How to use pressure cooker to make media:

Supplies:

6 – 8 quart pressure cooker
flasks/bottles and caps
sterile plates
media (trypticase Soy Agar, Nutrient Agar, Brain-Heart Infusion Agar)
measuring cylinder or cup
hot plate

1. Add water to pressure cooker (follow manufacturer's guidelines for canning). For an 8 quart cooker, this is usually 6 cups of water.
2. Place pressure cooker on hot plate to begin heating water while media is made. Do NOT put cover on.
3. Make sure the containers for bacterial media will fit into pressure cooker, will NOT block vents and will allow for proper closure of the cooker.
4. Determine volume for media to be made. Never make more than half of the volume of the container. For example, if you are using a 250 ml flask, the maximum amount of media that can be made is 125 ml.
5. Follow media maker's direction to determine appropriate measure of powder into container. Add water and gently mix (does not need to be dissolved!!). Try to avoid getting too much powder on sides of container.
6. Make certain that containers are NOT floating in water in pressure cooker. If necessary remove some of the water so that all of the media containers are flat on the rack. It is also best if the containers have at least some space between them (it does not need to be much).
7. Close top and place regulator on vent. Wait for pressure to get up (this will vary greatly with your hot plate and pressure cooker but averages about ~ 20 – 30 minutes for a 6 – 8 quart cooker). Cooker is at 15 psi when regulator rocks back and forth in a regular manner. Begin timing at this point (~20 minutes, depending upon how full the cooker is).
8. After time, remove from heat and let pressure drop on its own (~ 20 minutes).
9. Place pot in sink and rinse top with cold water (as a safety precaution), then open. KEEP FACE AWAY FROM STEAM AS YOU OPEN TOP!!
10. Place media in water bath (at 50^0C) or on bench.
11. Pour when cool to touch, using alcohol and paper towels to catch any run off.
    - surface should be clean, level and free from drafts in an area that is going to be free from traffic until the media solidifies (~ 1 hr)
12. Once solidified, wrap plates in plastic and store upside down in refrigerator.
    - thicker plates will last longer
    - if being used immediately, plates need not be refrigerated

Taken from BSCS Lab Block - Immunology
in Equipment & Techniques

III. EQUIPMENT AND TECHNIQUES FOR CULTURE OF MICROORGANISMS

Microorganisms have become increasingly prominent subjects of biological investigations since the early work of Pasteur, Koch, and others. From the information accumulated about the physical characteristics and chemical activities of microorganisms, investigators have been able to utilize some of the unique qualities of these organisms in the study of fundamental biological problems, such as nutrition, growth, genetics, etc.

In order to study microorganisms or use them to investigate biological problems, it is necessary to employ special equipment and techniques. This chapter is devoted to the discussion of some of the special equipment and technique problems associated with the utilization of microorganisms in the high school laboratory.

It has been found that the medium used for the culture of microorganisms may be a common household item such as an apple, pear, grape, etc., or it may be a complex mixture requiring a variety of organic substances. The use of commercially prepared dehydrated media often saves much time in preparation for laboratory work. Specific information on the choice of an appropriate medium and its preparation can be found in bacteriological manuals and in the references listed below:

Levine, M. 1954. Laboratory technique in bacteriology. 3rd ed. Macmillan, New York.

Levine, M., and H. W. Schoenline. 1960. A compilation of culture media for the cultivation of microorganisms. The Williams and Wilkins Co., Baltimore, Maryland.

Morholt, Evelyn, P. Brandwein, and A. Joseph. 1958. A sourcebook for the biological sciences. Harcourt Brace, New York.

Pelczar, M. 1957. Manual of microbiological methods by the Society of American Bacteriologists. McGraw-Hill, New York.

Sussman, A. S. 1964. Microbes: their growth, nutrition, and interaction. D. C. Heath and Company, Boston, Massachusetts.

A. Sterilization

The sterilization of media, transfer equipment, and culturing apparatus is essential for most studies involving microorganisms. If specific microbes are to be studied in a state free from possible interaction with others, then all foreign organisms present, either on the equipment or in the medium constituents, must be removed or destroyed.

Sterilization with heat is the most common method of eliminating foreign organisms. However, where conditions demand, chemicals, gas, or filtration can be used to achieve the same purpose.

1. *Steam Sterilization* (autoclaving)

Media are commonly sterilized with steam at 15-lb pressure for 20 minutes in an autoclave. The steam and pressure prevent undue loss of water from the medium, and the resulting 121°C (Centigrade or more correctly Celsius) temperature destroys most types of organisms and many of the resistant spores.

An ordinary pressure cooker (Figure III–1) will serve as a substitute for an autoclave. The 21-qt size will hold up to 28 Erlenmeyer flasks (125 ml) if they are stacked 14 to a tier. If stacked, however, the lower tier should be covered with a waterproof material (such as aluminum foil) to prevent excessive wetting of the cotton plugs.

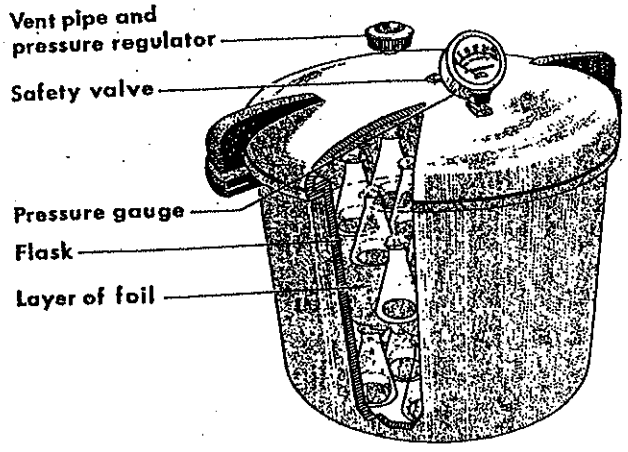


FIGURE III–1. Pressure cooker used as autoclave.

It should be understood that the time required to obtain sterilizing temperatures in an autoclave (pressure cooker) is dependent upon the rate of energy input of the heat source and the rate of heat loss from the autoclave.

If the sterilizing cycle is to be completed in a 50-minute laboratory period, it is necessary to have a heat source equivalent to a 1,200-watt hot plate. Any burner on a conventional gas or electric kitchen range will deliver this amount of energy.

If the following steps are taken, time can be saved without sacrificing efficient sterilization.

a. Have water in the pressure cooker at boiling temperature before adding materials to be autoclaved.

b. Have vent pipe (exhaust valve) open when placing the lid on the pressure cooker.

c. Keep vent pipe open until steam flows continuously from the valve opening. The desired temperature will not be obtained if air remains in the container. (Example: At 15 lb of steam pressure, the temperature will be 121°C; on the other hand, with *equal* amounts of steam and air, the temperature would be 110°C in the same container.)

Modifications by some manufacturers of pressure cookers automatically regulate the evacuation of air and steam. Directions regarding the use of a pressure cooker with an automatic air vent accompanies each unit when purchased and should be followed carefully.

d. Regulate the heat source to maintain 121°C or 15 lb of pressure.

e. After the desired temperature has been maintained for the prescribed time, remove the cooker from the heat. Do not open the exhaust valve until the pressure has dropped below 5 lb since dropping the pressure too rapidly may cause plugs in the flasks and tubes to be blown out. After a few trial runs, the exact time needed for autoclaving (including the placement of media in the cooker, the sterilization, and the removal) can be determined.

f. After each use, the pressure cooker should be cleaned and thoroughly dried before storing. This procedure will reduce the possibilities of pitting of the metal and greatly extend the service life of the cooker.

2. *Dry Sterilization*

If dry sterilization is to be successful, materials must be kept at 165°–170°C for a given amount of time. The amount of time required for sterilization will vary from one type of material to another and may have to be determined by trial and error.

Glassware and metallic equipment can be dry-sterilized satisfactorily in ovens at 165°–170°C in approximately 2 hours, with the length of time dependent upon the mass of material to be sterilized. With materials such as sand and soil, it is important that the center of the mass be at the desired temperature for the prescribed time. A meat thermometer can be used to determine the temperature in the center.

Various commercial ovens are available that will provide temperatures up to 400°C for the dry sterilization of glassware and other equipment. An apartment-size gas or electric range makes an excellent substitute for a commercial sterilization oven and also can serve as a heat source for pressure cookers (autoclaves). This type of oven or range is generally available secondhand at a low purchase cost. They can be placed against a wall or demonstration desk and connected to the 220-volt electrical outlet or to the gas supply by means of adapters.

To prevent contamination of glassware and equipment after sterilization, several layers of brown paper or foil should be wrapped around items before they are placed in the oven. After sterilization, the paper wrapping might be browned but should not be brittle. (In Figure III–2, petri dishes and pipettes are shown in an oven used for sterilization.)

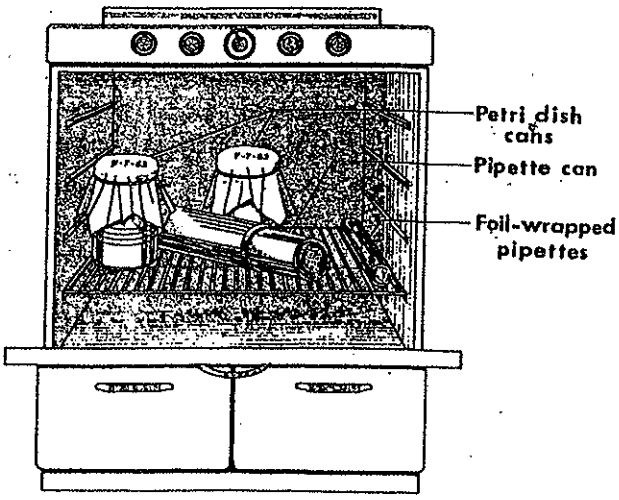


FIGURE III–2. Kitchen range used as hot air sterilizer.

IMPORTANT SAFETY INFORMATION

Cooking under pressure enables you to prepare food both quickly and deliciously. If used properly, your pressure cooker is one of the safest appliances in your kitchen. To insure safe operation, make sure you always observe the following simple rules whenever you use the pressure cooker:

1. Never overfill the pressure cooker.
2. Always look through the vent pipe before closing the cooker to make sure it is clear.
3. Always fully close the pressure cooker.
4. Never open the cooker when it contains pressure (when the air vent/cover lock is in the up position, there is pressure in the cooker).
5. Replace the overpressure plug when it becomes hard, deformed, cracked, worn or pitted, or when replacing the sealing ring. Replace the sealing ring when it becomes hard, deformed, cracked, worn, pitted or soft and sticky.

So that you understand the importance of these instructions, we would like to acquaint you with the reasons for them:

1. **Never overfill the pressure cooker.** —The pressure regulator is designed to maintain cooking pressures at a safe level. It relieves excess pressure through the vent pipe as it rocks back and forth. The overpressure plug is a secondary pressure relief valve which is designed to release excess pressure if something interferes with the pressure regulator's operation. Neither can perform their function if they are plugged or blocked. Plugging or blocking can occur if the cooker is overfilled. Many foods tend to expand when cooked. If the cooker is overfilled, expansion of food may prevent the pressure relief devices from functioning. Therefore, never fill the pressure cooker over ⅔ full.

2. **Always look through the vent pipe before using the cooker to make sure it is clear.** — If the vent pipe is plugged, the pressure regulator cannot rock back and forth and thus cannot relieve excess pressure. Pressure may then build to unsafe levels. To clean the vent pipe, see page 15.

3. **Always fully close the pressure cooker.** — The cover handle must be directly above the body handle. Your pressure cooker has specially designed lugs on the cover and body which lock the cover in place when the cooker is fully closed. However, if the cooker is not fully closed, the lugs cannot lock the cover onto the body. It's possible that pressure could build inside the cooker and cause the cover to come off and result in bodily injury or property damage. Always be sure the cover handle is directly above the body handle (see page 8). Do not turn past handle alignment.

4. **Never open the cooker when it contains pressure.** — The air vent/cover lock provides a visual indication of pressure inside the unit. When it is up, there is pressure. When it is down, there is no pressure in the cooker and it can be opened. If the pressure cooker is opened before all of the pressure is released, the contents of the cooker will erupt and could cause bodily injury or property damage.

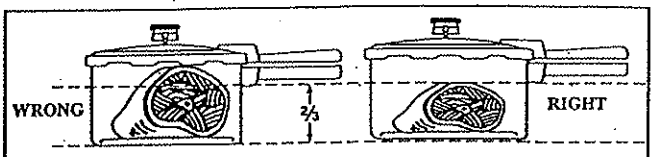
5. **Replace the overpressure plug if it becomes hard, deformed, cracked, worn or pitted, or when replacing the sealing ring. Replace the sealing ring if it becomes hard, deformed, cracked, worn, pitted or soft and sticky.** — The overpressure plug is a secondary pressure relief valve which is designed to relieve excess pressure by releasing from the cooker cover in the event that the vent pipe should become blocked. The overpressure plug is made of rubber, and when new, is soft and pliable. Over time, depending on the frequency and type of use, rubber becomes hard and inflexible. When hard and inflexible, the overpressure plug loses its ability to act as a secondary pressure relief valve. It should be

For most foods, it is safe to fill the pressure cooker up to ⅔ full. There are a few foods like rice, dry beans and peas, and soups which expand so much when cooking that the cooker should never be more than ½ full. Also, dry beans and peas must be presoaked (see page 51). And rice must be cooked in a bowl (see page 53). There are some foods that expand so much as a result of foaming, frothing, and sputtering that you should never pressure cook them.

Never pressure cook applesauce, cranberries, rhubarb, pearl barley, cereals, pastas, grains, dried soup mixes, or dry beans and peas which are not listed in the chart on page 52.

For your convenience, both the ⅔ and ½ full levels are marked by indentations on the side of the pressure cooker body. The upper marking indicates the ⅔ full level and the lower the ½ full level. In addition, in each section of the recipes you will find instructions on the maximum fill level for each type of food.

When cooking any food, do not let any portion extend above the maximum fill mark (see Fig. K).



Follow specific recipe for the amount of liquid that should be added to the pressure cooker. The liquid will not necessarily come up to the fill line.

Reminder: When cooking rice, dry beans and peas, and soups, the cooker should never be more than ½ full.

Fig. K

replaced immediately. Replace the sealing ring and overpressure plug at least every two years.

Should the overpressure plug ever be forced out of the cover due to excess pressure while cooking, it is important to call the Home Economics Department at 1-800-368-2194. **Do not attempt to replace the overpressure plug.**

Normally, the sealing ring does not become soft or sticky. However, if the sealing ring frequently comes in contact with oil, or foods which contain high oil or fat content, it may become soft and sticky. Do not apply cooking oil to the sealing ring. Hard, or soft and sticky sealing rings should be replaced immediately. Failure to replace could result in bodily injury or property damage.