



Sterile Culture Techniques

Successful control of contamination depends largely upon the operator's techniques in aseptic culture. You should always be aware of potential sources of contamination such as dust, hair, hands, and clothes. Obviously, your hands should be washed, sleeves rolled up, long hair tied back, etc. Washing your hands with 95% ethyl alcohol is an easy precautionary measure that can be taken. Talking or sneezing while culture material is exposed also can lead to contamination. When transferring plant parts from one container to another, do not touch the inside edges of either vessel. By observing where contamination arises in a culture vessel, you may be able to determine the source of contamination.

In plant tissue culture, small pieces of plant tissue are placed on or in a medium rich in nutrients and sugar. If bacteria or fungi come in contact with the plant tissue or the medium, the culture becomes contaminated. The contaminants (bacteria and fungi) will use nutrients from the medium and the plant, which quickly destroys the plant tissue. Our aim is to surface sterilize the plant tissue and put it on a sterile growth medium without any bacteria or fungi getting on the plant or medium. This is not easy because bacteria and fungal spores are in the air, on us, in us, and under us!

When you see sunlight shining in a window you can, from certain angles, see dust particles in the air. There are hundreds of bacteria attached to each dust particle. A horizontal laminar flow unit is designed to remove the particles from the air. Room air is pulled into the top of the unit and pushed through a HEPA (High Energy Particle Air) filter with a uniform velocity of 90 ft/min across the work surface. The air is filtered by the HEPA filter so nothing larger than 0.3 μm (micrometer) can pass through. This renders the air sterile. The flow of air from the unit discourages any fungal spores or bacteria from entering. All items going inside the unit should be sterile or sprayed with ethanol or isopropyl alcohol. They will remain sterile unless you contaminate them.

A transfer cabinet provides an enclosed environment that is not sterile but can be sterilized. A cardboard box lined with aluminum foil or plastic, or a well-cleaned aquarium, provides a shield to reduce contamination. A box that is 20-24 inches wide, 20-24 inches high, and 12-16 inches deep provides a good work area. Working inside any of these does not guarantee success. The following precautions are necessary for all work areas.

1. The room should be swept and if possible, mopped.
2. Each work surface should be washed with a 10% Clorox^R, Lysol^R, or other disinfectant solution.
3. Doors and windows should be closed.
4. Air conditioners and fans should be turned off.

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5. If possible, each technician must have a work space that can be properly treated against contamination. For example, the box or aquarium mentioned earlier, or a piece of poster paper lying on the table to indicate the student's sterile workspace.
6. Have spray bottles filled with 70% ethanol or isopropanol (never methanol) placed so each student has access to one bottle. Spray everything going into the sterile area.
7. Have a central supply area so all necessary items can be picked up and taken to the workspace as needed. Items can be returned to the central supply area after being used.
8. Sterile instruments will be needed for each person. One way to accomplish this is to have a ½-pint jar of 70 % ethanol for scalpels and short forceps. When tissue has to be positioned in a vessel, long 10-inch forceps are needed. The long forceps need to be placed deep enough in alcohol so that any part of the forceps that might come into contact with the vessel is sterilized. A 100-ml graduated cylinder can be used to hold the alcohol and long forceps. A ½-pint jar of sterile water is needed for dipping the instruments to remove the residual alcohol that might dry out plant tissues.
9. A sterile work surface is needed on which to place the sterile tissue to trim it. The easiest thing to use is a sterile petri dish. If you have glass ones, you can autoclave and reuse them. Pre-sterilized plastic dishes are used and discarded. Spray the bag of dishes with 70 % alcohol before you open it and place the desired number of unopened dishes at each station. Each dish has two sterile surfaces-the inside top and inside bottom.
10. Long hair should be tied back or covered. Hands should be washed, not scrubbed (scrubbing dries hands and creates flakes of skin that have bacteria) and sprayed with 70 % ethyl or isopropyl alcohol or coated with isopropyl alcohol gel. Gloves and masks provide extra protection. Do not talk while performing sterile operations. Do not lean over your work. Keep your back against the backrest of your chair. Try to work with your arms straight: this position may feel awkward, but it will reduce contamination. Do not pass nonsterile items over sterile areas or items. Reach around rather than over. Make your movements smooth and graceful so that you do not disturb the air more than is necessary. Work quickly though, the longer it takes to manipulate the tissues the greater the chance of contamination. Have only the necessary items in sterile work area. Remove items that are no longer needed as quickly as possible. Act out each step before beginning so that you understand what you are about to do.

Store cultures in a well-lit area (not in direct sunlight), and do not allow the temperature to exceed 80⁰ F where the cultures are stored. If you are placing cultures under lights, use only fluorescent light. The preferred schedule is 16 hours of light and 8 hours of dark. Check the temperature prior to placing the cultures under the lights because temperature will build even under fluorescent lights.

Check cultures every 3-5 days for contamination. Slimy areas mean bacterial contamination while fuzzy areas are due to fungal contamination. *Do not open containers that are contaminated.* The contaminants could be disease causing or pathogenic. The safest method of disposing of these cultures is to autoclave (or pressure-cook) them for 15 minutes at 15 psi.

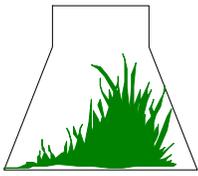
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Product Information Sheet

Contaminated plastic dishes can be placed inside a large can or autoclavable bag to be sterilized before discarding.

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