

# Standard Operating Procedure

## Title: Stock Suspensions of Micro-Organisms

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*Bacillus sphaericus* ATTC 4525

- 4.2.1. Remove the vial for the organism required from the freezer and place in a separate pre-frozen cryoblock.
- 4.2.2. Transfer using a sterile disposable plastic loop, in the biohazard cabinet, 2 beads onto 2 separate slopes. (Use RCA slopes for *Cl.Sporogenes* and TSA or Sporulation Agar (SA) slopes for *B.subtilis* and *B.sphaericus*).
- 4.2.3. Discard the loop.
- 4.2.4. Using a fresh sterile plastic disposable loop streak the bead onto the slope surface.
- 4.2.5. Remove the bead from the slope by tipping into Hibitane-containing waste container and pulling out with the loop.
- 4.2.6. Repeat for the second slope using a fresh sterile plastic disposable loop.
- 4.2.7. Incubate slopes at 32°C (±1.5°C) for 24 – 48 hours (*Cl.Sporogenes* under anaerobic conditions). If suitable growth has not been obtained after 4 days incubation, re-streak the organism from the beads onto a fresh set of slopes and incubate as above. Once substantial growth has occurred, place one slope in the fridge to be kept for further use, if required (spare). Label with the Organism Name, Date of Subculture, and the fact that it is a spare.
- 4.2.8. On the remaining slope perform a spore stain (see **MICLAB 065**). Approximately 80% of the cells must yield spores before continuing further. If not, re-incubate the slope at 37°C (-0.5 to +1.5°C) for 24 – 48 hours to shock the organism and perform a spore stain once again to determine the level of sporulation. The slope can be incubated for up to 1 week at 37°C (-0.5 to +1.5°C). If sufficient sporulation is not achieved after this time, return to 4.2.1.
- 4.2.9. Wash the growth off the slope with 5–10mL sterile Peptone Water and transfer aseptically into a sterile 20mL McCartney Bottle.
- 4.2.10. Centrifuge at approximately 4000 rpm for 10 minutes or until the suspension has spun down sufficiently.
- 4.2.11. Decant and discard the supernatant.
- 4.2.12. Add 5 – 10mL sterile Peptone Water to resuspend the centrifuged spores. Repeat steps 4.2.10 and 4.2.11 so that the spore suspension is washed three times in total.
- 4.2.13. After the third wash, decant the supernatant and resuspend the spores in 5–10mL sterile Peptone Water.

Fill an attest incubator with 200mL Distilled Water and heat the water until it reaches 56°C. Place the sealed spore suspension in the incubator and cover it with foil to trap the heat. Heat the suspension for 30 minutes to kill the vegetative cells. Store the heat-treated suspension in the refrigerator at 4°C(±1°C).

**NOTE:** Slopes can be kept for a period of 6 months. Upon expiry a fresh stock must be prepared. If the stock becomes contaminated prior to expiry, the spare slope can be used to produce another stock suspension.

*Aspergillus niger* ATTC 16404

- 4.2.14. Subculture *A.niger* from KWIK-STIK™ (plus Microorganisms) to produce organisms at the 3<sup>rd</sup> passage (see section 1). Transfer microorganisms to 2 SDA slopes and incubate for 5-7 days at 25°C (±1.5°C). This step produces organisms at the 4<sup>th</sup> passage. Once substantial growth has occurred, place one slope in the fridge to be kept for further use, if required (spare). Label with the Organism Name, Date of Subculture, and the fact that it is a spare.
- 4.2.15. Wash the growth from the other slope using 5-10mL sterile Peptone Water and transfer to a sterile 20mL McCartney bottle. Store the suspension in the refrigerator at 4°C(±1°C).