**FACT SHEET: Using Autoclaves Safely**

The primary purpose of the autoclave is to sterilize the items placed within the autoclave and keep these items free of microorganisms that could serve as “contaminants”. The autoclave is best used at both “ends” of the research project. On the “front end” the autoclave sterilizes glassware and lab instruments to ensure that the experiment is initiated free of biological contaminants. On the “back end” of the experiment, the autoclave is utilized to sterilize the waste products from the experiment and inactivate any microorganisms that may be contained within the waste disposal containers. The pressurized steam within the autoclave is sufficient for most contaminants, but this also presents several safety hazards to the person using the autoclave. The temperatures inside of the autoclave chamber are extreme, as a result, an autoclave can easily malfunction if it is not used and cared for properly.

Before using any autoclave for the first time, read and thoroughly understand the owner’s manual. The different makes and models of autoclaves ensure that each will have unique characteristics with respect to operation and maintenance.

**Modes of Operation**

The autoclave creates different patterns of high heat, vacuum and pressure to sterilize its load. The type of materials you sterilize will determine the type of sterilization “runs” you use. The general types of “runs” are “**Liquids**” for any type of water based solutions, “**dry goods with vacuum**” and **dry goods without vacuum**”. Autoclaves often have an additional “**drying cycle**” in which hot air is blown through the chamber to dry items before they are removed. These controls settings may be different for different brands of autoclave, so always refer to the instruction manual for guidance on loading, load sizes, and cycle types.

The “***liquids****”* run is longer than the other two modes, but it operates at lower temperatures so as to minimize the evaporation of the liquids being sterilized. Make sure seals on containers are loose so vapor expansion during the heating process will not explode the container. Never autoclave any flammable or volatile liquids!

The “***dry goods with vacuum***” run moves steam and heat into the deepest parts of large bags or bundles of material and produces the best conditions for killing persistent organisms. During this type of run, the chamber alternates between cycles of vacuum and high pressure. Then the chamber is pressurized with steam for a long period, followed by a short vacuum cycle. *It is important that steam and pressure be able to reach the entire load, so carefully loosen autoclave bag closures once they are in the autoclave.*

The “***dry goods without vacuum***” run simply pressurizes the chamber with steam for the duration of the cycle, and then returns to normal. This process is used primarily for items that have been cleaned, but need to be sterilized. Materials should be packed so that the heat and pressure can readily reach the whole load.

**Ensuring Complete Sterilization**

It is imperative to know that the autoclave has thoroughly sterilized its contents. Most autoclave bags or tapes are imprinted with a dye that changes color when the correct temperature has been reached. The problem with this check is that the dye is on the surface of the load and a positive reading does not ensure that the innermost parts of a large load are also sterile. However, an easy way to check this is to wrap something with autoclave tape and attach string to it as it is being inserted deep into the interior of the load to be autoclaved. Tape the other end of the string to the outside of the bag so that you can easily pull the indicator out. Recover the indicator after the run and confirm that it too has changed color.

**Autoclave Safety**

Autoclaves generate extreme heat and high pressure. All users should understand and respect the hazards these conditions create. Autoclave doors and their gaskets must be firmly locked into place before running the autoclave. To prevent a sudden release of high pressure steam most, *but not all*, autoclaves have safety interlocks that will prevent the unit from running if the doors are not securely closed. You should check your autoclave for interlocks and use extra caution if there are no interlocks present.

Be aware of the potential to sustain severe burns if you touch any of the hot elements of the autoclave. Interior components may be especially hot, so extra caution should be exercised around any autoclave with its interior components exposed or unshielded. Do not stack or store combustible material next to an autoclave (cardboard, plastic, volatile or flammable liquids). Always use heat resistant gloves when removing materials after sterilization and avoid touching the inner chamber surfaces.

Any burn to the face or covering extensive areas of the body should be treated as an emergency. Very minor burns may be treated yourself using standard first aid. Regardless of the degree of severity, report ALL burns to your supervisor or Principal Investigator as an injury.

**Acceptable Materials**

Obviously, the extreme conditions created by the use of high heat and pressure inside of the autoclave will require careful consideration of the type of material that goes into the autoclave. Information below should be considered as general information on this subject. For additional information with respect to suitability for autoclaving, consult with your laboratory supervisor, Principal Investigator, or refer to manufacturer’s technical services department.

***Glass***: In general, the only glass you should place inside the autoclave is Pyrex or Pyrex type material. Most vendor guides will indicate if a particular brand of glassware may be autoclaved. However, even Pyrex type glassware will explode if it contains a sufficient volume of liquid and the container is securely closed. *Never autoclave liquids in a closed container.*

***Polypropylene*:** Polypropylene is an inexpensive resin, which can withstand autoclaving temperatures. Polypropylene containers are available in a variety of shapes and forms including bags, pans and trays. The bags must be open to allow steam to penetrate. It is advisable to pour a small amount of water into the bag prior to autoclaving to facilitate heat transfer to items being sterilized.

***Latex or Vinyl:***These items will generally melt inside of an autoclave. Combustion is possible. However as waste items, placed inside of an autoclavable biohazards bag and with a steam setting, these items will melt slightly, but will not combust resulting in fire or smoke. Since biohazard bags inside of an autoclave often contain latex or vinyl safety gloves as well as paper and plastic culture plates, some melting should be observed with respect to these items. As paper is also frequently present always use a “wet” (steam producing) setting for autoclaving all bags of waste. *Failure to do so has resulted in fires*!

***Paper*:** Paper should not be placed inside of an autoclave except as a waste item inside of a red or clear biohazardous autoclave waste bag. Never sterilize waste containing paper on a “dry” setting. Always use “wet” (steam producing) settings to prevent a fire.

***Plastic pipette tips and culture plates****:* Some of these tips are plastic, some are high density polyethylene. The degree to which melting will occur will depend upon the setting of the autoclave, density of the material, and the type of plastic. In general, pipette tips are either autoclaved as waste inside of an approved biohazards bag or sterilized when clean before used in sterile experimental techniques.

***Metals****:* Most metals used in laboratories are designed for extreme conditions and are intended to be sterilized. With few exceptions metal objects may be autoclaved. Even small cages used in housing animals may be autoclaved, just be sure to remove any plastics, liners or other items that may melt or combust.

***Plant Material***: Plant material, especially transgenic plant material must be inside of a red or clear biohazard bag, with a cup (or more) of water added and with the bag unsealed.

**Unacceptable Materials**

It should go without saying, but every year campuses have fires or situations of excessive smoke production in an autoclave because someone forgot to be mindful of what was being placed inside the autoclave. There are a few items that under no circumstance should EVER be placed inside of an autoclave. These items include:

\* Radioactive material or any material that *may* be contaminated by radiation.

\* Combustible, flammable or volatile liquids.

\* Some types of plastics like polystyrene and polyethylene

\* Any liquid in a sealed container.

\* Any material contained in such a manner that it touches the interior surfaces of the autoclave.

**Correct Method of Packaging**

It is important to remember that the density of the load affects steam penetration. Therefore, if the autoclave chamber is not overloaded then the load should be properly sterilized.

**Testing the autoclave using biological indicator**

Testing the efficiency of an autoclave using a biological indicator is done by setting the proper time, pressure and temperature needed to render spores of *Bacillus stearothermophilus* unviable at 121 C. For this test, ampoules of *B. stearothermophilus* are autoclaved with a load. Upon completion of the cycle, the ampoules are incubated for 48 hours and then observed for any sign of growth which would indicate that the autoclave is not sterilizing properly. If growth occurs, the autoclaves are retested until a cycle time that allows for decontamination is found.

**Unloading the Autoclave**

When the cycle is complete, wait until the chamber pressure gauge reads ”zero” before attempting to open the autoclave. A waiting period of several minutes is required before removing the bag from the autoclave. This will allow the chamber and any residual liquids to cool, significantly reducing your chances of getting burned. Also be aware of molten agar that may have seeped from the bag and collected in the pan or tray during the cycle. Use special caution when autoclaving containers that may have become pressurized. Never autoclave a sealed container of liquids as this may result in an explosion of super heated liquid during the cycle or when the container is opened. *Always wear heat protective gloves when removing waste from the autoclave!*

**Causes of Autoclave Failure**

The reasons for autoclave failure (sterilization failure) usually fall into two categories – failure of the operator or mechanical failure due to breakdown or improper maintenance.

In any case of failure, report the incident to the principle investigator or the lab supervisor.