

Sterile Technique Primer

STERILE TECHNIQUE is a common sense system of practices used when handling microorganisms in culture to prevent contamination of the cultures and those working with them. These practices succeed only if the tools, culture vessels, and media being used are sterile to begin with, and if the worker understands how to prevent contamination of the cultures and workspace. Below we describe key aspects of sterile technique.

Sterilization Via Autoclaving

All materials and media used for culturing microorganisms (especially bacteria) must be sterilized before using. This is most efficiently done in a device called an *autoclave* that heats the material to high temperature (250 F) under pressure (~15 PSI) for a time long enough (15-20 min) to kill all microorganisms that may be present. Autoclaves are also used to sterilize old, contaminated materials prior to safe disposal via normal waste streams.

Have a work plan

Always work from a plan or a protocol. Knowing what you need to do and how you will accomplish it efficiently will minimize the time that cultures are exposed and vastly reduce the chances of making mistakes.

A Clean Workspace/ Protective Clothing

BEFORE and AFTER doing any work with microorganisms, it is critical to disinfect the bench top or hood with a disinfectant such as alcohol (minimum 70%; ethanol or isopropyl) or other available disinfectants. Lab coats and gloves should be worn if available. Your hands should be washed thoroughly and frequently with hot, soapy water.

Sterilization of Benchtop Tools

At the lab bench, tools may be quickly sterilized in a couple ways. Forceps and hockey stick spreaders should be dipped into an ethanol bath and then touched to flame. Allow the alcohol to burn off and the tool to cool before contacting bacteria. Inoculating loops and needles can be heated to red hot a flame to sterilize (Fig. 1a). ALWAYS allow flamed tools to cool before touching cultures – fried bugs! Ouch!

Protect Culture Media Against Airborne Contaminants

Culture tubes and Petri plates should never be exposed directly to the air column or your breath. Any time you must add or remove something from a culture tube, the cap should be removed and held right-side up to prevent contamination. Never set a cap on the benchtop. The rim of glass culture tubes should be flamed briefly before and after you go into the tube with a tool. Holding the tube at an angle also reduces exposure possibilities (Fig. 1c.) When using Petri plates, always keep the lid over the surface of the agar and raise it only enough to do your work (Fig. 2.)

Avoid Cross-Contamination of Cultures

If you are not paying attention carefully, it is easy to cross-contaminate cultures by forgetting to flame a tool or changing swabs. Never use the same tool in two different cultures without first flaming it to sterilize – when in doubt, flame it. With disposable swabs or loops, when in doubt, use a new one.

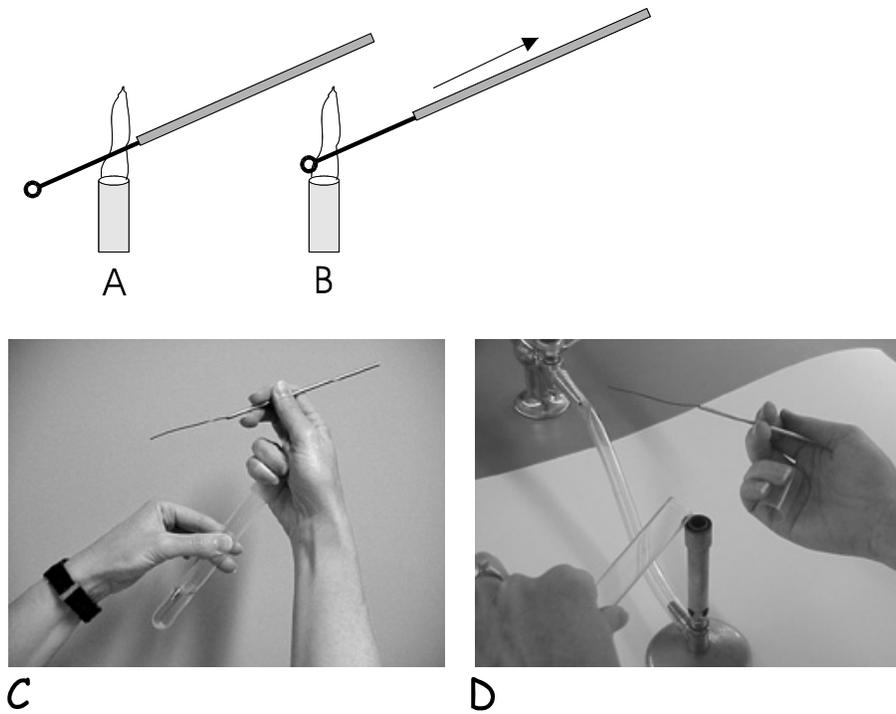


Figure 1. (A) Sterilization of inoculating loops/needles. Flame the loop to red hot starting at the base of wire and then (B) pulling it back through toward the tip. (C) Technique for holding the tubes and removing the caps to minimize airborne contamination. (D) Flame the tube mouth before and after intervention.

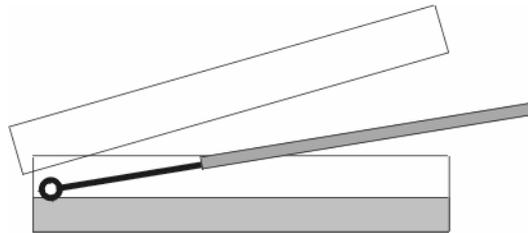


Figure 2. When working with Petri dish cultures, keep the lid over the agar surface at all times.