Aseptic Technique

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ABSTRACT

This chapter describes common laboratory procedures that can reduce the risk of culture contaminations (sepsis), collectively referred as "aseptic technique." Two major strategies of aseptic work are described: using a Bunsen burner and a laminar flow hood. Both methods are presented in the form of general protocols applicable to a variety of laboratory tasks such as pipetting and dispensing aliquots, preparing growth media, and inoculating, passaging, and spreading microorganisms on petri dishes. *Curr. Protoc. Microbiol.* 11:A.4D.1-A.4D.11. © 2008 by John Wiley & Sons, Inc.

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INTRODUCTION

Aseptic technique is a set of routine measures that are taken to prevent cultures, sterile media stocks, and other solutions from being contaminated by unwanted microorganisms (i.e., sepsis). While such actions are sometimes called "sterile technique," that terminology is appropriate only in reference to preventing introduction of **any** organisms to laboratory or medical equipment and reagents (e.g., during surgery). Since the goal of a biologist is to grow microorganisms or eukaryotic cells without introduction of extraneous organisms, aseptic techniques are crucial for accurate and meaningful experimentation.

One should always remember that a completely sterile working environment does not exist. However, there are a number of simple, common sense procedures that will reduce the risk of culture contaminations. Examples of aseptic technique are cleaning and disinfecting lab surfaces prior to use, limiting the duration that cultures or media are uncapped and exposed to the air, keeping petri dishes closed whenever possible, effectively sterilizing inoculating loops and other equipment that comes into contact with cultures or media, and avoiding breathing on cultures or sterile instruments. These precautions will become second nature after practical laboratory experience.

CAUTION: The information in this *APPENDIX* is meant as an introduction to basic aseptic technique and does not cover safety aspects necessary for working with specific organisms. It is important that the reader understand the appropriate methods of disposal and decontamination for the specific microorganism with which he or she is working. Refer to *UNIT 1A.1* and *APPENDIX 1B* for more information.

The Bunsen Burner

Probably the easiest way to create a relatively sterile environment on the laboratory bench is by using a simple gas-powered burner (see Fig. A.4D.1). This common piece of equipment burns a continuous stream of a flammable gas—usually natural gas (methane)—based upon a design made almost 150 years ago by the German chemist Robert Wilhelm Bunsen (1811-1899). A major purpose of the open flame in aseptic technique is to create a cone of hot air above and around the laboratory bench to reduce the viability of organisms on suspended dust particles. The ability of the Bunsen burner flame to heat things very quickly also makes it an ideal choice for sterilizing inoculating loops, warming glass bottle necks, or igniting alcohol on culture spreaders.

A Bunsen burner is not practical in some situations, e.g., within a laminar flow unit where the heat will disrupt airflow. A microincinerator may be used as an alternative. This consists of a circular heating element. Placing an inoculating loop or needle within the ring will quickly heat and sterilize the loop/needle. Note that a microincinerator does not provide other aseptic technique benefits of a lit Bunsen burner.

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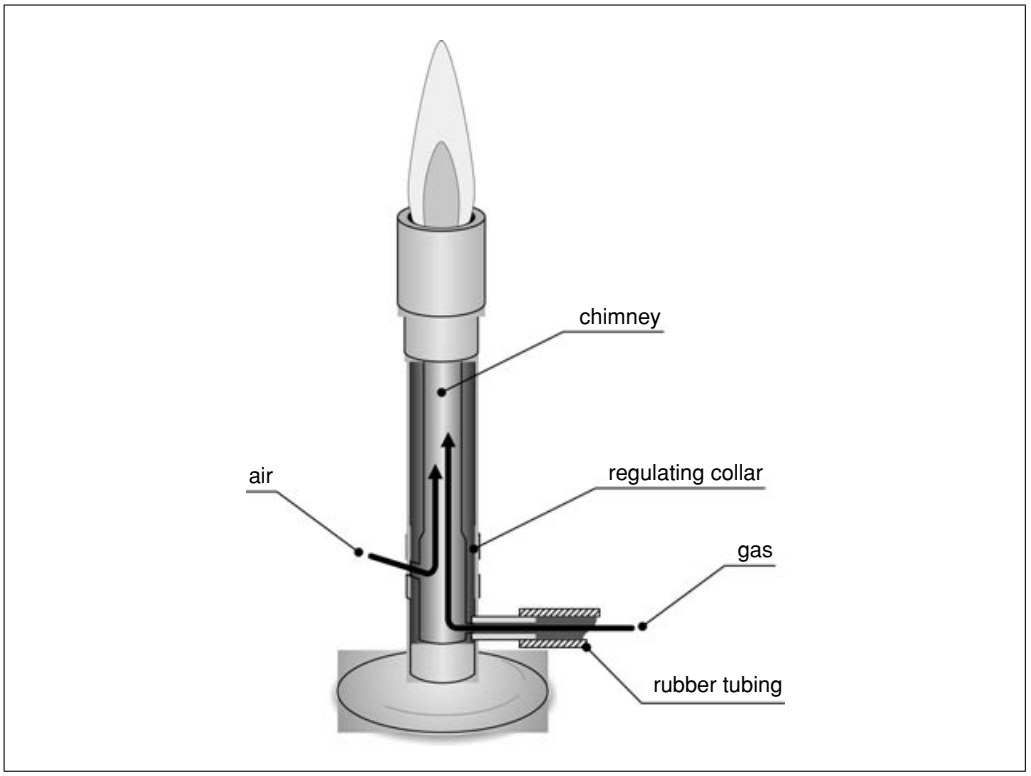

Figure A.4D.1 Bunsen burner. The gas burner consists of a vertical metal tube through which a narrow jet of natural gas is directed. Air is drawn in via air holes located near the stand. The gas/air mixture burns above the upper opening. The regulating collar can be turned to cover or partly cover the air holes, which allows regulation of the amount of air sucked in and hence the temperature and shape of the flame.

The Laminar Flow Unit

A laminar flow unit (or hood) is a sophisticated appliance that can further help prevent contamination of reagents and biological cultures. Used correctly, it provides the work space with clean, ultrafiltered air. It also keeps room air from entering the work area and both suspends and removes airborne contaminants introduced into the work area by personnel.

The most important part of a laminar flow hood is a high-efficiency bacteria-retentive filter, i.e., the HEPA (high-efficiency particulate air) filter. A certified HEPA filter must capture a minimum of 99.97% of dust, pollen, mold, bacteria, and any airborne particles with a size of >0.3 μm at 85 liters/min. The first HEPA filters were developed in the 1940s by the U.S.A. Atomic Energy Commission as part of the Manhattan Project (the development of the atomic bomb) to provide an efficient, effective way to filter radioactive particulate contaminants. HEPA filter technology was declassified after World War II, allowing extensive research and commercial use.

Laminar flow hoods are essential components of many biosafety level (BSL)-2 laboratories, where they help prevent spread of viruses and some bacteria. Consult *UNIT 1A.1* as well as the resources in *APPENDIX 1B* for additional information on biosafety and biocontainment.

STRATEGIC PLANNING

Contamination Risk Assessment and Space Organization

Localizing potential sources of biological contamination is an important part of arranging space when a room is used as a laboratory. Benches for aseptic work should be organized away from air conditioning vents, cooling fans, open windows, or blowers from heating or refrigerating systems. Containers used for the disposal of biological material may contain large numbers of viable microorganisms, even when emptied. Some contaminants, e.g., sporulating bacteria, yeasts, or fungi, are very difficult to eradicate from the working environment. Waste should be kept very well covered or, if feasible, in a different room, especially if using particularly harmful or sturdy microorganisms.

General Cleanliness

In principle, keeping high standards of cleanliness has to be a universally accepted norm in the biological laboratories. People should wear lab coats that are worn **only** in the laboratory. Researchers should wash their hands often during the course of the day, especially prior to any and all handling of biological cultures, media, or sterile supplies. Dust and stains must be removed regularly, and spills cleaned and decontaminated immediately. Bench tops and shelves should be washed immediately before all uses with 10% (v/v) household bleach (containing sodium hypochlorite). This solution will inactivate all viruses, bacteria, fungi, and other potential contaminants. Alternatively, a solution of antiseptic cleanser (e.g., Lysol), diluted according to the manufacturer's directions, is adequate. Organic disinfectants such as 70% (v/v) ethanol are generally less effective than bleach solutions because ethanol may evaporate too quickly to effectively sterilize surfaces and may not completely inactivate all potential contaminants. Outerwear may be covered with dirt or dust, and so should be kept in a cloakroom away from the work space. These simple rules of cleanliness will eliminate the bulk of potential contaminations from the work area.

Sterilizing Equipment and Reagents as a Prelude to Effective Aseptic Work

Starting aseptic work with material that is contaminated is a path to nowhere. Many reagents are supplied by their manufacturer as ready to use for cultures, while many others do not have to be sterilized at all (e.g., ethanol, phenol, or concentrated detergents). However, some require sterilization (i.e., the complete destruction or elimination of all viable organisms) before use.

People have developed a wide variety of sterilization methods based on heat and high pressure, liquid and gas chemicals, radiation, or physical removal of microorganisms that are used in the process of food storage, treatment of medical surgery equipment, or drug production. Although only a few, quite straightforward protocols are actually applied to the handling of goods in microbiology or molecular biology laboratories, they should be carried out very carefully, because there are no degrees of sterilization: an object is either sterile or not.

Filter sterilization

Reagents such as antibiotics, drugs, sugars, amino acids, vitamins, and complex media that are either flammable or would be denatured by heat are usually filter sterilized. A range of syringe-based or bottle-top filters of different pore sizes physically remove (exclude) living organisms and can be used according to the suppliers' instructions. Filtration devices may use either negative (vacuum) or positive pressure. An advantage of positive pressure in filtering is that it reduces clogging from precipitated salts.

Most living organisms are retained by a 0.45-μm filter. That size filter is often used as a prefilter because it also clears the liquid of larger particulates. However, because many bacteria can pass through 0.45-μm pores, a 0.22-μm filter should be used to ensure sterilization of the fluids. Viruses will pass through 0.22-μm filters, although they generally stick to the membrane. Some bacteria, such as saprophytic *Leptospira* spp. commonly found in domestic water supplies, may also pass through 0.22 μm filters. If such potential contaminants may cause difficulties, consider autoclaving water before using for solutions that need to be filter sterilized.

Filtering procedures are usually carried out in a laminar flow unit or on a bench equipped with Bunsen burner (see below) to avoid recontamination of just-sterilized material.

Autoclaving

CAUTION: Items containing volatile solvents or corrosive chemicals (e.g., phenol, trichloroacetic acid, ether, chloroform), or any radioactive isotopes **cannot** be autoclaved under any circumstances.

Autoclaving (using steam under pressure) is a rapid method for sterilizing almost anything except heat-labile substances. A temperature higher than the boiling point of water inside the autoclave (super-heating of liquids) is achieved because the system is under pressure. Typical autoclaving conditions of 121°C (250°F) for 15 minutes at 103 kPa (15 psi) are sufficient to kill virtually all forms of life, including bacterial endospores, and will inactivate viruses.

However, the time of the sterilization cycle should be modified according to the amount and type of loaded items. Examples of objects that have a slower surface heating or steam penetration and should be autoclaved longer are an unusually large bottle of media, a bulky bag of biohazard waste, or a beaker full of microcentrifuge tubes with aluminum foil covering.

General principles of loading an autoclave can be summarized as follows:

1. Polypropylene and polycarbonate can be autoclaved, but polyethylene and high-density polyethylene cannot. Initials imprinted on bottom of containers can help in identification of the material type (PP, polypropylene; PC, polycarbonate; PE, polyethlylene; HDPE, high density polyethylene).

2. The autoclave must not be overcrowded; the steam needs to be able to penetrate everywhere. All materials must be placed in an autoclavable tray (polypropylene plastic or stainless steel tubs) with sides at least 10 cm high to catch spills and eliminate possible damage to the machine. Dry items must be wrapped in aluminum foil to prevent them from being recontaminated after the procedure.

3. Liquids should be sterilized in a vessel of at least twice the volume of the liquid to be autoclaved, to avoid boiling over and spilling. To prevent bottle bottoms from breaking, the tub or tray holding them should be filled with 3 to 5 cm of water. Heat resistant borosilicate glass (Pyrex) vessels are preferred.

4. Containers must be loosely capped or stoppered to avoid shattering during pressurization and depressurization. For screw-cap containers, the lid should be hand tightened, then loosened one-half turn. Stoppers occasionally pop off during the autoclaving process, so loosely covering the stopper or cap with a piece of aluminum foil can help hold the cap in place.

CAUTION: Be aware that large bottles with narrow necks can simulate sealed containers if filled with too much liquid.

To optimize autoclaving of common laboratory goods, two general types of exhaust cycles has been designed:

1. The "gravity" or "fast exhaust" cycle is for all dry items, e.g., glassware, tips, flasks, tubes. The chamber is charged with steam, and a set pressure and temperature conditions are maintained for the desired period of time. At the end of the cycle, a valve opens, and the chamber rapidly returns to atmospheric pressure. Drying time may also be added to the cycle.

2. The "liquid" or "slow exhaust" cycle is used for liquids to prevent them from explosive boil-overs. Pressure is slowly released, which allows the super-heated fluids to cool gradually.

CAUTION: It is acceptable to autoclave dry items using the liquid cycle, but liquids should **never** be autoclaved using a fast exhaust cycle.

Sterilized goods can be denoted using a special indicator tape which contains a chemical that changes color when the appropriate autoclaving conditions have been met. Some types of packaging have built-in indicators on them.

Autoclaves should be tested periodically, using biological tests such as bacterial spore test kits. Users should consult with their institutions for guidelines on frequency of testing and record keeping.

Oven sterilization

Selected goods such as glassware, metal, and other objects that will not melt at temperatures between 121°C and 170°C can be sterilized with dry heat (in a hot-air oven). As the heat takes much longer to be transferred to the organism, a temperature of 160°C for ≥2 hr or 170°C for 1 hr is routinely used. The procedure certainly **cannot** be applied for liquids, rubber, or any plastic objects. However, the method has advantages; for instance, it can be used for powders and other heat-stable items that are adversely affected by steam, and it does not cause rusting of steel objects. It is also a method of choice for the treatment of glassware used for work with ribonucleic acid (RNA), since the process of baking not only kills organisms, but also inactivates any residual RNA-degrading enzymes (RNases).

General considerations

Note that a standard microwave oven (i.e., the household appliance), although capable of bringing water to boiling, **does not** provide a useful method for sterilizing laboratory goods and should only be used for warming up the liquids.

Sterilized solutions and other reagents will need to cool down before use, which takes time. Thus sterilization should be performed well before the actual experimental work begins.

It is best for each researcher in the laboratory to keep his/her own stocks of reagents (e.g., media, buffers, salts, glycerol) to prevent accidental contamination by a co-worker. In this manner, each worker is solely responsible for the condition of his/her reagents and for any impact on experimental results. This strategy can be facilitated by preparing media or solutions in several small bottles (aliquots), one for each researcher, with only one container per worker opened at a time.

Although growth media are more likely to produce noticeable biological contamination (by turning color, developing clumps, or becoming turbid), most other liquids used in the laboratories can also support the growth of organisms. Solutions should be checked before using by gently swirling and looking for the presence of cloudy material.

Table A.4D.1 Common Laboratory Items Useful for Aseptic Work

Material	Common use
Aluminum foil	Widely used in laboratories for packing goods for autoclaving. It protects them from recontamination and facilitates storage. Simple lids can be made of it for beakers, flasks or bottles. Wrapping boxes of disposable tips, centrifuge tubes, and bundles of glass pipets or spatulas with aluminum foil not only protects, but also helps to keep them organized by size or type.
Disposable rubber gloves	Should be worn at all times during aseptic work; usually made of latex rubber, PVC (polyvinyl chloride), or nitrile. Recently a range of mild to severe allergic reactions from exposure to natural rubber latex has been widely recognized. Providing the laboratory with both powder-free low-protein latex disposable gloves and nonlatex gloves is recommended. After removing latex gloves, hands should be washed with a mild soap and dried thoroughly.
Disposable wipes (lint-free)	Since they do not leave unwanted particles behind, they are considered very useful for wiping bench tops, interior of the laminar flow hood, or any other object during cleaning and disinfection.
70% (v/v) ethanol or other industrial methylated spirits (IMS; denatured alcohols)	Simple, flammable disinfectants used for cleaning items and surfaces from microorganisms and stains. They are also used for dipping culture spreaders. See Safety Considerations for usage instructions.
Eye protection and thermal gloves	Equipment for extra protection should be kept within reach and used during procedures such as opening an autoclave.
Flint-on-steel striker	Used to quickly ignite a Bunsen burner when needed.
10% (v/v) household bleach (containing sodium hypochlorite)	Chemical more effective for disinfecting surfaces than quickly evaporating ethanol. It should not be stored for longer than 1 month at room temperature, since sodium hypochlorite will degrade.
Laboratory coat	Clean, long coat of the right size used to protect the researcher against toxic substances and biohazard material. It also protects samples from contamination from human skin and apparel.
Spray or squirt plastic bottles	Very useful for dispensing ethanol, water, and other liquids for disinfection and cleaning of large surfaces and places difficult to reach with a wipe.

Useful Materials to Have on Hand

A list of simple materials that might be useful in aseptic work and should be kept on hand is presented in Table A.4D.1.

SAFETY CONSIDERATIONS

Autoclaving

Autoclaving produces steam, which can cause burns and requires a lot of caution in handling. The most important moment is opening the machine after completion of the cycle. Consider some basic rules that should be applied while opening autoclave:

Wear a lab coat, eye protection, loose fitting thermal (insulating) gloves, and closed-toe shoes.

Check that the chamber pressure is zero before attempting to open the door.

Stand behind the door when opening and use it as a shield. Slowly open a crack and

wait for steam to be released. Beware of a rush of steam. Open the door fully to remove tray.

After the slow exhaust cycle, open the autoclave door and allow liquids to cool 10 to 20 min before removing. Superheated liquids may suddenly boil over if the container is moved.

Do not move hot bottles if the liquid is bubbling or boiling.

When removing liquids from the autoclave, point the opening of the container away from yourself and other people because the liquid may suddenly boil and spray out the opening.

Working with Open Flame

Burners should be on only for as long as needed for a particular procedure. The blue flame of a hot Bunsen burner can be difficult to see. **Never** leave an open flame unattended, not even for a few seconds.

Since burns are among the most common laboratory accidents, making sure that face, clothing, and hair are not above or near the opening of the burner tube is a crucial safety measure. Temperatures in the hottest region of the burner flame approach 1500°C, and objects will heat extremely quickly. Any heated object, glass or metal, needs time to cool down before it can be safely touched.

It is also absolutely necessary to remove flammable and combustible materials from the vicinity of the flame. Nitrocellulose membranes, commonly used for blotting techniques, are extremely flammable.

Ethanol used for dipping culture spreaders is an exception to the rule regarding flammables, but only small volumes (≤20 ml) in glass beakers should be used at a time. An accidental alcohol fire in a beaker can be quickly extinguished simply by covering the top of the beaker with a piece of aluminum foil. Keeping the alcohol beaker covered with such a piece of foil when not in use will help reduce accidental fires and will slow evaporation.

A fire extinguisher should always be kept nearby.

The tubing connecting the Bunsen burner to the laboratory gas supply should be checked regularly. Latex rubber tubing tends to harden and crack after exposure to air, making it a poor tubing choice. Silicone tubing (e.g., Tygon) is generally more durable.

Never use a Bunsen burner in a Class II laminar flow hood. The heated air may disrupt air flow and possibly damage the hood.

Ultraviolet Light

Many laminar flow hoods are equipped with ultraviolet (UV) lights, which can help reduce contamination by inducing DNA damage to potential contaminants. Such light is also harmful to laboratory workers. Never look directly at a UV light, as that can cause eye damage. The UV light can also cause skin burns (sun burns) to anyone in the room. Consequently, UV lights should always be turned off whenever the room is occupied.

TECHNIQUES FOR MAINTAINING ASEPTIC CONDITIONS

Usually, there is no need to equip the laboratory with expensive paraphernalia just for culture of hardy bacteria such as *Escherichia coli*, since that bacteria's rapid growth rate generally allows it to out-compete any contaminants. A typical laboratory bench supplied with a Bunsen burner is sufficient. Working aseptically while using a cone of heat produced by the burning gas has been the practice in laboratories worldwide since the 19th century. It is straightforward and does not require considerable financial outlays.

However, a laminar flow hood is the method of choice when a more rigid aseptic technique is required. The rich media used for eukaryotic cell cultures and many microorganisms, coupled with their slow growth rates, necessitates high levels of security from contamination. For some organisms, work must always be performed inside a laminar flow unit to meet biosafety standards and to protect personnel against potentially harmful microorganisms or viruses.

Any aseptic work should be completed as quickly as is comfortable to minimize the risk of contamination. Consider that working in an aseptic manner may take longer than when being less cautious. Reserve extra time to avoid being rushed, which may result in spilling or breaking important samples, dishes, or solutions.

Bench-Top Aseptic Work Using a Bunsen Burner

Before starting work, locate the gas and air controls on the burner. They can be in different places on different models. In some cases, it is necessary to use the laboratory gas valve to control the gas flow rate. Once a Bunsen burner has been adjusted for optimal air and gas flow, those settings can be left in place, making future usage more efficient.

1. Close the burner's air control. If the burner has its own gas control valve, shut it and then open it allowing only small amount of gas to get into the burner tube.

2. To ignite the gas using a flint-on-steel striker, hold it 2 to 3 cm above and slightly to the side of the burner tube top. Squeeze and release striker repeatedly until a spark ignites the flame. If using a match, light it and slowly bring it up from the bottom along the side of the burner tube until the flame ignites. If a match is stuck in the middle of the gas stream, the high-pressure gas stream often blows out the flame before the burner can light.

3. If the initial flame is low on oxygen, it is bushy orange in appearance and is not very hot (Fig. A.4D.2A,B). Open the air control vent until the central blue cone of the flame forms and a slight buzzing sound is audible (Fig. A.4D.2C). If the flame is too small, gradually increase both the supply of gas and air flow to the burner. The hottest part of the flame is just above the central blue cone.

4. When finished using the burner, turn it off at the laboratory gas valve. Do not leave a lit burner unattended.

CAUTION: Never leave a lit burner unattended.

Handling and Pipetting Liquids

All growth media, cultures, and sterile reagents should be manipulated inside the cone of heat that is created above and around a lit Bunsen burner or within a laminar flow hood. Bottles and test tubes should be arranged near the burner but not directly within the flame. Brief (1 to 2 sec) flaming of the lips of tubes and flasks should only be done when caps are removed during transfer of liquids and cultures. This is especially important when the content is to be poured from a container (e.g., pouring petri dishes or aliquots of large volumes of liquid).

The purpose of flaming is not to actually sterilize, but to warm the opening and create

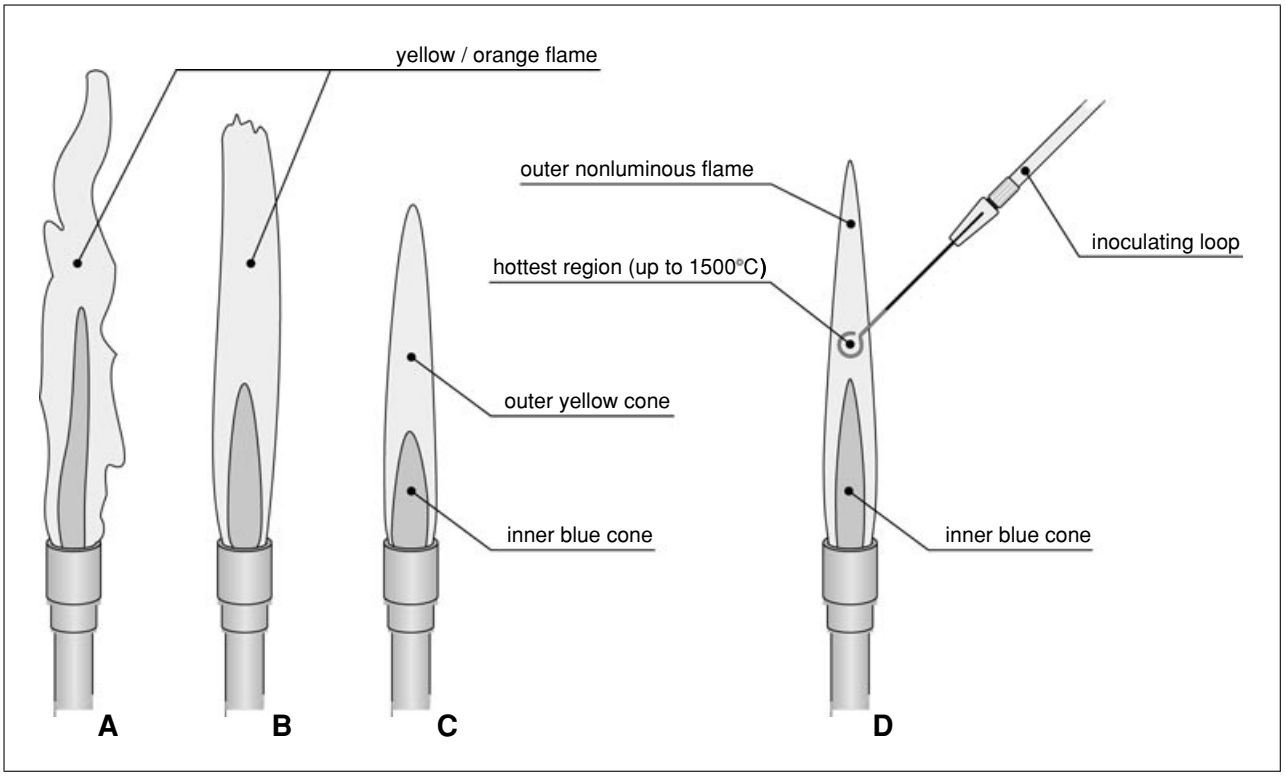

Figure A.4D.2 Adjusting the Bunsen burner and heating an inoculation loop. A lighted Bunsen burner with its air holes closed produces a cool, yellow-orange flame (**A**). It is not particularly useful for heating things. The more the air holes are open, the more blue and fierce the flame becomes (**B,C**). A properly adjusted Bunsen burner gives a blue flame containing two cones: an outer, nonluminous pale blue cone and an inner, deeper blue cone (**D**). There is no combustion inside the central cone. Temperatures in the part of the flame just above the central inner cone (where the inoculating loop is heated) approach 1500°C. See text for more details.

air convection currents up and away from the opening. This canopy of warm, rising air helps prevent the entrance of dust particles. Flaming should be performed immediately upon opening and just before closing tubes and bottles. Lips of disposable plastic containers and containers of flammable solutions should never be flamed. Autoclaved beakers containing sterile wooden toothpicks do not have to be flamed.

NOTE: The following instructions assume the experimenter is right-handed. In the case of a left-handed individual, switch the hands in the instructions from right to left, and vice versa.

Manipulating vessels containing liquids

Most manipulations of cultures or sterile reagents in tubes, bottles, or flasks should be performed as follows:

1. Loosen closures (i.e., lids, caps, etc.) of all containers prior to any manipulations. This will ensure that a procedure will not have to be stopped midway.
2. Hold the container in your left hand at a 45 degree angle, so that dust cannot fall in when it is open.
3. Hold the instrument to be used for manipulation (inoculating loop, pipet, needle, toothpick, etc.) in the right hand.
4. Grasp the container closure using the little finger of your right hand, and lift from container. Do not set the container closure down. Doing so increases the risk of it becoming contaminated from the bench top. Remember that even a cleaned bench top is not sterile.
5. While continuing to hold the closure with the little finger, lightly flame the container opening.
6. Quickly manipulate the instrument into the container, and then withdraw it.
7. Lightly flame the container opening again.
8. Replace the container closure immediately.

Transferring liquids from one test tube to another

1. Hold both tubes in the left hand.
2. Remove the closures from both tubes with little finger of right hand.
3. Flame the lips of both tubes.
4. Take culture, reagent, etc., from first tube.
5. Add culture, reagent, etc., to second tube.
6. Flame the lips of both tubes again.
7. Replace both closures.

Storing and Using Pipets

Cans of sterile glass pipets should be kept in horizontal positions, to reduce introduction of dust and other airborne contaminants. Pipets should be removed in a manner that prevents the tips from contacting any potentially contaminated surfaces. Remove pipets by inserting as little of the fingers as possible, and as the pipet is withdrawn, hold it away from any surface to prevent its contamination (including the ends of other pipets, since they have come into contact with your hands, and are therefore considered nonsterile).

Individually wrapped, presterilized plastic pipets are an alternative to glass pipets, generally inexpensive when purchased in small quantities, but expensive over long terms. Remember that the outsides of the wrappings are considered contaminated. Open from one end, and then peel the wrapping backward, allowing removal of the pipet without contact with the outer surface. Bulk-wrapped, presterilized pipets are somewhat less expensive, but it is often difficult to maintain sterility of the large quantities of pipets in each package.

Using an Inoculating Loop

Before each use, an inoculating loop must be sterilized using a burner, as follows:

1. Place the junction between the loop wire and the handle just above the inner blue cone (hottest point) until the wire turns red.
2. Slowly draw the wire through the blue flame, making sure that every part of the wire is heated to glowing red. The loop tip is heated last.
3. Cool the loop by making contact with another sterile surface, e.g., an unused section of an agar plate. **Do not** blow on the loop or wave it in the air to cool it. Such actions will contaminate the loop.
4. The loop is now ready for immediate use. **Do not** put the loop down or touch it to a nonsterile surface before using.
5. Flame the loop again immediately after use before setting it down.

As an alternative, presterilized inoculating loops may be purchased. These are particularly handy for use within a laminar flow hood, where a Bunsen burner should never be used. Plastic, disposable loops are available either individually wrapped or packaged in bulk.

Using Petri Dishes (Plates)

Before pouring medium into sterile petri dishes, first remove the plates from their container (e.g., plastic sleeve), and arrange them on the lab bench convenient to the burner. Flame the lip of the container of liquefied medium immediately before pouring the medium into the plates, and again after each

5 to 10 plates that are poured. Remove petri dish lids only when needed for pouring the medium, and close immediately after. When the lid is removed, it should be held over the plate as a shield and never placed on the bench top

For all manipulations of cultures in petri dishes, lids should be lifted for as short a time as possible. Lids are never to be placed on the bench top. Do not walk around the room with an open plate. As with all other media, do not breathe on open plates.

Working in the Laminar Flow Unit

An additional layer of security against contamination is the use of a laminar flow hood. Although several modifications of the two major flow hood design types (horizontal and vertical) are commercially available (see Fig. A.4D.3), the vertical hood design is probably the most commonly encountered nowadays. The major design concepts of both types of hoods are similar. Room air is taken into the unit and passed through a prefilter to remove gross contaminants (lint, dust, etc.). The air is subsequently compressed and channeled through the HEPA filter, which removes nearly all of the bacteria from the air. Purified air flows out over the entire work surface in parallel layers at a uniform velocity with no disruption between the layers. A cluttered hood or poor technique can easily overcome the desired airflow and reverse currents, potentially introducing contaminants into the work area.

Thoroughly read through the laminar flow unit supplier's manual for detailed information on the operation of a particular appliance, especially with regard to the position of air ducts and the track of laminar flow.

Cleaning, disinfecting, and arranging the space inside hood (before use)

Before starting work or re-entering the hood, remove jewelry from the hands and wrists, tie long hair back, button up the laboratory coat, and roll up the coat sleeves. Wash your hands and forearms and don fresh disposable gloves. Spray the exterior of gloves with disinfectant and then rub the hands together before putting them inside the hood. Keep hands within the cleaned area of the hood as much as possible and do not touch hair, face, or clothing. Hand cleanliness is reduced each time bottles and other nonsterile items are touched, so the disinfecting procedure should be repeated periodically.

To maintain efficient air flow, the Class II laminar flow hood should remain turned on

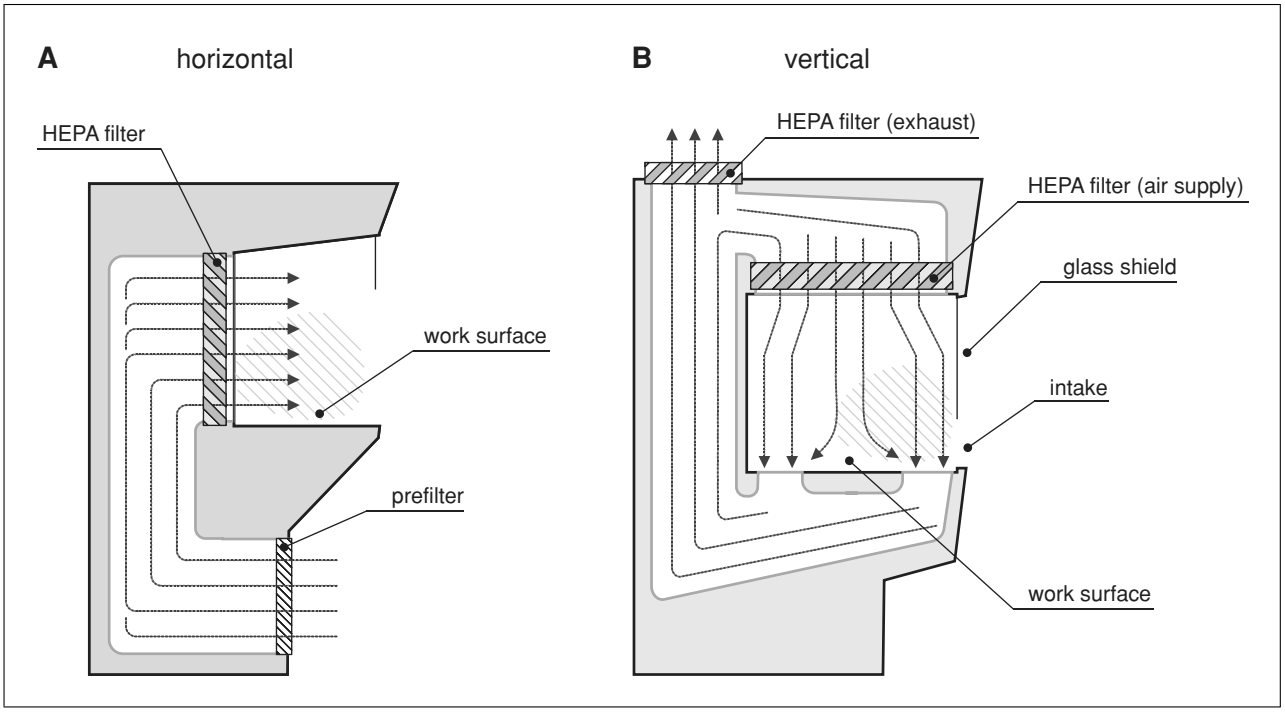

Figure A.4D.3 Laminar flow hoods. Hoods with vertical (**A**) or horizontal (**B**) flow are the two major types of laminar flow units. See text for details.

24 hr a day. If turned off for any reason, it should be turned on at least 30 min before use. The hood should be thoroughly cleaned before any use. Spray the working surface liberally with 70% (v/v) alcohol solution (denatured ethanol or other industrial methylated spirit) and swab immediately with lint-free wipes.

Any object that is introduced into the laminar hood environment, even glassware or plastic containers marked as sterile, should be disinfected by liberally spraying the outside with 70% ethanol. Always remove outer pouches and wraps from disposable equipment (e.g., pipets, loops, spreaders, cell scrapers) at the edge of the work area just before the sterile contents are pulled into the hood. To help reduce introduction of contaminants from outside the hood, always keep a set of clean pipettors inside for exclusive use.

Leave a wide, clear space in the center of the hood (not just the front edge) as a working surface. Arrange the area to have easy access to all of it without having to reach over one item to get another (especially over an open bottle or flask). Reaching over items increases chances of knocking things over, or transferring contamination through the workplace

Never place large objects near the back of the hood or clutter the area. Not only can these objects contaminate everything downstream, but they also disrupt the laminar flow pattern of air, which normally suspends the contaminants and removes them from the area.

A common mistake is to keep waste, old cultures and media, empty boxes, notebooks, manuals, protocols, pencils, and other unnecessary items inside the hood. Such objects should never be kept inside the laminar flow unit.

Working in a Class I or II laminar flow hood

Perform all work at a distance of no less than 6 in. from the front edge of the work surface. At a lesser distance, laminar flow air begins to mix with the outside air and contamination is possible.

To ensure the proper air flow, maintain a direct path between the filter and the area inside the hood where the manipulations are being performed (nothing should touch the filter). Undesired turbulence may also be produced by coughing or sneezing into the hood, quick movements, rotating, talking, etc. To help keep air disturbances to a minimum, the hood should be located out of the stream of traffic in the lab.

Remember that a laminar flow hood is **not** a completely sterile environment. All the rules of aseptic work on the bench top apply to work in a hood. Minimize the time bottles or plates are open, avoid touching extraneous surfaces with pipets, etc.

In case of any spill, clean the work surface immediately with sterile, distilled water and follow by spraying liberally with alcohol. Use a side to side motion, starting at the back of the hood and working forward.

If you fully comply with all the above instructions, chances for the introduction of sepsis into the cultures or reagents are low. Never become so engrossed in your work that you forget these basic rules.

Cleaning and disinfecting the space inside hood (after use)

When finished using the hood, remove all unnecessary items, and then clean all surfaces by liberally spraying with 70% alcohol. Most laminar flow hoods are equipped with a UV lamp, which helps kill introduced microorganisms by irradiation. Since UV light is also harmful to humans, do not switch on the light when anyone is working in the laboratory. Turn on the UV light at the end of the work day to help keep down levels of potential contaminants.

IMPORTANT NOTE: Before switching on the UV light, remove all important samples of living organisms to a sheltered location, so they are not affected by the harmful conditions.

TROUBLESHOOTING

Most lapses in aseptic technique will become apparent by contaminations of cultures or supposedly sterile media and reagents.

A simple method for checking the quality of your aseptic technique is to perform manipulations of medium without intentionally adding bacteria. Incubate the medium overnight at 37°C, and then observe for signs of contaminant growth (e.g., turbidity).

A small amount of contamination is not always evident until the medium is incubated at an appropriate temperature or atmosphere conditions.

Some bacterial cultures may grow briefly, then lyse. This may be due to bacteriophage contamination as a result of poor aseptic technique. If this is suspected, thoroughly clean all laboratory benches and equipment with disinfectants, and strengthen use of aseptic techniques.

Many contaminations occur through the poorly designed or defective air handling systems, possibly transferring microorganisms from neighboring laboratories. Your facility's physical plant management should be consulted if you suspect such possibilities, as your laboratory's air handling system may need to be re-engineered.