



uOttawa

A GUIDELINE FOR THE SAFE USE OF AUTOCLAVES



University of Ottawa
Office of Risk Management

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PURPOSE

The purpose of the “Guideline for the Safe Use of Autoclaves” is to inform potential users and their supervisors of the issues that must be considered to ensure the autoclave process is undertaken in a safe, effective and efficient fashion.

It is important to recognize that specific faculties and departments may have more stringent operational requirements in place to meet their own needs. In addition, many of the autoclave facilities have dedicated individuals who are delegated the task of ensuring the autoclave is used properly, maintained regularly, quality assurance standards are met, and users are trained. You are therefore required to meet with these individuals prior to using an autoclave. If you have any difficulty identifying the appropriate individual in your faculty or department, contact ORM for assistance.

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1. INTRODUCTION

This guideline is designed to assist you in understanding the intent and limiting factors associated with the use of an autoclave when decontaminating biohazardous or potentially biohazardous material, or sterilizing solids and liquids.

An autoclave is a specialized piece of equipment designed to deliver heat under pressure to a chamber, with the goal of decontaminating or sterilizing the contents of the chamber. **Decontamination** is the reduction of contamination to a level where it is no longer a hazard to people or the environment. While **sterilization** is the total destruction of microorganisms present in the material.

Decontaminating or sterilizing is achieved because heat damages the cell's essential structures, including the cytoplasmic membrane, rendering the cell no longer viable. This will only occur if the material in the chamber is heated to a specific temperature under a certain pressure for a given period of time by the penetration of steam/heat. This guideline outlined the key factors of operating autoclave safely and efficiently in greater detail, as each step in the process is discussed.

2. PERSONAL PROTECTIVE EQUIPMENT

Due to the fact that autoclaves utilize steam, heat and pressure, there is a potential risk of personal exposure to extremely high temperature. Therefore one must wear the appropriate protective equipment. In addition, material to be loaded contains potentially infectious material, so the standard laboratory protective equipment must be worn.

- **Safety eye and face protection**

Face shield minimizes the risk of facial steam burns.

- **Gloves**

Latex or nitrile gloves prevent contact with contaminated material, while heat resistant gloves (asbestos free) must be used when loading and unloading the autoclave.

- **Lab coats**

Long sleeves must be used to protect wrists and forearms, plus an apron if a spill hazard exists.



Figure 1. Personal Protective Equipment for Autoclaving

Note: Remember that although the autoclave trays may be cool, the door and walls of the chamber may still be hot enough to cause a burn!

3. TRAINING

Training is absolutely required prior to using the autoclave. Not only will this minimize the risk of personnel being harmed, but it is essential to ensuring a successful decontamination or sterilization of

your material. Training will also help minimize the risk of damage to the equipment. This guideline is one tool to assist in training. In addition, you must receive training specific to the autoclave you will be using. This will assist you identifying problems with your own load or previous loads, i.e., interpretation of cycle log records. Thus training will help promote:

- Your safety
- Your research quality
- Optimal use and care of equipment

At the University of Ottawa, Autoclave Safety Training is offered by the Faculty of Science and the Faculty of Medicine several times per year. Users can register the training session on the UOttawa Online Registration website. As different faculties may have more stringent operational requirements to meet their own needs, users work at main campus are recommended to attend the training happen on main campus (usually DRO 214) while users work at the RGN building are recommended to take the training at RGN.

All principal investigators and supervisors should document that this training has been received by their staff (students and support staff) that will be using the autoclave.

(A quiz have been used for training was attached as Appendix 2)

4. ITEMS FOR AUTOCLAVING (THE CANS AND CAN NOTS)

Although autoclaving provides an economical way of sterilizing and decontaminating items, not all material can be autoclaved. Some materials present specific hazards when they are autoclaved; such as the generation of toxic /noxious gas. To help you identify what may or may not be autoclaved a general list of items has been included in this guideline.

4.1. Items CAN be Autoclaved

- Media, tissue culture buffers, stable non-volatile substances
- Cultures and stocks of infectious material
- Culture dishes and related devices
- Discarded live and attenuated vaccines
- Contaminated solid items such as petri dishes, Eppendorf tips, pipettes, gloves, paper towel

4.2. Items CANNOT be Autoclaved

- Materials containing: solvents, volatile, chlorinated compounds (HCL, bleach) or corrosive chemicals (such as: phenol, trichloroacetic acid, ether, chloroform) etc.
- Material contaminated with chemotherapeutic agents
- Radioactive material (without prior approval)
- Some plastics.

5. LOADING AN AUTOCLAVE

This section will address the various steps to be undertaken when preparing and autoclaving the

material to be decontaminated or sterilized. The following factors will be discussed: the packaging (primary and secondary), the identification requirements (labelling) and the fundamentals of loading an autoclave to maximize steam penetration. Each factor plays a critical role in ensuring a successful decontamination /sterilization.

Prior to loading an autoclave, verification should be undertaken to ensure the autoclave has been functioning correctly and has been meeting the validation requirements. This verification can be performed by:

- Reviewing the previous cycle log record,
- Examining the results of the regular verification record,
- Speaking to the individual responsible for the autoclave.

(For more information, please refer to Chapter 12. Quality Control)

5.1. Packaging

As the success of the decontamination/sterilization is dependent upon the penetration of heat; how material is initially prepared will greatly affect the outcome. Consideration must be given to the primary container (containing the contaminated waste), volume of liquid, amounts of material, and the secondary container (containing the primary container).

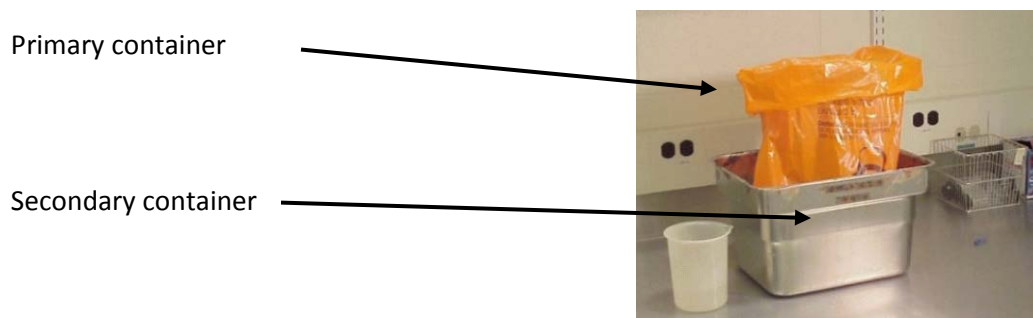


Figure 2. A Sample of Primary Container and Secondary Container for Autoclaving

5.1.1. Choose the Right Material for Container

The structural integrity of the container is an important consideration. Not all containers withstand the demands placed on them during the autoclave process. Desirable characteristics are heat resistance, good thermal conductivity, puncture proof and impervious to water.

Good Choices:

- Borosilicate glass (Pyrex) has very low thermal expansion property and therefore resistant to breaking due to heating
- Polypropylene (PP) and polycarbonate (PC) are heat resistant plastics
- Stainless steel is a good heat conductor and thus facilitates sterilization

Poor Choices:

- Polystyrene (PS)
- Polyethylene (PE)

- High density polyethylene (HDPE) – do not resist heat well

Most containers will identify the type of plastics identified on the base of the container with the appropriate initials imprinted (PP, PC, PS, PE, HDPE, LDPE...). If there is a risk of material melting, ensure they are placed in a secondary container which is resistant to heat.

5.1.2. Primary Container

The primary container is the container which comes into direct contact with the contaminated material of fluid. This may include such items as: flasks or vials holding liquids (either media or infectious material), wrapping paper or muslin protecting instruments, and biohazard bags containing waste.

This packaging must permit heat/steam penetration, and ensure pressure differentials are not created as this will result in breakage. (Containers must **NOT** be sealed when placed in an autoclave!) This may be accomplished by using techniques such as:

- Loosening screw caps or using self-venting caps.
- Capping open containers for sterilization with aluminum foil.
- Using envelope folds for wrapping craft paper or muslin. (This adds the benefit of protecting contents from contamination during the opening process.)
- Opening plastic bags slightly prior to loading them into the autoclave.

5.1.3. Autoclave Bags

At the University of Ottawa, the orange autoclave bag (Figure 3, they are also available in other colors) is used for **decontamination of biohazardous waste**. This bag is made from heat resistant materials hence no other regular garbage bag should be used for autoclaving. To facilitate the steam transfer, leave the bag open prior to autoclaving and can add water to the contents to generate steam from within. Do **NOT** fill the bag beyond the **75%** of the holding capacity.



Figure 3. Autoclave Bag

Note: To obtain the autoclave bags, you can request through the service or faculty/department technical support or purchase them at the Sciences Stores at D'Iorio Hall.

5.1.4. Secondary Container

The secondary container with the sufficient high sides (15 or 30 cm) is used to contain any spill that may occur. Extreme care must be taken to ensure they are capable of withstanding the demands of the autoclave procedure. Age of pans can often cause them to become brittle or to soften when heated.

Regular inspection of the secondary container will help identify compromised containers. In addition, they should be made of material that facilitates the transfer of heat to compensate for the restricted airflow within the chamber caused by their presences.

5.1.5. Separate Material Properly

Prior to autoclaving, it is necessary to separate material for re-use from that which will be disposed. This will have the benefit of: preventing re-handling and therefore minimize the risk of contamination, reducing the risk of personal injury due to sorting of sharp objects, and finally avoiding having to separate items that have become stuck together during the autoclaving process.

5.1.6. Weight and Volume Restriction

When packaging the material, consideration must also be given to the final weight of the items, taking into account possible water absorption during the autoclave cycle. Total weight must not exceed the load bearing restrictions of autoclave, or cause ergonomic injuries as a result of transferring this material in and out of the autoclave.

As volume and density will impact the heat transfer and steam penetration it is important **NOT** to fill the containers beyond the **75%** of the holding capacity. This also allows for liquid expansion, preventing overflow. Similarly with solid material, the additional available volume will allow the contents to shift during transfer into a secondary container or the autoclave without spilling out of the bag. Avoid packing or compressing the contents to achieve volume limits as this will restrict steam penetration.

5.1.7. Moisture Adding

It is recommended that water to the depth of 2.5 cm be added to the pans containing glassware. This will fill insulating air pockets that may be generated when the glassware is placed in the pan. (This apparently is not such a concern for metal pans.) In addition to insulating properties, these pockets are believed to play a role in glassware breaking. The addition of water will also help minimize the quantity of material that may be baked on to the surface.

Add 250 ml of water to the contents of bags containing solids (more if the bag is large). This will create additional steam which will displace the dry air from the bag, increasing the rate of heat penetration. It is best to allow the water to drain down the sides of the bag, taking care to avoid splashes and/or spillage.

5.1.8. Temperature Sensitive Tape

Temperature sensitive tape must be affixed to bags and each individual item. When the temperature sensitive tape has been exposed to high temperature, dark (usually black) lines will appear. Thus it may be used to indicate that the labeled material has been autoclaved.

Note: *It is not proof that the autoclave cycle was successful at decontaminating or sterilizing the contents.* A biological indicator or other means must be used to act as a quality control validator. (See Chapter 12. Quality Control)

5.2. Labelling

It is necessary to label all material being placed in the autoclave. This is important for the following reasons:

- Material may be subsequently sorted and returned to the appropriate laboratories.
- Once problem or concern arises regarding the function of the autoclave the individuals potentially affected may be informed.
- Once a spill occur the risk may be assessed based on the contents involved

- You may not be aware of a problem until much later in your research project, for example: contaminated cell cultures.

5.2.1. Label Content

The following information must be affixed to the primary containers:

- Name of responsible person
- Contact information
- Identification of contents (including volume of liquid contained)

5.2.2. Autoclave Bag Labelling

Same to all the hazardous waste, the autoclave bag must be labelled with the 'University of Ottawa Hazardous Waste Label' (Figure 4). The following information must be completed:



- Professor name
- Building, room identification and phone number
- Contact person
- Date
- Content of waste

Figure 4. University of Ottawa Hazardous Waste Label

Once waste is decontaminated successfully by using an autoclave, the biohazardous symbol on the autoclave bag must be defaced by the University of Ottawa 'Autoclaved Biomedical Waste-Non-Hazardous' label, which indicates the bag of waste is disposed of in the regular waste stream. (See Chapter 8. Autoclaved Waste Disposal)

5.3. Transport Material to Autoclave

When transporting material to be autoclaved, use a cart with guard rails. Ensure the use of a secondary container to collect any spillage should the cart be knocked or jarred during transport. Use a direct but not heavily populated route. Decontaminate the surface container prior to transport (for waste may double autoclave bag), unless there is no risk of contamination.

Waste should be restricted to the laboratory zones and not areas used by the general public. If this not be possible contact the ORM for advice.

5.4. Fundamentals of Loading to Ensure Success

As much attention must be applied to loading the autoclave, as was given to packaging. Again the determining factor is ensuring heat/steam penetration. Therefore care must be given to avoid overloading the chamber, placing bags/containers in the chamber which are too large, and adding too much weight which will tax the design elements of the autoclave. Consideration must also be given to ergonomic factors.

Simple measures can be used to ease the flow of heat and steam thorough and into the contents of the containers. Here are some fundamental rules:

- Load the material in such a fashion to present the least resistant passage of air exchange through the load, from the top of the chamber to the bottom.

- Avoid crowding or stacking.
- Place packages on their edges to enhance steam penetration, place a rack or other item against these items to prevent them from slipping.
- For loads which are mixed (fabric and hard goods) place the hard goods on the bottom to prevent possible condensation from dripping on to the fabric.
- Place empty flasks, test tubes or other non-porous containers on their sides with loose covers. This provides a horizontal pathway and prevents trapping air pockets.
- Ensure containers do not touch each other, this will ensure all surfaces are sterilized.
- No items should touch the top or sides of the autoclave container as the container is pushed inside.
- Liquids and dry goods are processed separately as they require different cycle selections.
- A load of liquid filled containers should be of similar size, shape, content and volume; because exposure time is based on these characteristics.
- Run material to be sterilized separate from those to be decontaminated.

6. OPERATING AN AUTOCLAVE

6.1. Autoclave Technology and Cycles

There are three technological processes associated with autoclaving material and are based on the following design elements: gravity, pre-vacuum, and isothermal.

- Gravity
The gravity design is utilized for sterilizing non-porous heat and moisture stabilized goods. These included liquids and media and solids such as laboratory waste.
- Pre-Vacuum
The pre-vacuum increase the speed and efficiency associated with sterilization. It expands the capability of the gravity design by its ability to sterilize porous heat and moisture stabile materials; such as animal bedding.
- Isothermal
This design addresses the need to sterilize at lower temperatures for the heat sensitive and heat coagulable material: such as infant formula or media. The isothermal process is usually used for pasteurization, inspissation, or fractional (intermittent) sterilization.

Each process has a variety of cycles that may be employed and are dependent upon the process, or options associated with each autoclave. These include: heat, gravity, pre-vacuum, sterilize, exhaust, and dry. Exhaust may either be undertaken rapidly or slowly. The rate of exhaust will depend upon the nature of the load. Dry material will permit a fast exhaust, while liquids require slow exhaust to prevent boiling over of super-heated liquids. Refer to the autoclave manual to obtain a complete description of the cycles available.

6.2. Factors to Consider

Each autoclave will have specific instruction for its own use. It is important to follow the manufactures

recommendation and each user must receive hands-on training on its use. Although many factors must be considered when determining the length of time required some general guidelines can be provided.

Factors that will affect your cycle times will be:

- If the autoclave will be used for sterilization or decontamination
- Manufactures recommendation for media sterilization
- If the material is primarily solid or liquid
- Volume of liquid
- Shape and size of containers used
- Thermal conductivity properties of the container
- Viscosity of the liquid
- Density of the material
- Position of the load within the autoclave chamber

To be effective the autoclave must reach and maintain a **temperature of 121-123 °C** for **at least 30 minutes**. This is achieved by using saturated steam under **at least 15 psi of pressure**.

It is important to recognize the length of time required to achieve this temperature will be dependent upon the factors stated above. In addition the sensing device which records this temperature is not located within the load of material being autoclaved. Therefore additional time may be required to ensure the centre of the load has achieved this temperature, for examples with samples that have a very high biological load the contact time must be extended to 60 minutes.

6.3. Determining Correct Cycle Time

As the cycle time will vary with the composition of the load, it is important to determine the appropriate time requirement. Assuming the minimal time of 30 minutes, may prove to be a very costly mistake.

- Select the typical bag size and contents
- Place the biological indicator in the centre of the load. Attach a string or tape to it to allow easy retrieval.
- Run the autoclave using the typical cycle selection
- Retrieve the biological indicator and process as per manufactures instructions
- If the indicators turn yellow the autoclave process has FAILED
- Increase the cycle time by 15 minutes and retest
- Retest until the indicator proves a successful autoclave cycle. PASS is indicated by no colour change, and hence no bacterial growth.

6.4. Cycle Failure

If the autoclave cycle fails to be completed, the load must be re-autoclaved. This may be achieved by switching the autoclave to the manual cycle (if available). If the failure was due to a power outage the

cycle may not be initiated again until power is restored throughout the whole grid system or emergency power has been activated. Refer to the manual for detailed instruction specific to your autoclave.

7. UNLOADING AN AUTOCLAVE

The greatest risk of personal injury occurs during the process of unloading the autoclave. Not only is the risk of burns or scalding significant, but one may also be exposed to the vapours and gases generated by the inadvertent autoclaving of volatile chemicals. Super-heated liquids also pose of risk of exploding if they are shaken or moved during the cooling process. In addition glassware can break if the autoclave door is opened too quickly, and sufficient time is not provided for them to approach room temperature. *Consequently, extreme caution must occur during this final stage.*

When you unload an autoclave, ensure following the procedures below:

1. Wear all necessary personal protective equipment.
2. The chamber pressure gauge of the autoclave should be zero before opening the autoclave's door.
3. Crack door slightly and stand back to allow steam to escape. To minimize the risk of accidents caused by steam escape, the person who opens the autoclave door should stand directly behind it.
4. Slowly open autoclave door. Opening the autoclave door too quickly may result in glassware breakage and/or steam burns to the skin.
5. If boiling or bubbling is present, wait until it subsides. Assess the risk of super-heated liquids.
6. Over exposure of saline or water is not a critical factor (as it is with media), so these liquids may be allowed to cool (for 10 min.) in the autoclave after cracking the door to release the steam. This is also recommended for all other items.
7. Bring the autoclave trolley to the chamber.
8. Using heat resistant gloves carefully transfer the containers (pans) to the trolley. Be careful not to jolt the containers as it could result in breakage.
9. Move the containers to a draft free area.
10. If not already cool wait 10 minutes, prior to storing sterilized material or preparing autoclaved waste for disposal.
11. After every use, it is advised to close the autoclave door but do not seal the door as this will shorten the life span of the rubber gaskets on the door.
12. Verify that the temperature sensitive tape has changed color, or diagonal lines or the words "autoclaved" has appeared. If no change appears on the tape, the load is required to be re-autoclaved after placing new tape on the material. Also verify the cycle log recorder to check the temperature and time attained. If minimum time and temperature is not attained on the second cycle, users should contact the person responsible for maintenance of the unit to initiate repairs. Leave a note on the autoclave stating the problem and that it cannot be used until problem has been resolved.
13. The container that could be contaminated by a liquid splash (due to boil over) or by direct

contact with contents of the waste bag (such as melted agar) should always be washed after each use.

8. AUTOCLAVED WASTE DISPOSAL

Once the waste has been successfully autoclaved, the waste is no longer considered biohazardous. It may now be disposed of to the appropriate municipal waste stream.

It is now important to alter the hazard awareness signage and symbols to reflect this. This should be undertaken in two ways:

- Using a large felt marker deface the biohazard symbols.
- Apply the University of Ottawa label which indicates that this waste is “Autoclaved Biomedical Waste - Non- Hazardous” (Figure 5 left).

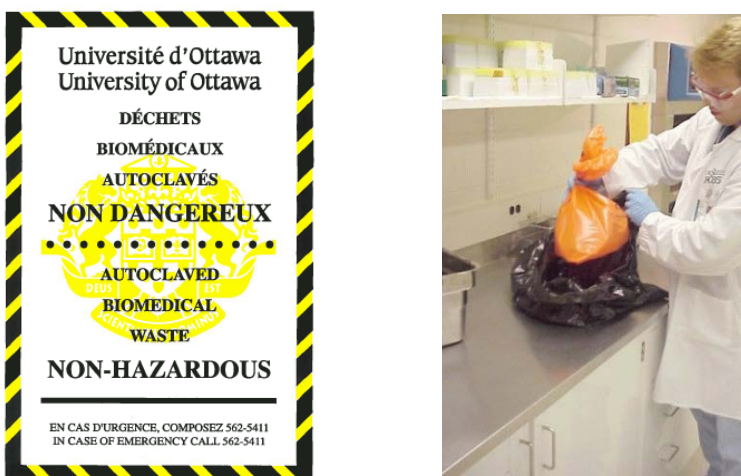


Figure 5. ‘Autoclaved Biomedical Waste - Non- Hazardous’ Label (left); Dispose Autoclave as Regular Waste (right)

Once this has been accomplished the defaced and labeled autoclaved waste bags should be placed inside a plastic trash bag (black) designed for domestic applications (Figure 5 right). It is greatly recommended to place only one or two biohazardous bags inside a single plastic trash bag (domestic applications) to prevent an overload and to minimize the risk of breakage. Ensure the black bag is securely tied close.

9. WASTE STORAGE

Often it is necessary to wait for the previous autoclave cycle to end prior to autoclaving your own load. When this is the case, the waste should be stored in a safe, dry and secure place. This is to minimize the risk of personnel and animals being exposed, and to avoid the inadvertent disposal of the waste prior to autoclaving.

The waste **must be appropriately labeled** (name, telephone number, content). A secondary container must be used to collect any seepage that may occur. The bag must be sealed and placed in such a fashion to prevent it tipping over. **Do not seal the bag** in such a fashion as to prevent it being re-opened (slightly) to permit steam penetration during autoclaving.

Waste should be stored for the minimal length of time in an autoclave facility, a couple of days at the

maximum. If significant bio-degradation or strong odours are likely to occur, another storage venue must be identified. If waste not be autoclaved within 2-3 days contact the individual responsible for the autoclave or ORM.

10. RECORD KEEPING

Records are a vital component of the autoclave process. These records act as historical proof that the autoclave has been meeting the regulatory requirements and/or industrial standards. Four types of records are to be kept:

- **Autoclave Training Records** which indicate the personnel has learned how to safely operate an autoclave process and understand the potential risk.
- **Autoclave Log** which identifies the users, nature of the load, cycles used and exposure times. (See Appendix 3)
- **Validation Records** which record the results of the validation activities undertaken. This would include the results of the biological indicator undertaken every six operating days. (See Chapter 12. Quality Control and Appendix 4)
- **Performance Records** which record the dates of problems were encountered, remedial action taken and any service calls required. (See Appendix 5)

In addition, **annual service reports** (if applicable) should be kept. This record will permit a general assessment of the condition of the autoclave and help to answer the question “Should we be considering replacing this autoclave in the next 3-5 years?”

Note: As being regulated by the *Ministry of the Environment and Climate Change* and being required by the *Canadian Food Inspection Agency*, record of validation test using biological indicators shall be retained at the operation facility for a **minimum of 5 years**, and must be in a format that is available for inspection by Ministry Environment staff.

11. PREVENTATIVE MAINTENANCE

Preventative maintenance will greatly increase the efficiency of your autoclave, reduce downtime and save costly repairs. It may be considered as a scheduled inspection, which could result in minor adjustments or repairs, major repairs, or premature replacement of parts. It is designed to maintain safety, and delay or avoid an emergency.

The work associated with this activity is greatly reduced when: the autoclave is used properly, the loads are packaged and loaded in an appropriate fashion (to minimize the risk of spills and explosions, and material touching the chamber walls), and secondary containers are used to collect spills.

11.1. Daily / Weekly Maintenance Activity

Although the manual will provide specific instructions some general guidelines can be provided. Steps that should be taken daily or weekly, depending upon use pattern and control during packaging and loading, are:

- Disinfect exterior surfaces of the autoclave
- Check door gaskets for wear

- Clean interior walls with trisodium phosphate, other mild detergent or as recommended by the manufacturer. Never use a strong abrasive or steel wool. Rinse with tap water after cleaning.
- Clean screen leading to discharge line
- Check primary and secondary containers for integrity (stress fractures, cracks, chips etc.)
- Consult manual to determine other recommended activities

11.2. Yearly Maintenance Activity

An annual inspection and maintenance program should be undertaken by qualified individuals.

12. QUALITY CONTROL

A number of tools are available to assess the performance of the autoclave; these include physical, chemical and biological indicators. It is important to note that these indicators will only respond to time, temperature and moisture conditions, and not to organic load.

12.1. Physical Indicators

Physical indicators are pressure and temperature recording devices. Thermocouples can be placed inside the load to determine the temperature achieved in the bag itself.

12.2. Chemical Indicators

These indicators change colour after being exposed to specific temperatures, for example: heat sensitive tape. Upon exposure to the given temperature the change will occur; it is not time related. Therefore these indicators can only attest to the temperature attained and not to exposure time and hence success of sterilization.

12.3. Biological Indicators

Biological indicators are used in the efficacy testing of the autoclave process to effectively sterilize the contents being treated. Spores of *Bacillus stearothermophilus* are used, as they are the most resistant organism to steam autoclaving. To determine the effectiveness of the autoclave process the biological indicator must be placed in a typical test load (solid or liquid) and exposed to the typical cycle conditions. Biological validation test is the standard method of validating the effectiveness of your autoclave procedures.

Note: Testing using biological indicators for waste treatment **MUST** be undertaken at least once in **every six operating days** to confirm that the equipment is capable of meeting the requirements that the Spores of *B. stearothermophilus* will be reduced within the waste by **a level of 6 Log₁₀ (99.9999%)**.

(See C-4: *The Management of Biomedical Waste in Ontario*)

12.3.1. Biological Validation Test Procedures

1. Read and follow the supplier's instructions.
2. Place *B. stearothermophilus* in centre of representative test load.
3. Process autoclave in a normal fashion.
4. a) Extract and incubate *B. stearothermophilus* sample as instructed by manufacturer.

- b) Use another ampoule (same lot #) not autoclaved to act as a positive control.
- 5. Check for colour change at regular intervals during the incubation period (8, 12, 24, and 48 hours). If media is yellow and turbid the autoclave process has FAILED. Immediately upon noting yellow colouration, re-run all samples with new biological indicators.
- 6. If failure continues to be noted, either increases time of exposure or initiate repairs to the autoclave. Note the autoclave cannot be used again until validations procedure indicates that autoclave is now adequately sterilizing the material.
- 7. Record all results. (Positive and Negative)

12.3.2. Biological Validation Test Records

- Record biological indicator information: brand, lot #, expiry date.
- Record the date, operator, cycle time, cycle temperature of the test run.
- Record results: colour change noted (Failure), no colour change to yellow (Pass).

(See Appendix 3 and refer to Chapter 10. Records Keeping that this record must be kept for at least five years.)

13. MISTAKES IN AUTOCLAVING

A number of events (operator and mechanical) can cause the autoclave cycle to fail, but most tend to be directly related to the packaging and loading consideration taken. This may result in having to re-autoclave material, or modify the cycle conditions (length of exposure time or temperature).

Unfortunately, lack of diligence during the autoclave process can be very costly in terms of:

- Personal injury
- Down time
- Lost experimental data
- Expensive and/or length repairs
- Inappropriate disposal to landfill resulting in regulatory violation and an inquiry/fine

Typical mistakes:

- Insufficient personal protective equipment (resulting in injury)
- Rushing (resulting in burns, spills etc.)
- Inappropriate material selection (resulting in glassware breakage or unexpected or undesired melting of containers)
- Sealing containers (resulting in pressure buildup, explosions, and lack of steam penetration)
- Lack of use of secondary containers (resulting in the chamber becoming contaminated)
- Over-filling containers (resulting in liquids boiling over and loss containment of solids)
- Poor loading practices (resulting in lack of steam penetration through the load)

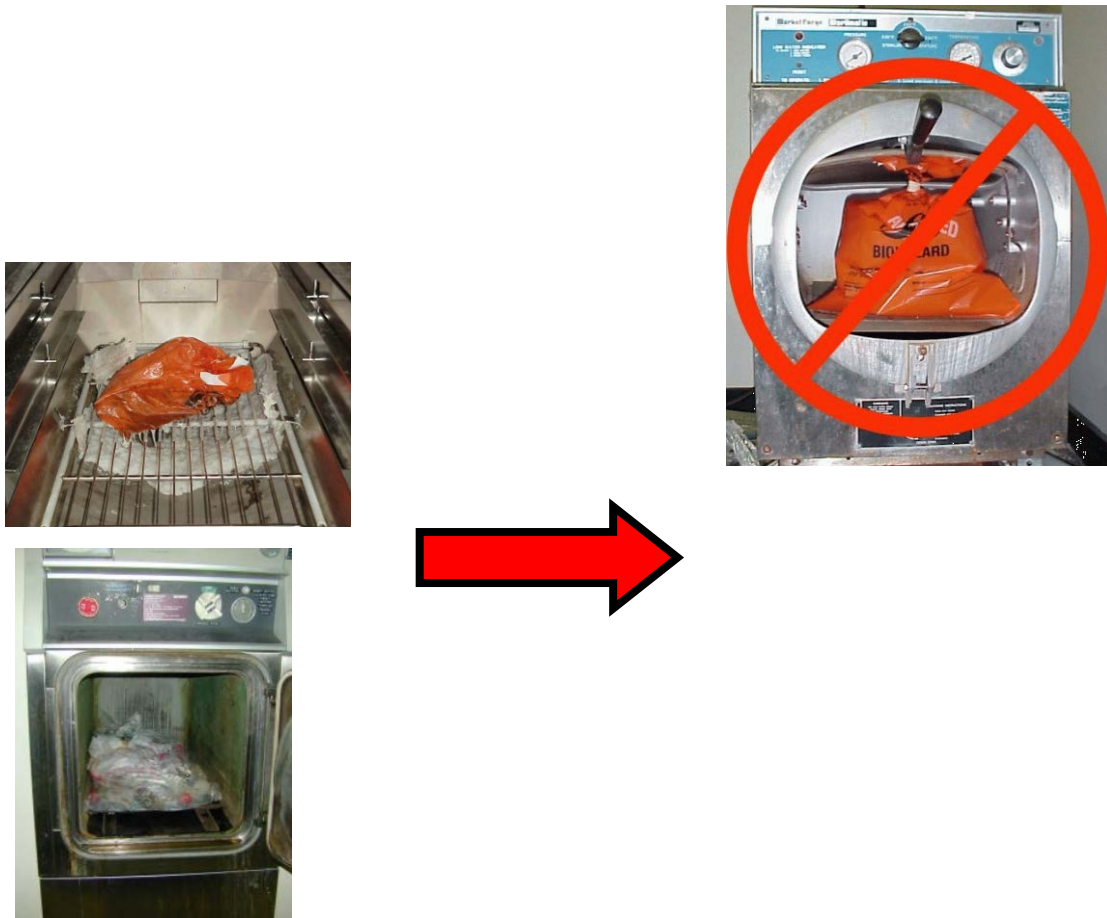


Figure 6. An Example of Autoclave Process Failure Caused by Sealing the Autoclave Bag

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Appendix 1 THE 1, 2, AND 3'S OF AUTOCLAVING

WEAR PERSONAL PROTECTIVE EQUIPMENT

Eye and Face Protection, Gloves (including heat resistant gloves), and Lab Coat (long sleeve apron)

1. Packaging and Loading

- Prepare and load material to ensure steam penetration
- Ensure all containers including bags are vented
- Do not overfill containers (prevent spill and boil over)
- Ensure sufficient water in load to allow steam penetration or add 250 mL water to bags
- Containing solids to ensure steam penetration
- Use secondary containers
- Label all material (name, contact telephone number, contents)
- Ensure material is permitted to be autoclave
- Do not mix clean and contaminated material in the same load
- Complete “Daily Autoclave Log”
- Do not allow bags to touch or strap sides of autoclave
- Use approved autoclave bags

2. Operating an Autoclave

- Ensure the autoclave is operating properly before commencing
- Determine the appropriate exposure time for the load, consider the many factors effecting
- Exposure time
- Ensure the autoclave attains the desired temperature (normally 121°C) and pressure (minimum 15 psi) for the desired time (minimum 30 min.)
- Record information in “Daily Autoclave Use Log”
- Undertake validation test using a biological indicator (*B. Stearothermophilus*) every six operating day
- Record results on the “Biological Test Indicator Results” form

3. Unloading an Autoclave

- Wait until the chamber pressure gauge reads zero before opening
- Open slightly to allow steam to escape (protect yourself from the steam)
- Wait 10 minutes for the contents of the autoclave to cool
- Remove the waste in manner reduce the risk of spillage, use a trolley
- Verify temperature and duration of exposure has been met

Appendix 2 TRAINING DOCUMENTATION

This document is to attest to the fact I, _____ (first and last name), have read the “*Guideline to the Safe Use of Autoclaves*” and am aware of the issues that may affect the effectiveness of autoclaving, issues to minimize personal exposure, and the requirements for documentation. In addition I have been trained with regards to the correct use and recommendations associated with the use of the specific autoclave I will be using. I have answered correctly the following questions which confirm my basic knowledge of autoclave safety related issues.

Circle the correct answer.

- | | | | |
|----|--|---|---|
| 1. | The effectiveness of autoclaving is based to a large degree on the appropriate packaging and loading. | T | F |
| 2. | To prevent spillage autoclaving, seal all autoclave bags. | T | F |
| 3. | The technique used to load the autoclave does not greatly influence the success of the cycle. | T | F |
| 4. | All material can be autoclaved. | T | F |
| 5. | A change in colour of temperature sensitive tape does not indicate the material has been successfully autoclaved. | T | F |
| 6. | It is not necessary to use a biological indicator to test the efficacy of the autoclave if it consistently achieves the selected temperatures. | T | F |
| 7. | As the material has been autoclaved there is no risk associated with unloading the autoclave upon completion of the cycle. | T | F |
| 8. | The personal protective equipment that must be worn includes eye and face protection, gloves (including heat resistant gloves), lab coat (plus apron if spill risk). | T | F |

Signature of Autoclave User

Date

Signature of Supervisor

Date

Appendix 3 DAILY AUTOCLAVE USE LOG

Model: _____ Serial#: _____ Room #: _____

Date	Name of Generator	Contact Telephone No.	Description of Contents	Name of Autoclave Operator	Cycle Selected	Temperature Achieved (°C)	Length of Time Autoclaved

BIOLOGICAL INDICATOR TEST RESULTS

Make: _____ Model: _____ Serial #: _____ Room #: _____

Biological Indicator: _____ Type: _____

Date of Autoclaving & Incubation	Operator	Cycle Selected & Temperature (°C)	Cycle Time	Duration of Incubation (hrs)	Biological Indicator Expiry Date & Lot #	Results Pass (P) Fail (F)

Autoclave Make: _____ Model: _____ Serial #: _____

Date	Problem	Remedial Action	Comments	Description of Service Call