# 2. Laboratory equipment

The choice of laboratory equipment must take into account national regulations and the technical premises that determine the requirements for its appropriate use. The more complex an instrument is, the more the user will depend on the support of a supplier for maintenance; it is therefore pertinent to foresee the magnitude of the costs that may be involved in its use. Quite often, the costs for use and maintenance of an instrument will exceed the costs of purchase.

It is also important to foresee the problems that may arise in case of failure of the instrument. Sometimes it may be advisable to purchase an instrument from a company that offers a guaranteed service, instead of another, perhaps cheaper, instrument for which a local maintenance service is not available.

The operating manual describing the function, installation, and use of an instrument should be provided free by the supplier and read by the user prior to any purchase. This avoids any subsequent misunderstanding. For example, it should be assured that an adequate power supply, as recommended for the instrument, is available locally, otherwise the purchase may be useless, or the cost of adaptation will increase the effective purchase price considerably.

# Autoclaves, ovens, incubators, and water-baths

Autoclaves, hot-air ovens, incubators, and water-baths are devices for heating air or water. The heat is usually generated by an electrical module, but in some instruments heat is generated by fire, or there may be a heat-storage block, such as a separate water reservoir in solar systems or a metal block controlled by a thermostat.

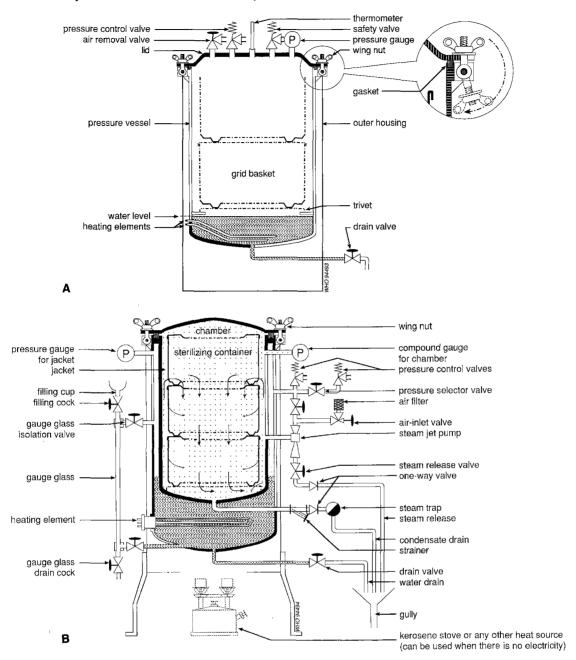
### **Autoclaves**

Autoclaves generate steam from water at a temperature above 100  $^{\circ}$ C in a closed chamber (Fig. 2.1). At these temperatures, the steam is above atmospheric pressure, and the conditions are optimal for the sterilization of laboratory equipment, medical devices, and media used for microbial culture. Bacteria cannot survive in such an environment, but viruses are not necessarily killed. The temperature can be kept 30–40  $^{\circ}$ C lower than in dry-air ovens, so that temperature-sensitive materials can also be sterilized. However, autoclaves need careful handling and must be inspected regularly; they can be dangerous and cause serious injury if steam accidently escapes from the equipment.

Two types of autoclave are available. The non-jacketed autoclave, which exists in vertical and horizontal versions, is simpler and has some practical disadvantages, but it is cheaper than a steam-jacketed autoclave with automatic air and condenser discharge.

Sterilization of porous materials, like laundry and bandages, is more difficult, since the air in these materials must be replaced by steam. This replacement is improved by evacuating the closed chamber of the autoclave containing the materials to be sterilized. With modern autoclaves, the chamber can be repeatedly evacuated so that the pressure in the chamber falls to 5.5 kPa. The chamber is then heated to evaporate water for sterilization.

Fig. 2.1. A: Non-jacketed autoclave. B: Steam-jacketed autoclave.



The main factors influencing steam sterilization are:

- saturated steam,
- temperature, and
- time.

The materials can be exposed to steam in a single heat cycle. However, this method of sterilization is less effective than intermittent exposure in three cycles over three days, which may kill all vegetative forms of sporulating microbes.<sup>1</sup> The cycle conditions may be shortened by increasing the pressure, and hence the temperature of the steam (Fig. 2.2). As with hot-air sterilization, effective steam

 $<sup>^{1}</sup>$  In hospital microbiology, spores are defined as cells of microorganisms that withstand heating to 75  $^{\circ}$ C for 20 mins.

Fig. 2.2. Relation between steam pressure and temperature at constant volume.

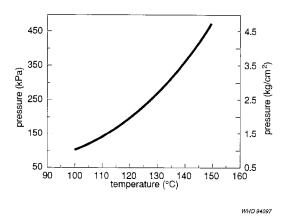
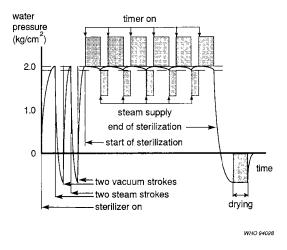


Fig. 2.3. Temperature variation during autoclave sterilization.



sterilization starts when the autoclave has reached the appropriate temperature (Fig. 2.3).

Suitable temperature and pressure regimes for operating autoclaves are shown in Table 2.1.

Table 2.1. Operating regimes for autoclaves

Sterilizing temperature (°C)	Appropriate pressure (kPa)	Minimum holding time (min)	Overall time (min)
115	75	30	50
122	115	15	40
128	150	10	30
136	225	3	20

### Use of autoclaves

- 1. Prepare the material for autoclaving with indicator paper.
- 2. Fill the bottom of the autoclave with demineralized water, up to the support.
- 3. Place the material to be sterilized in the autoclave, close the lid and make sure that the rubber washer is in its groove. Screw down the clamps firmly.
- 4. Open the air outlet valve.
- 5. Turn on the heating (electric element, gas, burner, kerosene stove). Do not leave the autoclave unattended.
- 6. Close the outlet valve when a constant jet of steam issues from it. Reduce the heat supply so that it does not heat too guickly.
- 7. Once the expected temperature is reached, reduce the heating to maintain the temperature.
- 8. Do not touch the drainage tap or the outlet or safety valve while heating under pressure.
- 9. When the required time is up, turn off the heating completely.
- 10. When the temperature falls below 100°C, open the outlet valve slowly. Do not leave the outlet valve unopened for too long.
- 11. Never unscrew the lid clamps and open the lid until the hissing sound has stopped.
- 12. Leave the sterilized material to cool before removing it from the autoclave.
- 13. Check whether the autoclave tape (used for packaging of the material to be sterilized) has turned black and the covering paper has turned brown (not yellow or black).

# Periodic inspection and cleaning

- Door gaskets should be kept clean and regularly checked for cracks and pitting due to deterioration.
- Door clamps and door locks should be checked for proper operation, and lubricated with high-temperature grease. The proper operation of the pressure locking device should be determined.
- Valve discs and seats must be inspected for signs of wear or cutting.
- Pressure gauges and thermometers should be checked periodically against a known standard.
- Adequate functioning of autoclaves should be checked weekly by the use of a biological (spore suspension) or chemical indicator. For biological testing, WHO recommends the use of *Bacillus stearothermophilus* ATCC 1953. For thermal control, commercially available chemical test strips that check the time and temperature exposure can be used.
- The function of manometers must be checked every 3 months.

### Hot-air ovens

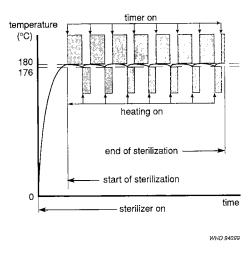
Hot-air ovens are used mainly for drying laboratory equipment and surgical devices in dry air. There are two types of hot-air oven, with and without internal circulation of dry air. Only small hot-air ovens can work without internal air circulation. Sterilization in dry air is less effective than steam sterilization, despite the higher temperatures applied (Table 2.2).

Although bacteria are killed at the higher temperatures, some spores can survive; also bacterial endotoxins are only partially inactivated. The temperature must be monitored in at least two areas of the sterilization chamber—preferably areas where the conditions for sterilization are most difficult to achieve. It is important to remember that the timing of sterilization should begin when the air in the oven has reached the required temperature (Fig. 2.4).

Table 2.2. Dry air sterilization in a hot-air oven

Temperature (°C)	Time (min)
160	180
170	120
180	30

Fig. 2.4. Temperature variation during hot air sterilization.



# Use of hot-air ovens

- 1. Set the thermostat to the required temperature.
- 2. If there is a fan, check that it is working.
- 3. Allow to continue heating for the appropriate time after the temperature reaches the pre-set value.
- 4. Switch off the heating when time is up.
- 5. Wait until the temperature falls to 40 °C before opening the door.

# Incubators

Incubators are used mainly for bacterial culture, but have additional uses within the laboratory. The incubator must maintain a constant temperature ( $35\pm2\,^{\circ}\mathrm{C}$  for bacterial culture). Temperature in incubators should be recorded daily. Like all laboratory instruments, incubators must be cleaned regularly (at least every 14 days) and immediately after any infective material is spilt. Make sure that the actual temperature corresponds with the thermostat control when the instrument is used. In carbon dioxide incubators used for microbial culture, the concentration of carbon dioxide should be maintained at  $5-10\,\%$  and the humidity at  $50-100\,\%$ .

### Water-baths

Water-baths are used for investigations at 25 °C, 30 °C, 37 °C, 42 °C, or 56 °C. It is important that the water-bath maintains a constant temperature within a narrow range (  $\pm$  0.1 °C) during the investigation. Incorrect adjustment of the temperature and insufficient temperature stability will seriously affect the results of measurements.

### Use of water-baths

- 1. The level of water in the water-bath must be above the level of the solution to be incubated.
- 2. Open containers, vials, or tubes must be incubated in a water-bath with the lid of the water-bath open to avoid contamination and dilution of the incubated material by condensed water.
- 3. The water in water-baths must be changed regularly to avoid the growth of algae and bacteria.

### Periodic inspection and cleaning

- Circulators should be regularly disassembled and cleaned to remove scale and algae.
- Thermometers must be checked when they are received from the suppliers, and thereafter every 3 months, against a known standard (i.e., ice/water mixture or boiling water).
- The functioning of manometers must be checked every 3 months.

### Balances

Balances are used to measure the weight or mass of a substance. If a comparison is made between two objects, one known and one unknown, then the measurement is mass. If the measurement is made against gravitational pull, then the measurement is weight.

There are two main categories of balance:

- mechanical balances,
- electromagnetic balances.

Balances that are based on other principles of measurement (e.g., piezoelectric balances, magneto-elastic balances, gyrodynamic balances, string balances) are less frequently used and will not be discussed here.

A number of factors influence the weighing processes. They become more important the smaller the mass of the substance to be measured. Therefore, the weighing of small quantities is more prone to errors than the weighing of large quantities.

The following influences can cause errors in measurement:

- temperature,
- moisture (atmospheric humidity),
- electrostatic effects.
- magnetism,
- gravitational forces,
- air.
- vibration.

Many analytical balances, and particularly those measuring in the microgram range, are constructed to minimize the effects of as many of these factors as possible.

The collective term "analytical balance" describes a balance suitable for chemical analysis. The weighing range of certified analytical balances is between 10  $\mu g$  and 50 kg. They may be mechanical or electronic. Optical balances are mechanical balances equipped with an optical read-out.