Appendix 1: Aseptic Techniques for Handling Microbial Culture

The use of aseptic technique in the transfer of microorganisms is vital in microbiological experiments, to ensure there is no contamination of cultures by microorganisms from the environment. Also, the environment is not contaminated by the organisms being handled.

1. The sterile field:

- All microbial work should be carried out within the sterile field around the flame where air convection currents (an updraft) are maintained.
- Light the Bunsen burner. Allow it to burn for 20 seconds before carrying out a procedure, to ensure sufficient time for sterilising the surrounding air.

2. Sterilising the mouth of the vessels:

- When removing the lid of a vessel (e.g. bottle, broth tube or culture flask), hold it at ~45^{o} or a slant as far as possible without spilling the contents (Fig. 1). This prevents airborne particulates from entering. While holding the lid in one hand after opening, pass the mouth of the vessel over the flame in a circular motion 3–4 times with the other hand, to burn off any plausible contaminants (Fig. 1).

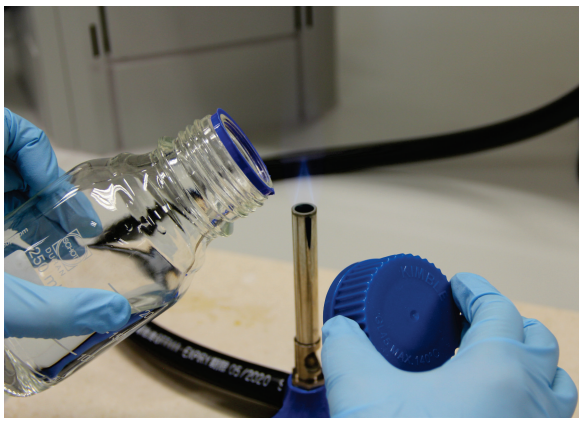

Fig. 1

- When adding or removing liquids, hold the vessel at a slant.
- Flame the mouth of the vessel again. Allow it to cool slightly, and recap the vessel.

3. **Transferring microorganisms:**

- When transferring microorganisms to a broth tube or culture plate, do so in as short a time as possible, to reduce the time of exposure.
- When handling culture-ware with a wide opening, such as a Petri dish, raise the lid of the dish at ~45° or with as little exposure as possible (Fig. 2).

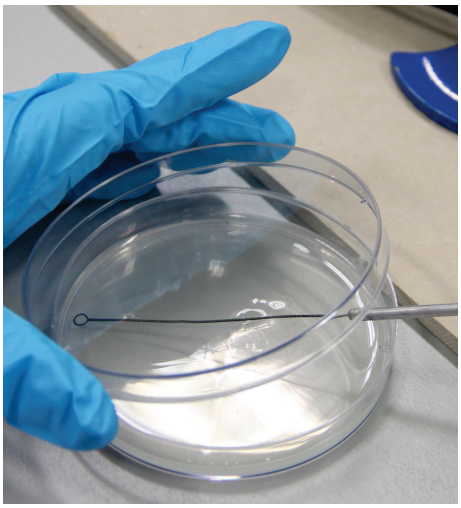

Fig. 2

4. **Sterilising the inoculating loop:**

- When using a reusable inoculating loop, place it at the tip of the blue cone (Fig. 3). This is the hottest part of the flame. Flame the loop to red hot (Fig. 3) and allow it to cool. Disinfect the loop after each use by flaming it to red hot. The loop can be placed in storage after cooling.
- When using a disposable inoculating loop, use a new one for handling different samples. Disinfect it with 10% chlorine bleach before disposal.

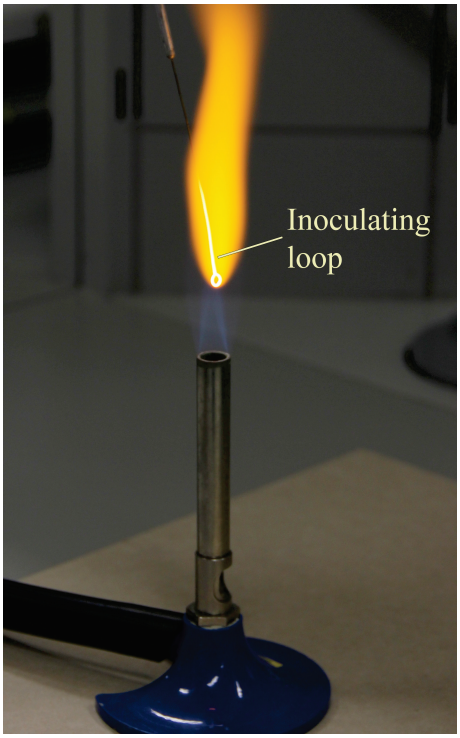

Fig. 3

5. **Sterilising the spreader:**

- When using a reusable spreader (glass or metal), immerse it in a beaker of 70% ethanol*. Pass it through the flame gently to burn off any clinging microorganisms (Fig. 4). Allow it to cool. Disinfect the spreader after each use by the mentioned procedures before placing it in storage.
- When using a disposable spreader (i.e. a plastic spreader), use a new one for different samples. Disinfect it with 10% chlorine bleach before disposal.

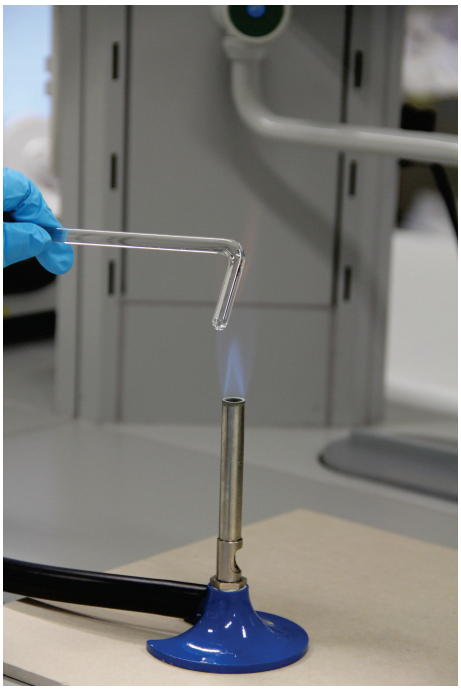

Fig. 4

***Caution: Do not put a hot spreader into ethanol, as the ethanol may catch fire. If the ethanol catches fire, cover the beaker with a fireproof board (larger than the beaker) to cut off the oxygen supply, thus extinguishing the fire.**