

Heat shock / Heat Activation

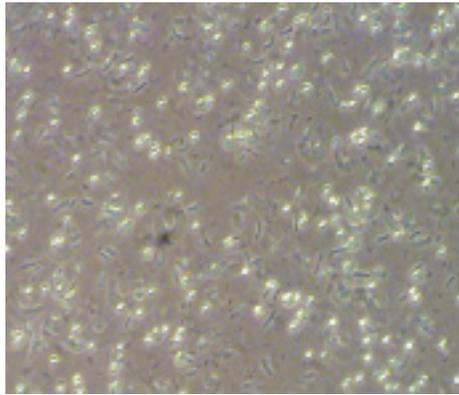
By Kurt McCauley

Verifying the population of a lot of incoming biological indicators is a common and recommended practice. Care should be taken to follow the manufacturers' methods, which almost certainly will include a heat treatment step, often referred to as a "heat shock". Depending on the organism contained in the BI, the conditions of the heat shock will vary. For example, USP chapter <55> states that thermophilic spore formers should be treated at 95°C to 100°C for 15 minutes, while non-thermophilic spore formers should be treated at 80°C to 85°C for 10 minutes.

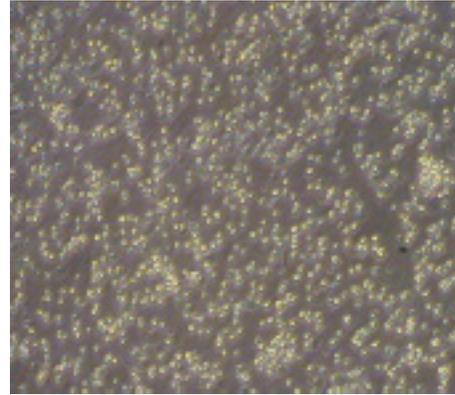
What is the purpose of the heat shock? Heat shock is simply the controlled heat treatment (time at a stated temperature) under which a microbial population is subjected. From a microbiological standpoint, heat shock serves two main purposes:

- 1) The activation (or "breaking of dormancy") of spores inducing them to germinate. This is a well-studied effect and certain species (or strains of the same species) seem to be more responsive to activation via the heat shock treatment.
- 2) The isolation of spore formers from a sample by the elimination of vegetative cells through thermal destruction. The spores, being more robust, will survive the heat treatment while the vegetative cells will be eliminated from the viable microbial population. A boil test could be considered a type of this heat treatment.

The spores used in biological indicators should be clean and free of vegetative cells as well as all other cellular debris. Therefore, the purpose of the heat shock in the population assay of a biological indicator is primarily the heat activation of the spores.



Spores Prior to Cleaning

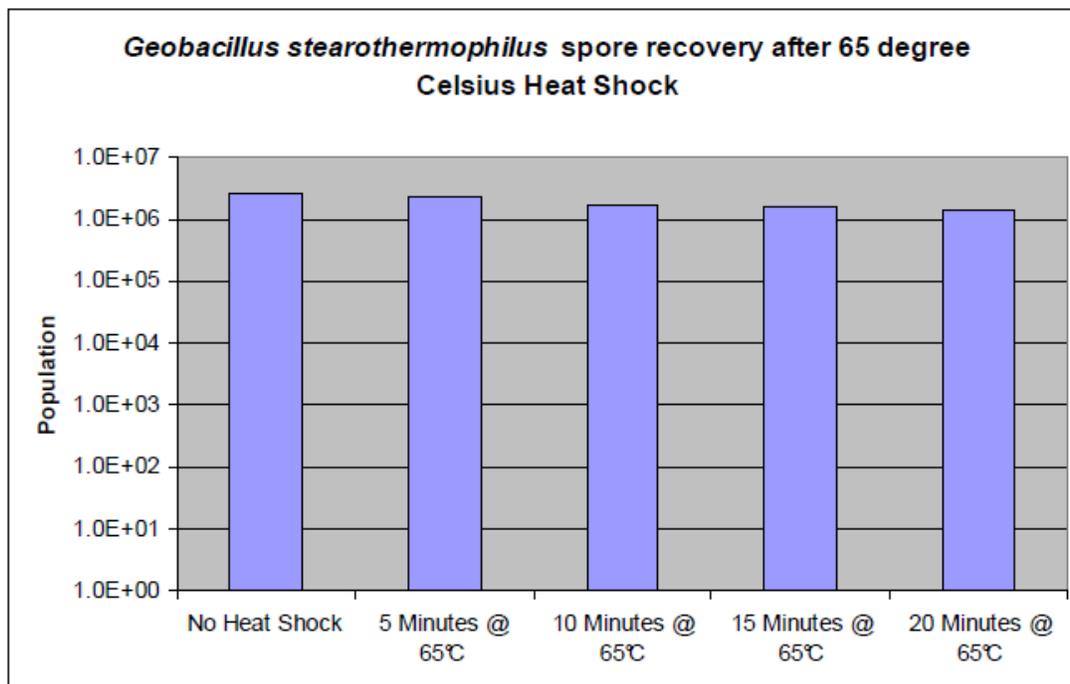


Spores After Cleaning

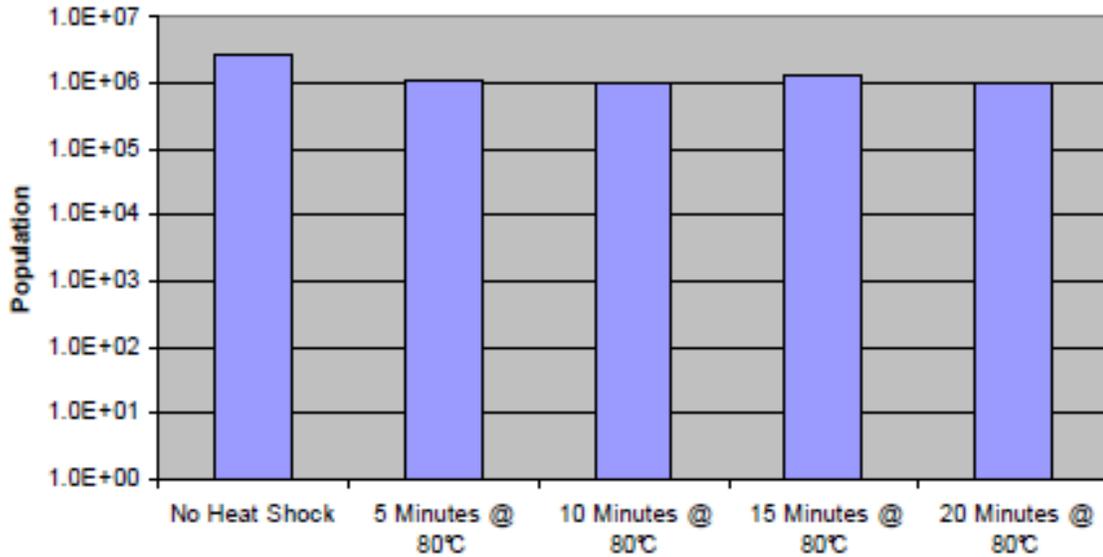
An example of the heat shock effect can be viewed in the series of charts below (data were obtained from an internal Mesa study). Under these tests, there was little change in the population of *Geobacillus stearothermophilus* spores in any of the heat shock treatments as compared to the untreated sample. Other strains of the same organism may show a different result.

The *Bacillus atrophaeus* spores showed an increase in recovery after heat shock with the optimal conditions being 10 minutes at 80°C. This is the same time-temperature combination for heat shock stated in the USP for this organism.

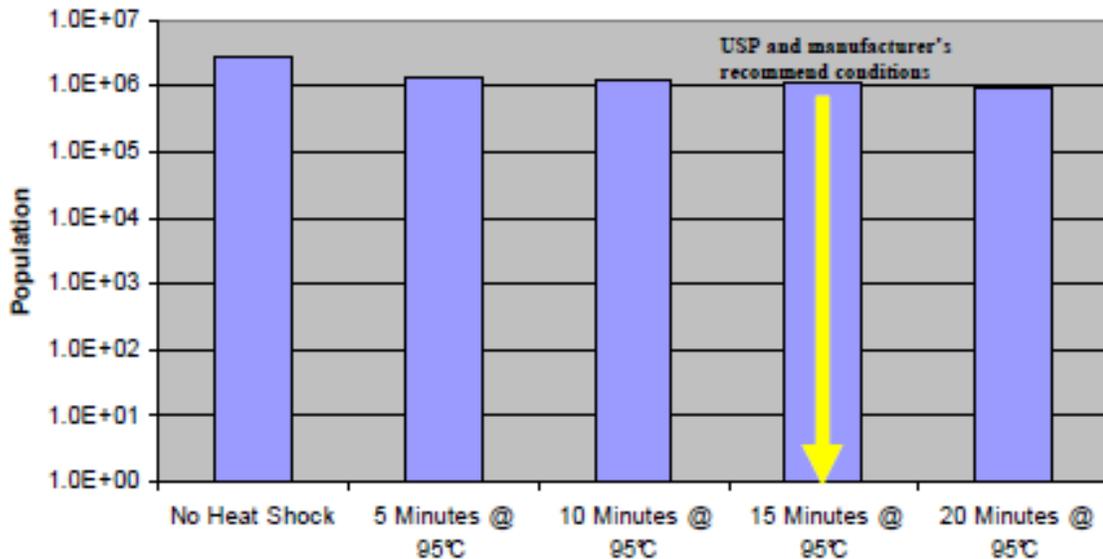
When performing a population assay on a lot of biological indicators there are many variables that can lead to results different from the certified value, and heat shock is only one of those variables. It is important to follow the manufacturers' instructions as closely as possible in order to successfully verify the population.



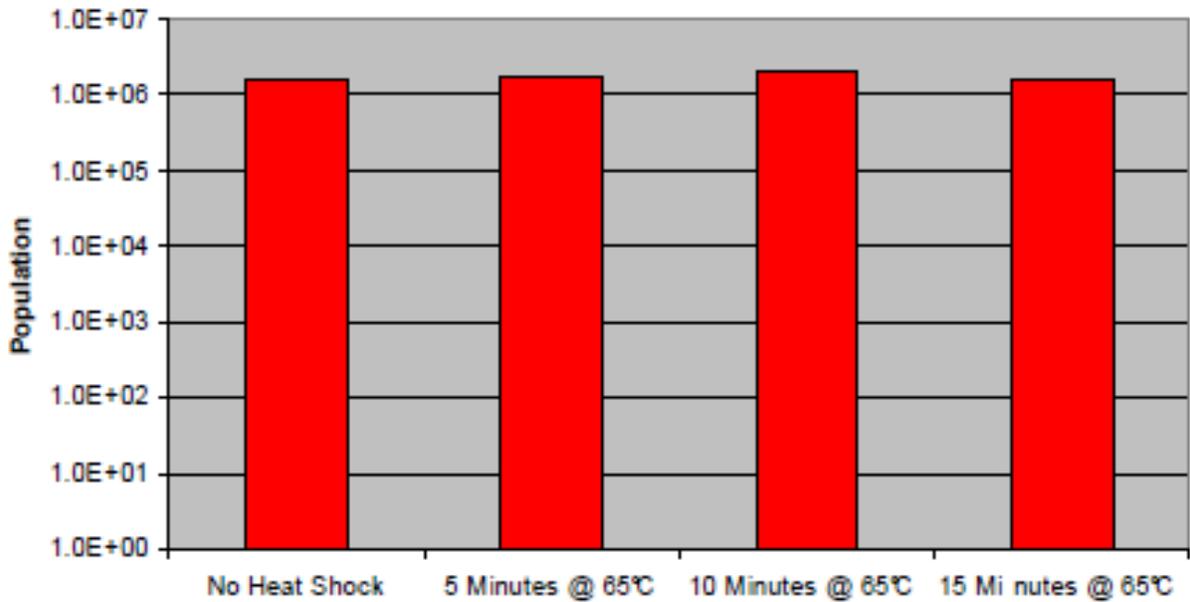
Geobacillus stearothermophilus spore recovery after 80 degree Celsius Heat Shock



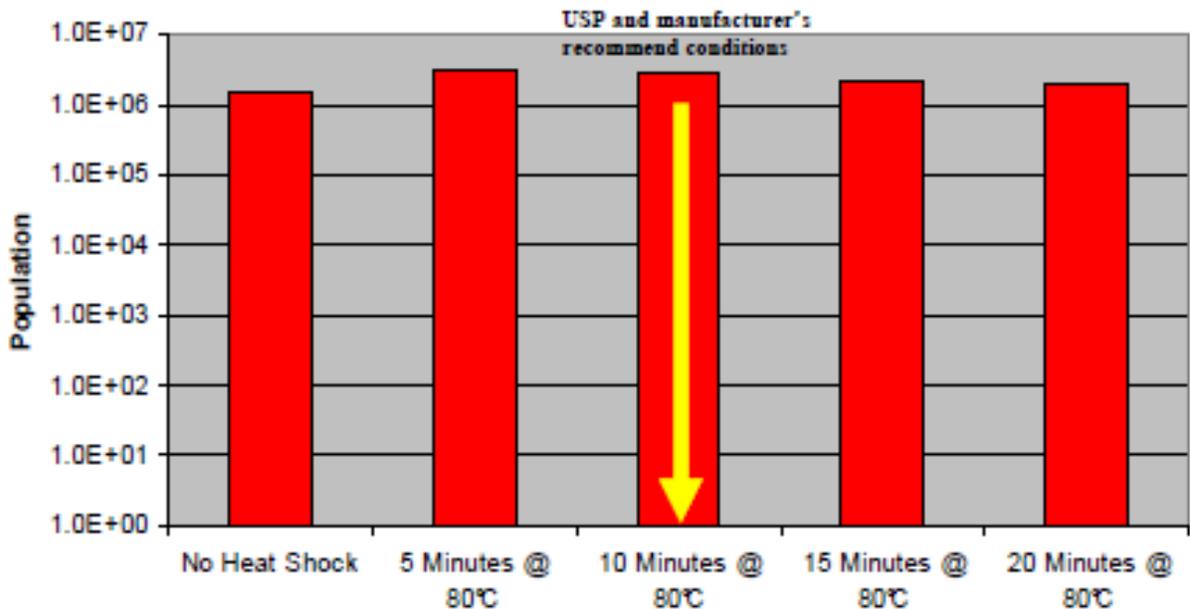
Geobacillus stearothermophilus spore recovery after 95 degree Celsius Heat Shock

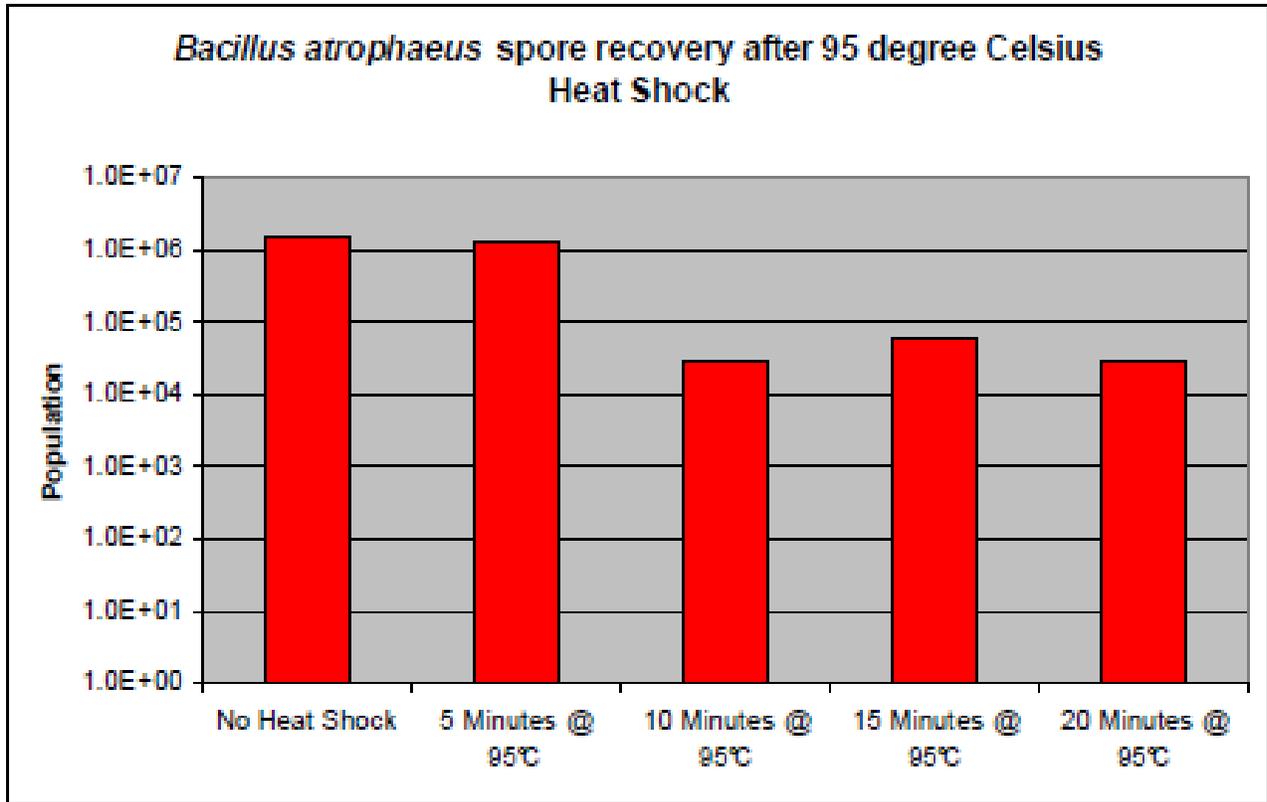


Bacillus atrophaeus spore recovery after 65 degree Celsius Heat Shock



Bacillus atrophaeus spore recovery after 80 degree Celsius Heat Shock





Kurt McCauley has a B.S. in Microbiology and is a Product and Applications Engineer in the Sterilization and Disinfection Control Division of Mesa Laboratories. He began work at Mesa Labs in 1995 and has been involved with all aspects of biological indicator production and development. Mr. McCauley currently serves as Co-chair for AAMI Working Group 91 (Resistometers), and is an active member of both AAMI/ISO Working Groups 4 (biological indicators) and 6 (chemical indicators).