Standard Operating Procedure
Spreading Liquid Cultures of Bacteria on Agar-Media Plates

Spreading liquid bacterial culture onto agar-plates is a standard technique in biology. However, care must be taken when plating cells as an open flame and ethanol are used in order to maintain a sterile environment and equipment. A thoughtful lab-bench design is a must: ensuring that the alcohol is not too close to the open flame and that the open flame is positioned in a place which limits the extent to which your hands will pass near the fire. It is also crucial to know how to use all fire prevention equipment and their location in the lab: including emergency shower and fire extinguishers.

A) Work Area Preparation

1) Put on Personal Protective Equipment (close-toed shoes, eye goggles, hair restraints, and Flame-Resistant lab coat). Also, remove any clothing or personal items that may pose a specific threat (eg. loose and dangling jewelry or clothes or any synthetic fabrics that could melt or burn in a fire). Although a need for sterile a environment is of the utmost importance, latex gloves are not suggested since the presence of latex would greatly increase the severity of any burns received, much like the melting of synthetic fabrics. Hands should simply be washed with warm soap and water to remove possible contaminants.

2) Clean your work bench and remove all unnecessary items from the immediate work area, especially flammable items and chemicals.

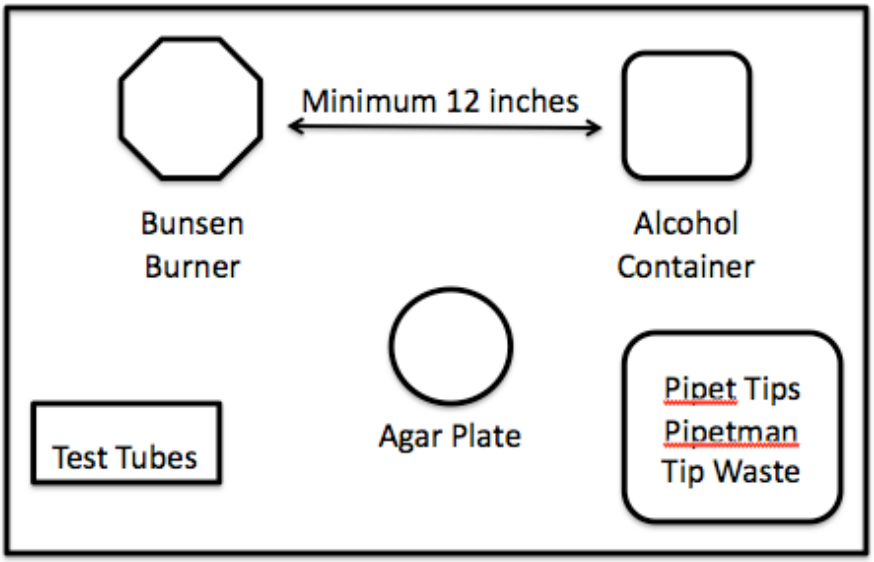
3) Pour ethanol into a secondary container made of GLASS or METAL, wide enough to dip the spreader into. Plastic is not appropriate since it would melt if the alcohol ignited, preventing fire containment. The container should have an easily replaceable lid so that any fire may be smothered if the alcohol ignites. Only a small volume of ethanol is needed, 1 cm or less in depth, which in a standard container should be less than 10 mL in volume. An example of an ideal container is a Pyrex Glass Petri Dish (FisherSci Cat# 08-747B).

4) Wipe up any ethanol that spilled while pouring from a stock bottle into container described above.

5) Arrange liquid culture rack, petri dishes, alcohol container, Bunsen burner, and any other materials necessary to plate cells. The alcohol container should

be kept as far away as is reasonable from the Bunsen burner. The liquid cultures and petri dishes should be kept reasonably close, but not so close as to risk burns to the hands when manipulating plates and test-tubes. Keeping cultures and plates near the flame will aid in maintaining a sterile environment by utilizing the updraft of air created by the Bunsen burner to reduce the risk of spores falling into petri-dishes or test-tubes.

This is an example of a bench set-up arranged for someone who would pipet with their RIGHT HAND. It is best to keep the flame near the hand you use less, as this will decrease the likelihood of your working hand passing too close to the flame.



B) Plating Cells

1) Ignite the Bunsen Burner (a 3-5 cm flame is sufficient).
2) Remove desired culture from the test-tube and place on an agar plate.
3) Dip the glass spreader into alcohol, allow excess to drip off, and return lid to ethanol container.
4) Touch spreader to the flame to ignite the alcohol, and remove. Slowly turning the spreader will prevent burning drops from falling while the alcohol burns.
5) Spread culture around plate and recover petri-dish.
6) Repeat steps 2-6 as necessary,
7) When finished, immediately turn-off flame, then procede with clean-up of other materials.