Activity One: Inoculating a Fungal Culture

The Plate
Always bring the culture to room temperature before plating the sample. If using the round DTMs, tape must secure the lid to the base on two sides to decrease possible contamination.

The name of the animal and the date when the culture was plated is written on the bottom of the base of the culture. The DTMs are stored upside-down in the incubator, so by labeling them on the bottom, it is easier to remember to keep them upside-down when taking them in and out of the incubator.

Sampling
The DTM is plated by using the Mackenzie toothbrush sampling method. Individually wrapped toothbrushes are sterile when unopened. A separate toothbrush is used for each individual DTM, never re-use a toothbrush.

*If the animal has an apparent lesion* Brush the animal from head to tail tip 15-20 times avoiding the lesion and paying special attention to the feet and face (if there are not lesions already there). After about 20 brushes from all over the cat, brush the suspected lesion about 5 times, ensuring that there has been some hair collected in the toothbrush.

*If the animal does not have a lesion* but is fostered with, housed with or part of a litter where one member has apparent ringworm lesion, all members who have been exposed MUST have a DTM sample taken. To take a sample of an animal with no lesion, brush the animal 20-25 times with the toothbrush over the whole body paying special attention to the feet ears and face.

Once the toothbrush samples have been collected, set up a clean area to inoculate the culture by placing an Acell wipe on the surface where the DTM will be plated. This avoids any environmental contamination that might get on the plate. Hold culture plate upside-down with the medium side up. Gently stab the tips of the toothbrush into the media, starting at 12 o’clock on the darker, more opaque side of the media and systematically move to the 6 o’clock position. Repeat on the lighter side of the culture plate and cover the whole plate in a consistent pattern.

Once a DTM is fully plated, store upside-down in the appropriate bin in the incubator.
Activity One: Storing the Fungal Culture

Creating an in-house incubator to store the fungal cultures is an important step to identifying a ringworm infection. Be creative when setting up an incubator. The most important things to remember are:

- Keep temperature between 80-85F degrees
- Keep out of direct sunlight
- Keep vented to avoid too much moisture

**Simple Tupperware Incubator**

1. Start with a shoe box sized Tupperware, create a small hole on one side for venting.
2. Place a towel above and below incubator
3. In between layers of towel below incubator place small constant low-setting heating pad (like ones found in heated pet beds) that can safely remain on.
4. Monitor temperature with cheap reptile thermometer that can be found at any pet store.
5. If the thermometer is reading high temperatures, try adding a small box to elevate the cultures away from the heat source

6. Monitor cultures daily and record color change and growth

4 day old culture  |  6 day old culture  |  9 day old culture