Basic Sterile Technique

A. Contamination Potential: Cultures and Media.

1. Anything from non-ambient temperature and humidity is subject to condensation and hence is a source of contamination. This includes:
   a. Refrigerated items such as medium.
   b. Cultures from incubators.
2. Medium and other reagents, with very few exceptions, do not need to be warmed to 37°C prior to use. In fact, pre-warming to 37°C can shorten the half-lives of many medium components, as well as trypsin. Furthermore, water baths, no matter how frequently they are cleaned, are a common source of bacterial and more particularly, fungal contaminations.
   a. For these reasons, we recommend that medium and other liquid reagents be warmed to room temperature only.
   b. When warming reagents to room temperature, it is recommended that they be protected from fluorescent light by wrapping them in aluminum foil. Fluorescent light causes breakdown of some medium constituents into toxic by-products.

B. Cleaning of the BSC.

This should be undertaken at the beginning of the work day and at the discretion of the user in between cultures.

1. Remove and dispose of all waste.
   a. We prefer using Virkon rather than chlorox.
      i. It rapidly inactivates potential hazards.
      ii. It has a colored potency indicator.
      iii. It does not eat stainless steel like bleach.
      iv. Dilute bleach solutions have a very short life.
      v. The draw backs include cost and powder-induced irritation.
2. Thoroughly wipe down BSC surface with 70% EtOh.
   a. It is advisable to move items such as tube containers and pipet tip boxes so that the surface underneath them can be wiped as well.
3. Lower the hood sash.
4. Immediately turn off the blower.
5. Turn on the UV light, irradiate BSC for 10 minutes only.

a. Longer irradiation is ineffective and only serves to shorten the life of the UV bulb. It is not apparent to the eye when the UV bulbs are delivering ineffective doses of UV irradiation.

6. Turn off the UV light.
7. Turn the blower back on.
8. Immediately raise the sash to the appropriate level.
9. Let the blower run for 10-15 minutes before initiating work in the BSC.

C. Wipe-down of Reagents and Cultures:

1. Prior to bringing reagents and cultures into the BSC, they should be sprayed down with 70% EtOh.
   a. Care should be taken with vented flasks such that the membrane in the flask cap is not wetted.
2. After placing them in the BSC, reagents and cultures should be wiped, top to bottom, with an EtOh-soaked Kim-wipe.
3. Fresh plates from unopened or tightly sealed containers or bags do not need to be wiped down.
   a. opened bags of flasks etc. should be tightly resealed with either rubber bands or tape.

D. Movement and Positioning within the BSC

1. The BSC maintains a “sterile” internal environment by blowing a curtain of HEPA-filtered air. Disturbing this curtain breaches the integrity of the environment. For these reasons, we recommend that movement in and out of the BSC be restricted.
   a. Minimize clutter within the BSC-don’t have unnecessary items inside.
   b. Do not cover the front grill with pipette wrappers, papers, etc.
   c. Do a rough calculation of the number of pipettes you’ll need and bring them into the hood with you. Lay them to one side.

E. Starting to work:

1. It is recommended that you always wear gloves when handling cultures. We also recommend the use of either CLEAN lab coats or disposable sleeves.
2. Spray your hands with 70% EtOh.

3. Place your hands into the BSC and hold them motionless for about three seconds. This permits the air barrier to reform.
4. Try to conduct your work in the middle of the BSC. Do not work right in front of the grill, or even worse, over the grill.
5. Movements should be slow and deliberate. Jerky movements disturb the air barrier.

F. Actually Working:

1. Loosen, but do not remove, the caps of solution bottles and flasks.
2. Open individually wrapped pipettes as follows:
   a. open at the top
   b. peel back both sides like a banana.
   c. do not let the dirty outside come in contact with the sterile inside.
   d. discard the wrapper in a out-of- the way location in the BSC.
3. Remove the cap from the bottle.
   a. It is preferred to hold the cap in your hand away from the top of the bottle/flask.
      i. Alternatively, the cap can be placed upside down on the BSC surface-this is not preferred.
4. Insert the pipette into the bottle, making sure not to contact the mouth of the bottle.
5. Aspirate the required volume of medium.
6. Withdraw the pipette, again making sure that the pipette, especially the tip, does not contact the mouth of the bottle.
7. Place the cap back on the bottle.
   a. It is important to minimize the time containers are open.
   b. It is also important to never pass anything (including your hands) over the mouth of an open container.
8. Transfer the pipette contents into the recipient flask/bottle:
   a. Do not allow liquid to fall great distances, as this creates splash-back and aerosols.
   b. It also creates excessive bubbles which are generally bad for cells.
   c. The preferred method is to:
      i. Dribble the contents gently down the side of the container.
      ii. Gently discharge the contents at the bottom of the container.

9. Remove the pipette from the container, taking care not to touch the tip of the pipette to the mouth of the container.
10. Pipetmen are required for many applications. The following guidelines will help you use them safely:
    a. Always use blocked, sterile tips.
    b. Wipe down the pipetman with 70% EtOh prior to use.
    c. Do not let the barrel of the pipetman touch any surfaces of culture flasks or medium containers.
    d. As an alternative to inserting a pipetman into a culture flask:
        i. using a 1 ml pipette, transfer a small volume of culture to a single well of a 96 well plate.
        ii. The correct sampling volume can then be safely removed from the well.
    e. As an alternative for using pipetmen to make additions to flasks:
        i. transfer a small volume of solution to a small, sterile tube.
        ii. Use the pipetman to make the additions to the tube.
        ii. transfer the tube contents back to the flask/bottle.
11. Continue using these practices until you are done!

G. Clean-up

1. Remove and return cells to incubator.
2. Return medium to proper storage.
3. Discard all waste in the appropriate receptacles.
    a. The university considers pipettes and tips as sharps, and hence they must go in puncture-proof sharps containers.
4. Spent culture medium should be disinfected by transferring the liquid into the disinfectant solution (Virkon or bleach).
5. Generally speaking (unless you are dealing with known or suspected pathogens) culture flasks can be placed, un-rinsed, in the orange biohazard bags.
6. In general, gloves should go into the orange biohazard bags as well, although apparently this is not a hard university policy.
7. The hood should be thoroughly wiped down with 70% EtOh at the end of procedures.
8. As a kindness to your labmates, restock pipettes and flasks.

H. Miscellaneous Items:

1. Do not mouth-pipette. Ever.
2. Only one person at a time should work in a BSC- no buddying up.
3. While working, conversation should be kept to a minimum.
4. It is strongly advised that only one culture be in the BSC at a time, to minimize cross-contamination.
    a. Some authorities state that each culture has its own dedicated reagents, but that is often impractical.
5. Although this is not often done in practice, some authorities caution against use of antibiotics in culture medium, as they may mask low-level contaminations.
    a. We carry our cultures in antibiotic-containing medium, but at regular intervals (we try for every three months), we passage them in antibiotic-free medium to ensure their sterility.
6. Cell lines should be obtained from reputable vendors such as the ATCC, not from the lab down the hall.
7. Until demonstrated otherwise, new cell lines should be considered as potentially contaminated and if space is available, they should be kept in a quarantine incubator.
    a. suspect cultures have their own reagents, and are handled only at the end of the day, to minimize contamination.
8. Once demonstrated to be clean, cell lines should be rapidly expanded and many aliquots frozen.
9. Cross-contamination of cell lines is a relatively frequent phenomenon. If your research centers heavily on one or a few cell lines, it is a good idea to have their identity verified.
    a. Short tandem repeat analysis (STR: DNA fingerprinting) is probably the best way to have this done.
10. Pouring, as opposed to pipetting, is discouraged, due to:
    a. the potential for contamination from back-splashing;
    b. residual remaining liquid on the vessel lip can form a bridge for contaminants.
11. Flames in BSCs are a bad and dangerous idea.
12. Sharing medium is a bad idea. Wherever practical, selfishness is a virtue in cell culture.

13. It is a good idea to examine your cultures, macroscopically and microscopically, on a frequent basis (pretty much every day) to learn what “normal” behavior is. This makes spotting abnormal behavior much easier!

14. Cultures cannot regulate their own temperature and pH. Therefore, being out of a 37°C/5% CO_2 environment is stressful. This should be kept in mind when working with cultures or observing them. Time out of the incubator should be minimized.

15. Finally, biohazards:
    a. Reputable suppliers of cell lines will tell you the BSL class under which you should handle specimens.
    b. Universal safety precautions should always be observed.
    c. If you are dealing with primary human source material (or suspected infected material of any source), you should consult EHRS.

A great reference:

Culture of Animal Cells: A Manual of Basic Technique, by R. Ian Freshney, 4th Edition, Wiley-Liss, 2000.