAREFULLY!

It is mandatory to validate the effectiveness of waste sterilization monthly for every autoclave using *Geobacillus stearothermophilus* indicator vials. EHS is now monitoring that these tests are happening.

Procedure:

1. Monitoring is to be done **monthly**.
2. Self-contained biological indicator vials (SCBI) containing *Geobacillus stearothermophilus* and medium with a pH indicator to detect growth are available for Common Biology autoclaves through Biology Receiving (N3/17).
   a. Current ones (acquired June 2013) are Self-Contained Biological Indicators (SCBI) from NAMSA, Supplier No: SCS-05; Purchased through VWR, VWR Cat No: 95029-702
   b. Storage 15-30°C. Do not refrigerate/freeze or expose to direct sunlight or UV light.
3. A typical load for waste disposal is to be one 24” x 30” autoclave bag. The bag should be **no more than half full**. The bag should be tied loosely, leaving the top open a few inches to allow steam to circulate within the bag.
4. **Exposure**: Tie a long string to the biological indicator vial & insert it into the middle of the load to be sterilized (the string will allow easy removal after autoclaving). Don’t forget to label the vial with date, autoclave & cycle used.
5. Autoclave the load for a **minimum of 75 minutes** sterilization time.
6. **Activation**: At the end of your cycle, remove the biological indicator vial from the load & ‘activate’ (instructions are those for NAMSA’s SCBI’s noted above – if using a different brand, follow manufacturer’s instructions):
   a. Each SCBI contains a spore disc and culture medium contained in a crushable glass ampoule.
   b. Seal the cap by pressing down firmly until the medium ampoule is crushed.
   c. Inspect to make sure spore disc is completely saturated with medium. If not, hold the vial vertically and tap gently on hard surface to make disc go into medium.

**Incubation**: Place the autoclaved biological indicator(s), plus an activated, non-autoclaved, control vial of biological indicator in a vertical position in an incubator at 55-60°C for 48 hours.

7. **Monitoring**: Examine the biological indicators daily during their 48 hour incubation & record your observations. They will need to be filled in on the on-line log (see below).
   a. If sterilization has worked, the medium will remain purple after 48 hours.
   b. If sterilization has failed, the medium will turn yellow due to acid released during bacterial growth and turbidity will also be visible. Tubes with bacterial growth should be removed as soon as they are detected and disposed in the autoclave waste in your own lab. (yellow indicator can change colour if left to long – in control tubes, growth should be obvious after 24 hours incubation).
8. **Recording**: EHS has developed a ‘Biological Indicator (BI) Test Results Log’ that requires the following information. A custom Biology Department web form is available at [https://www.mcgill.ca/biology/autoclave-monitoring-results](https://www.mcgill.ca/biology/autoclave-monitoring-results)

Your results will be added to cumulative log sheets that will be posted on the relevant autoclave(s). Items you need to record in the on-line form include:

   a. Date  
   b. Name of Operator  
   c. Autoclave – e.g. N5 Right or Left - select from drop-down box.  
   d. Cycle [type – gravity vs. liquid] & Temperature  
   e. Cycle Time  
   f. Type of load (e.g. petri dishes)  
   g. Biological Indicator Used - NAMSA, SCBI in the current case  
   h. Biological Indicator Expiry Date & Lot # [written on vial]  
   i. Results (Pass/Fail) for Autoclaved Indicator & Control Indicator. Pass = sterilized (medium remained purple); Fail = not sterilized (medium turned yellow & cells grew – the control should always fail, if not, the indicator lot has problems)

9. **Disposal**: Used vials of biological indicators should be disposed of in your lab’s biological waste for autoclaving