Appendix 10
Protocol for Transfer and Culture of Spore Strips

Appendix 10: SPORE STRIP TRANSFER PROTOCOL
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12/08/2001

Preparation for Transfer

1. In the biological hood, turn on the UV light and laminar flow fan for one hour. Any instruments that you use (Bunsen burner, test tube racks, forceps) should be in the hood during this time so that they, too, are sterilized. DO NOT put the spore strips in the hood at this time because the UV light will kill the spores and invalidate the experiment.
2. Label the media tubes.
3. Prepare a small jar of 95% ethanol. Change the ethanol daily or with each set of experiments.

The Transfer

1. Ignite Bunsen burner.
2. In front of the flame, and six inches inside the hood, place the test tube rack where the transfer will occur. Keep the rack with the majority of the media tubes well off to the side. Arrange a small number of tubes on the transfer rack and loosen their caps.
3. Dip two sets of forceps in the ethanol and flame them in the Bunsen burner flame (an alcohol flame is not hot enough to kill spores). Remove the (blue) glassine envelope from its larger envelope with the flamed forceps.
4. To open the glassine envelope, there are flaps at either end that can be peeled apart. You may have to tease the end of the envelope to expose the flaps.
5. Peel the envelope open gently until the white spore strip is exposed. While holding the glassine envelope with one set of forceps, reflame the other forceps. Remove the spore strip from the glassine package with the freshly flamed forceps.
6. Inoculate the media by lifting the cap straight up off of the tube and dropping the spore strip into the growth medium. Be careful to not touch the tube with the spore strip, as the outside of the media tube is not sterile.
7. Tighten the cap on the media tube immediately.

Incubation and Observation

1. Incubate the media tubes upright at 37^{o}C (*B. subtilis* and *B. thuringiensis*) for seven days. For *B. stearothermophilus* incubate the media tubes upright at 60^{o}C for seven days. Every 24 hours, record the growth status (pH indicator and turbidity) of each tube.
2. The media tubes have a pH indicator that will change color (purple to yellow) when growth occurs. Score tubes as follows: (+) = growth (yellow), (-) = no growth (purple), (+/-) = intermediate growth (any color in between yellow and purple).