STERILIZATION AND ASEPTIC TECHNIQUES

Sterilization Techniques

AUTOCLAVING: This is a very reliable method for sterilizing most materials. However, it is not suitable for materials that are damaged by high temperatures. Some autoclavable substances begin to breakdown with sustained sterilizing temperatures. Extended sterilizing of an agar medium can result in pH changes of up to 0.5 unit, carbohydrates can be partially hydrolysed, proteins can be denatured and inhibitory compounds formed by the combination of amino acid and glucose units. Therefore the duration and temperature should be suited for the application.

The autoclave sterilizes the contents by raising the temperature to a point where contaminating microbes and spores are killed. Increasing the atmospheric pressure inside the autoclave allows the temperature to be raised above the normal boiling point of water without boiling occurring. Steam can be used to quickly raise the internal temperature of the autoclave, heat conduction is rapid and has great penetrating power. The temperature and duration required to sterilize a flask of media with steam is shorter than that required by a 'dry' heat sterilizing treatment.

The duration of the heat treatment is important because it is essential that ALL contaminants are killed; partial sterilization may leave viable microorganisms on lab material or in media. REMEMBER: Check to see that the temperature and duration are set correctly. Frequently a common setting is used if the volumes being autoclaved don't vary greatly. However, larger volumes require longer autoclaving times to allow heat to penetrate to the core of the liquid (Table 1).

TABLE 1. Minimum exposure time at 121^{o}C for a full load of the following volumes in appropriate sized flasks.

Volume (ml)	Time (min)
75	25
250	30
500	40
1000	45
1500	50
2000	55

Less than full loads will requires slightly less time. Longer times are required for heat to penetrate to the core of larger volumes.

Bottles should be LOOSELY capped to allow for the equalization of air pressure during sterilization, otherwise internal pressure may cause bottles to break. Media flasks should be plugged with a foam stopper to allow equalization of air pressure and the foam plug and flask neck wrapped with tinfoil.
Wrap lab utensils and equipment in tinfoil so that when they are removed from the autoclave they will remain sterile.

IMPORTANT: When opening the door of the autoclave after a sterilization run, allow the temperatures between outside and inside the chamber to equalize for a short period of time by unsealing the door slightly. DO NOT SWING THE DOOR WIDE OPEN, the sudden escape of hot air from the chamber will drop the internal pressure and the hot liquids will boil over in the reduced atmospheric pressure (you can break glassware, lose media and the cleanup is difficult).

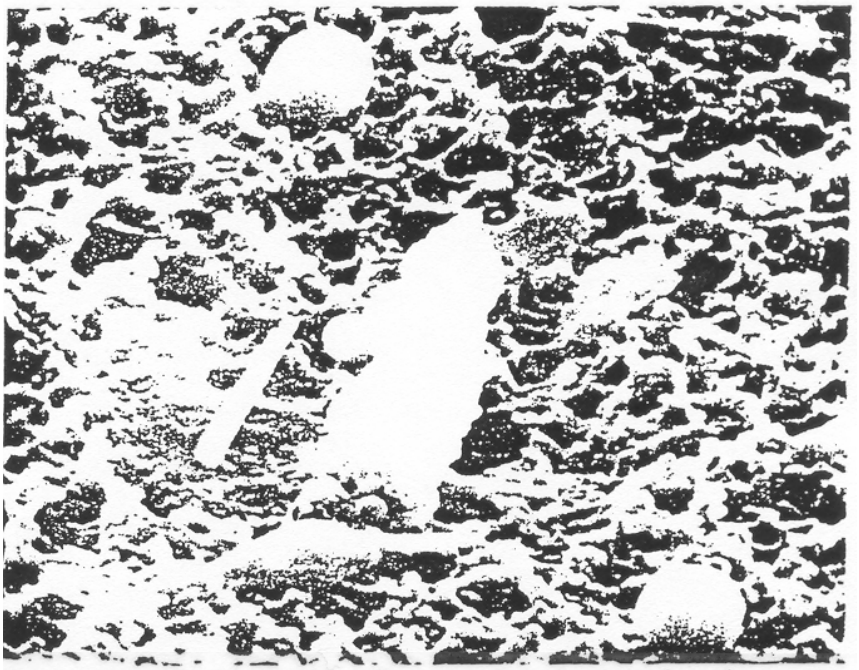
MILLIPORE FILTRATION: Heat labile substances such as amino acids, vitamins, hormones and antibiotics will be destroyed in a normal autoclaving cycle. These products can be sterilized at room temperature by using a membrane filter.

The surface of the filter has very fine pores (see figure) that can prevent bacteria from passing through. A 0.22-0.25 μm pore size will exclude all bacteria, yeasts and fungal spores from the filtrate. (see relative sizes of common contaminants on next page)

The filtrate can be added to autoclaved material if desired once the autoclaved material has cooled.

The Millipore filters can be bought in different pore sizes and filter diameters according to need and can be bought pre-sterilized or unsterilized, in which case they must be sterilized by autoclaving prior to use.

There are re-usable filter chambers available that can have the filters replaced and there are disposable filter chambers that can be used for small volumes such as syringes.



Photomicrograph showing particles and bacteria collected on the surface of a microporous membrane filter.

REMEMBER: The filtrate must be collected in a container that is sterile.

ULTRAVIOLET STERILIZATION: UV sterilization is used for materials that otherwise cannot be treated (light plastics, paper products etc). Ultraviolet sterilization is a surface effect that requires direct illumination. Thus an object to be sterilized should not be in the shadow of another object. The materials should also be as clean as possible and dust free before treatment

because some bacteria can survive in the 'shadow' of dust particles.

The high energy radiation from the UV lamp (from 220-300 nm) is absorbed by many biomolecules including DNA, RNA and proteins causing damage to these molecules which in turn result in death to the micro-organisms. Exposure times vary for different micro-organisms ranging from 9 seconds to 4 hours (Table 2).

CAUTION: High intensity UV radiation can generate ozone gas which can be uncomfortable in a poorly ventilated room. Long term exposure to ozone gas may be harmful to your health. Damage to exposed skin can occur in as little as 90 seconds in the presence of a UV light. **NEVER** look at a ultraviolet light source with the naked eye. **Always wear protective glasses or a face shield** when working with UV light.

DISINFECTANTS: Non-porous work surfaces can be disinfected with a number of products. Of the more popular ones, 70% ethyl alcohol is effective for both table tops and on hands/forearms. Wiping non-porous surfaces down with alcohol is effective and very common when you want to work in a contaminant free space such as a flow hood.

CAUTION: Do not spray alcohol onto work benches in the presence of open flames. Avoid inhaling the vapours. Ethanol fumes may cause headaches.

Other products such as SAVLON, which is sold as a germicidal soap for skin care, is also effective. The work surfaces, hands, and forearms can be washed with savlon. Use distilled water and paper towels to clean the area you wish to work on. Savlon does not dry out the skin as much as ethanol.

Relative Sizes of Small Particles

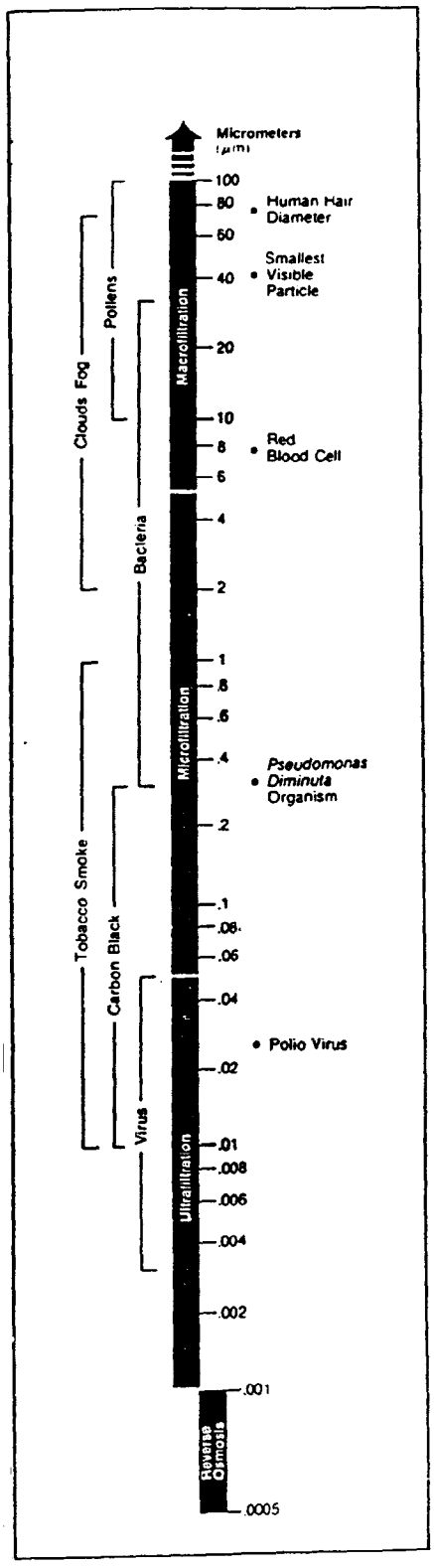


Table 2. ULTRAVIOLET RADIATION LETHAL DOSES

MICRO-ORGANISM	Lethal Dose For 180μW/cm^2 Radiation Intensity at Work Surface		
	90% Kill	99% Kill	99.99% Kill
Clostridium tetani	27.4 sec	54.8 sec	1.82 min
Bacillus anthracis (Spores)	25.1 sec	50.2 sec	1.67 min
Corynebacterium diphteriae	18.7 sec	37.4 sec	1.2 min
Staphylococcus aureus (Haemolytic)	14.4 sec	28.8 sec	57.7 sec
Escherichia coli	13.6 sec	27.2 sec	54.4 sec
Serratia marcescens	12.2 sec	24.4 sec.	48.9 sec
Streptococcus pyogenes	12.0 sec	24.0 sec	48.0 sec
Eberthella typhosa	11.9 sec	23.8 sec	47.7 sec
Streptococcus salivarius	11.1 sec	22.2 sec	44.4 sec
Streptococcus albus	10.2 sec	20.4 sec	40.9 sec
Spigellla paradysenteriae	9.3 sec	18.6 sec	37.3 sec
Yeast (Average)	22.2 sec	44.4 sec	1.48 min
Brewer's Yeast	55.6 sec	1.6 min	3.7 min
Fungi (Moulds)	2.8-28 min	6-56 min	12-114 min
Protozoa	5.6-9.3 min	11.2-18.6 min	22.4-37.2 min
Algae, Blue-Green	28-56 min	0.9-1.9 hrs	1.9-3.7 hrs

Aseptic Techniques

Laminar flow hoods are designed so that a positive flow of filtered (sterile) air passes over the material in the hood. The filters (pre- and HEPA filter) are designed to eliminate particles of 0.3 microns or smaller (which eliminates most bacteria and particulate matter).

REMEMBER: Hands and forearms should be disinfected with 70% ethanol or Savlon before working in the flow hood.

The working areas should be kept clean and free of particles.

The flow hood should be allowed to run for a few minutes prior to working with open sterile media etc. so that the filters are passing sterile air across the bench.

The working area should be kept clear of unused items, do not place unsterilized objects 'upwind' of open sterile media or critical items because contaminants may be blown onto the media.

Be aware that exposed skin is a source of contamination, skin cells are constantly being shed. Try to avoid reaching over critical areas or exposed media to reach for objects. Do not allow lab coat sleeves to drag across the bench.

Always place work away from you when talking (speak softly so that you do not spread bacteria into the flow hood area). When sneezing or coughing turn away or remove yourself from the flow hood area.

Remember to work as far INTO the flow hood as possible where the air stream is the strongest. The positive pressure at the outside edge of the hood may not be enough to prevent contamination from air borne particles.

Utensils such as scalpels, forceps etc, should be stored in 90% ethanol and flamed prior to use. **Be careful not to burn yourself - alcohol flames are invisible and very hot!**

Do not flame a utensil and then place it back into the alcohol beaker if it is still flaming! This can happen if you are not paying attention. If glassware containing alcohol should ignite, don't try to pick up the beaker or the utensils, smother the flame with a large beaker placed over top of the fire.