Sterilization Guidelines

Introduction

Sterilization techniques are critical when working with any microorganisms in the laboratory. Proper sterilization of all materials before and after the experimentation is key to a safe and successful experiment.

Concepts

- Sterilization
- Autoclaving
- Dry heat

Background

Sterilization is defined as the death of all living things, including spores, in or on an object. Achieving this task is much more difficult than is commonly realized. It is difficult to guarantee total sterility, and even the most severe methods often fail. However, for practical purposes sterilization can generally be achieved using dry heat, steam, incineration, gamma radiation, or by using some of the more severe chemicals. The most commonly used chemicals are the gases of formaldehyde or ethylene oxide and the liquids formalin, glutaraldehyde, hypochlorite and chlorite solutions. In all cases, the key to success is good penetration by the sterilizing agent. This is particularly important for large objects where the center may be unpenetrated even after a long period of time.

In the presence of water, most microbes are destroyed by a temperature of 80 °C maintained for 10 minutes. There are exceptions, however, and thus the need for more severe measures. Fungal spores and various viruses are particularly resistant and in boiling water at 100 °C an exposure of 20 hours may be necessary to guarantee the inactivation of the most resistant spores!

In the school laboratory setting three methods are generally used to sterilize materials—dry heat, filtration, and autoclaving. Generally, the methods of incineration, gamma radiation or severe chemicals are not employed. These methods are generally used commercially to produce pre-packaged sterile materials.

Safety Precautions

Autoclaves present hazards of high temperature and high pressure, and require full compliance with the manufacturer's safety instructions. A suitable pressure/temperature/time cycle for one autoclave may not be appropriate for another. Be sure to read and educate yourself about the microorganisms to be utilized in your experiments. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Procedures

Dry Heat

Dry heat requires the use of a temperature-regulated oven such as a normal kitchen stove. It is limited, however, to materials that will not degrade at the required high temperatures. Plastic and wood items generally are not sterilized using dry heat. Items sterilized using dry heat are placed in a pre-heated oven at 160 °C (320 °F) and baked for at least 2 hours. For very small items, such as slides or filter papers, an infrared lamp may be used. Ten minutes exposure is usually sufficient.

Boiling Water

Where facilities are limited, boiling water can be used for simple experimentatal procedures. A minimum boiling time of one hour is recommended, unless spores or resistant pathogens are likely to be a problem. In these cases, several hours (even up to 20 hours) may be required. If these problems are known, autoclave sterilization is required.

As an alternative to prolonged boiling, the technique of tyndallization can be used. Materials are boiled in a covered container for 30 minutes and then allowed to cool and stand for 24 hours. The container is again brought to a boil for 30 minutes, allowed to cool and stand overnight and then boiled again. It is important that the container is kept covered during the complete process.

Steam Autoclaving

The active agent in autoclave treatment is hot, wet steam. Autoclaves operate at pressures usually above atmospheric pres-

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sure, which increases the boiling point of water and hence the temperature. If an autoclave (or pressure cooker) runs dry, the items should not be considered sterile. If cooked dry, the items have been treated the same as dry heat. Be sure to check the specifications of each individual autoclave to know when the items are actually up to proper temperature and pressure. Most autoclaves and pressure cookers, which operate at 15 lb/in^2 (15 psi) pressure and a temperature of 121 °C require 20 minutes for complete sterilization. Length of sterilization often varies with the volume of the material being sterilized.

Filtration

Microbiological membrane filters provide a useful way of sterilizing materials such as vaccines, antibiotic solutions, animal sera, enzyme solutions, vitamin solutions, and other solutions that may be damaged or denatured by high temperature or chemical agents. Pores small enough to prevent passage of microbes but large enough to allow organism-free fluid to flow through exist in the filters. The sterile liquid is collected in a sterile flask or other sterile container.

Sterilization Guidelines for Some Common Items

Item	General Method
Agar	Autoclave*
Apparatus (glass)	Autoclave
Disposable tips—Micropipet	Autoclave
Filters—Millipore	Autoclave
Glassware (Petri dishes, slides, coverslips)	Dry Heat**
Glass bottles with caps	Autoclave
Heat-Sensitive Compounds (Amino acids, antibiotics, vitamins)	Filtration***
Instruments (Scalpels, forceps)	Dry Heat
Magnetic stir bars	Autoclave
Non–heat sensitive compounds (Bacto-peptone, EDTA, nutrient broth)	Autoclave
Pipets (glass)	Dry heat
Silicone tubing	Autoclave
Stoppers—Rubber, silicone	Autoclave
Test tubes	Dry heat
Toothpicks	Dry heat
Water	Autoclave (preferred)

* Autoclave — (15 lbs/in^2) 121 °C for 20 minutes or as per autoclave instructions.
** Dry Heat — 160 °C (320 °F) for 2 hours
*** Filtration — 0.45 µm pore size

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Microbiological materials require careful disposal. Sterilization of used materials is paramount prior to disposal.