

Bacillus Ampoule Test Procedure, for Autoclave Monitoring

The following testing will be conducted on a monthly basis and results will be recorded in an autoclave log. Log records to be kept for a minimum of three years. Please contact EH&S to supply spore ampoules if needed (ext. 24244).

Initial Process:

1. Retrieve two spore ampoules (Bacillus Stearothermophilus).
2. Identify the indicators (spore ampoules) by labeling them with the proper information. Such as date, autoclave number, control, test, etc.
3. Place the spore ampoules in a horizontal position with representative materials to be sterilized. Both ampoules should be located in the part of the load that would be considered most questionable for sterilization. (remember to attach a piece of autoclave tape onto the load)
4. Select appropriate cycle to process the load (usually 30min for liquid or dry cycle).
5. Once the cycle has come to a finish allow autoclave to decompress and cool down (min. 10 min).
6. Remove load from autoclave and allow it to further cool down (10 - 15min).
7. Retrieve the spore test ampoules from the load.
8. It is common to see a color change from the spore ampoules from purple to black; usually this distinguishes indicators that have been exposed to steam to those that have not.

Incubation process:

Use any incubator that is adjusted to a temperature between 55⁰C and 60⁰C. Take both indicator ampoules from the load that was run and gently squeeze the plastic crusher to break the glass ampoule and place those in the incubator. Take a third spore ampoule (label control ampoule) that was not run in the load proceed to break the glass ampoule and also incubate in the same incubator, allow incubation and verify all three spore ampoules after 24 hours.

Interpretation:

1. Examine indicator ampoules for any color change after 24 hours. The presence of yellow color change indicates a fail test and positive bacterial growth. No color change indicates a passing test and proper sterilization of sample. The control ampoule should have a yellow color change.
2. If a fail test is confirmed run the test again and discontinue normal use of the autoclave for the time being. If the second test round fails again, inform all appropriate personnel as well as Environmental Health & Safety (Biosafety Specialist ext. 24244). Discontinue full use of autoclave machine until approved for use.
3. If a passing test is confirmed from the first round or second round, you can proceed with regular use of the autoclave.
4. Record all results in your monthly autoclave log.
5. Ensure to dispose of all used Bacillus ampoules by autoclaving first before discarding.