

Autoclave Safety



Introduction

- This self-guided workshop serves as a basic introduction to general autoclave safety measures and is intended as a starting point for the workplace-specific training that is to be provided by your supervisor and/or other University services.
- To successfully complete this awareness workshop, you must complete the knowledge assessment. Only then will your grade be entered in the system

Key Concepts

- Purpose of autoclave safety training
- Principles of autoclaving
- Quality control of autoclaving cycles
- Preparation for autoclaving
 - Items that **can** and **cannot** be autoclaved

Responsibilities

- In a workplace, people at all levels must ensure the health and safety of:
 - Themselves
 - Other individuals
 - The environment
- The safe use of autoclaves requires training (online and site-specific), supervision and supervisor authorization.



What Can Go Wrong?

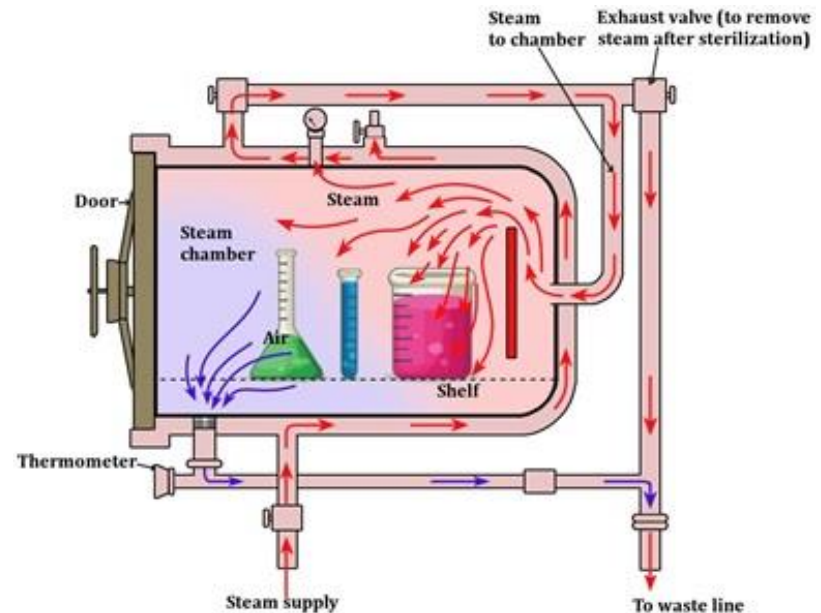
- Users and staff can be injured if biohazards are not properly handled or if prohibited samples are processed. This can cause:
 - Lacerations from sharps put in biohazard bags
 - Respiratory distress from toxic fumes released when autoclaving prohibited substances (e.g., phenol or formaldehyde)
- Equipment can be damaged if prohibited samples are processed.
 - The internal autoclave walls can be corroded by certain substances, like bleach.

Principles of Autoclaving

- What is autoclaving?
- How does an autoclave work?

"An autoclave is a machine that is used to eradicate biohazardous waste from the surface of tools or instruments. It was invented by Charles Chamberland in 1884. Autoclaves sterilize or disinfect through physical means by using pressure, temperature and steam. They are often referred to as steam sterilization machines."

www.scienceabc.com



Sterilization vs. Decontamination

- Semantically different
- Technically identical
- Legal requirements are different depending on the work environment.
 - Hospitals
 - Pharmaceutical industry
 - Universities

What's the Difference?

Sterilization:

- Refers to the **total destruction** of contaminants.
- The absence of potential microbiological contaminants is necessary in preparation for experimental protocols.



Decontamination:

- Refers to the **reduction of contamination** to a level where it is no longer a hazard to people or the environment.
- Waste treatment technique



Moist Heat is Better than Dry Heat

Moist heat

- Lower temperature and high-pressure of water (steam)
- Coagulates/denatures proteins more rapidly and at lower temperatures
- ~~Also membranes, ribosomes, DNA/RNA~~
- Better penetration and more efficient transfer of energy

Dry heat

- Dry air of higher temperature and for a longer time
- Oxidation, dehydrates (protoplasm): slower process
- Proteins, DNA/RNA
- Proteins are more stable under dry heat

Moist Heat Autoclave

- Pressurized vessel allowing for moist heat at temperatures above 100°C (121°C, 132°C)
 - 15 psi gives 121°C
 - Heat is the key element, not pressure.
- **Important factors to consider:**
 - Temperature and duration
 - Complete replacement of the air with steam
 - Microorganism, media and material heat tolerance
 - Material must be packaged to maximize heat penetration as a function of load characteristics (volume, density). Example: sterilization of 1L of culture media in a 2L flask will take longer than 4 x 250 ml in individual 500 ml flasks).



Autoclave Cycles

- Different materials
- Different cycles
- Different sterilization times

Pre-Vacuum Cycle

- **Conditioning phase:** Vacuum pulses (3 or 4) alternating with steam injections in the chamber allow for removal of air pockets.
- Used for materials with good tolerance to heat and vacuum (e.g., glassware, glass pipettes in metal canisters, waste in autoclave bags), allowing for higher temperature and shorter time as well.
- Sterilization temperatures: 121 °C, 132 °C, 134 °C (100 °C to 141 °C)

Pre-Vacuum Cycle (cont.)

- Allows for shorter cycle time (even at 121 °C) because of better steam penetration.
- Offers drying phase, depending on the setting.
- Not all items can withstand vacuum when heated (e.g., delicate plastic rings).

Gravity Cycle

- For glass, plastic or metal items.
- Air and steam do not mix readily.
- Air is heavier than steam and is displaced to the bottom of the chamber and forced out through the drain (cold air and non-steam-saturated pockets remain).



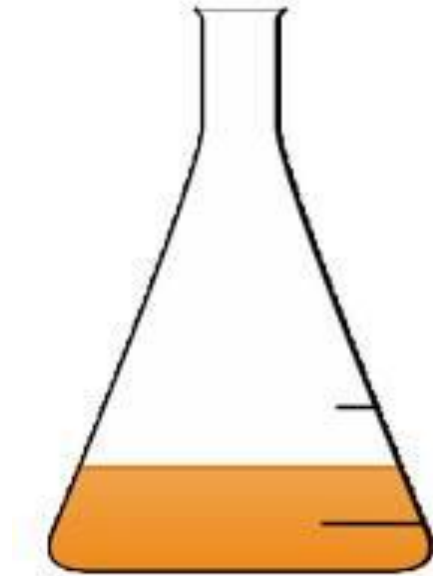
Gravity Cycle (cont.)

- Not for liquids because exhaust is too fast.
- Sterilization temperature: 121°C.
- Offers drying phase, depending on the setting.
- Requires longer sterilization time than pre-vacuum cycle.



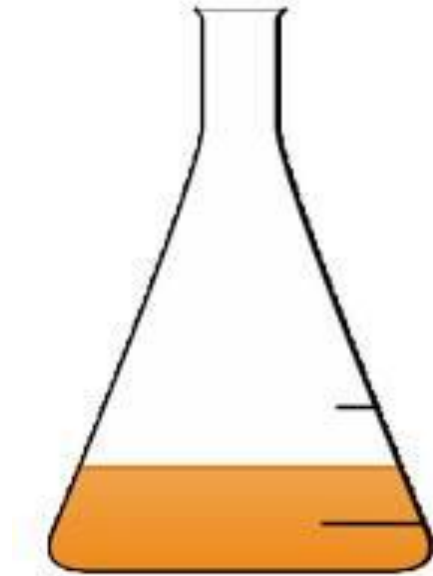
Liquid Cycle

- For liquids and media in vented borosilicate glass or metal containers.
- Sterilization temperature: 121°C.
- Features **slow exhaust phase** to prevent spillover of liquid. **Avoid overfilling of flasks: 50-60% max volume.**



Liquid Cycle (cont.)

- Offers milder conditions for sensitive materials (even solid material).
- More condensation at the end because there's no drying phase.



Preparation for Autoclaving

How to facilitate heat penetration:

- Vessels should be loosely capped. Biohazard bags should be loosely sealed.
- Aluminum foil will block penetration.
- Do not overfill containers or waste bags:
 - Low density: <75% of holding capacity
 - Liquids or higher density: 25% to 60% of holding capacity
- Follow faculty procedures to autoclave.

Validation of Cycles

HOW?

- Heat sensitive tape (on every single item)
- Biological indicators (once a week)
- Sterilization integrator (immediate readout monitor)
- Load probe and chamber probe (temperature sensing)

WHY?

- Legally required for decontamination (6 log reduction)
- Quality assurance for both sterilization and decontamination

WHEN?

- Performed weekly
- Records kept for two years (environmental regulation)

Heat-Sensitive Tape

- Must be affixed to bags and to each individual item (e.g., biohazard waste bags, holding baskets, surgical packs).
- Used to indicate that the autoclaving temperature has been reached.
- Marking will usually appear at 121°C and is ***only an indicator, not proof that the autoclave cycle has successfully decontaminated or sterilized the contents.***



Biological Indicator

- *Geobacillus stearothermophilus* spores (gram-positive thermophilic bacterium)
- Due to its high resistance to heat, it is a suitable biological indicator of microbe life after a sterilization cycle.



Biological Indicator (cont.)

- Log reduction value (1 log reduction = 90% reduction in colony forming units).
- $\geq 10^5$ to $\geq 10^6$ spores per ampoule
- We use a sterility assurance level of a 5 to 6 log reduction (99.9999 %)
 - Environmental regulations for waste are $\geq 10^6$.
 - Mandatory weekly liquid and solid waste documentation maintained for two years.



Sterilization Runs

Typically include the following:

- Water
- Media solutions
- Cell culture buffers
- Stable, non-volatile substances
- Surgical instruments
- Glassware
- Stainless steel
- Culture dishes and related devices
- Pipette tips
- Test tubes

Decontamination Runs

Typically include the following:

- Liquid media waste (in glass or autoclave-compatible polypropylene flasks)
- Biohazard-contaminated solid waste (in orange biohazard bags)
 - Petri dishes
 - Cell culture dishes/plates/flasks
 - Microcentrifuge (e.g., Eppendorf tubes)
 - Centrifuge (e.g., Falcon, FroggBio) tubes
 - Pipette tips
 - Gloves
 - Paper towels
 - Animal food and bedding

The \$64,000 Question

**Can my materials or
waste be autoclaved?**

Items That **CAN** Be Autoclaved

- Media, tissue/cell culture buffers, stable non-volatile substances
- Cultures and stocks of infectious material
- Culture dishes and related devices
- Discarded live and attenuated vaccines
- Biohazard-contaminated solid items (Petri dishes, tubes without chemical hazards, pipettes, tips, gloves, paper towels)



Items That CANNOT be Autoclaved

Do not place the following items into orange biohazard bags:

- Sodium hypochlorite (e.g., **BLEACH, JAVEX**) or other chlorinated products
- **ORGANIC SOLVENTS** or other unstable organic materials that generate fumes when heated (e.g., formaldehyde, formamide, *b*-mercaptoethanol)



Items That CANNOT be Autoclaved

- **ACIDS** and **ALKALI**
- **FLAMMABLES**
- **OXIDIZERS**
- **PHENOL** or **TRIZol** (guanidinium thiocyanate-phenol-chloroform)

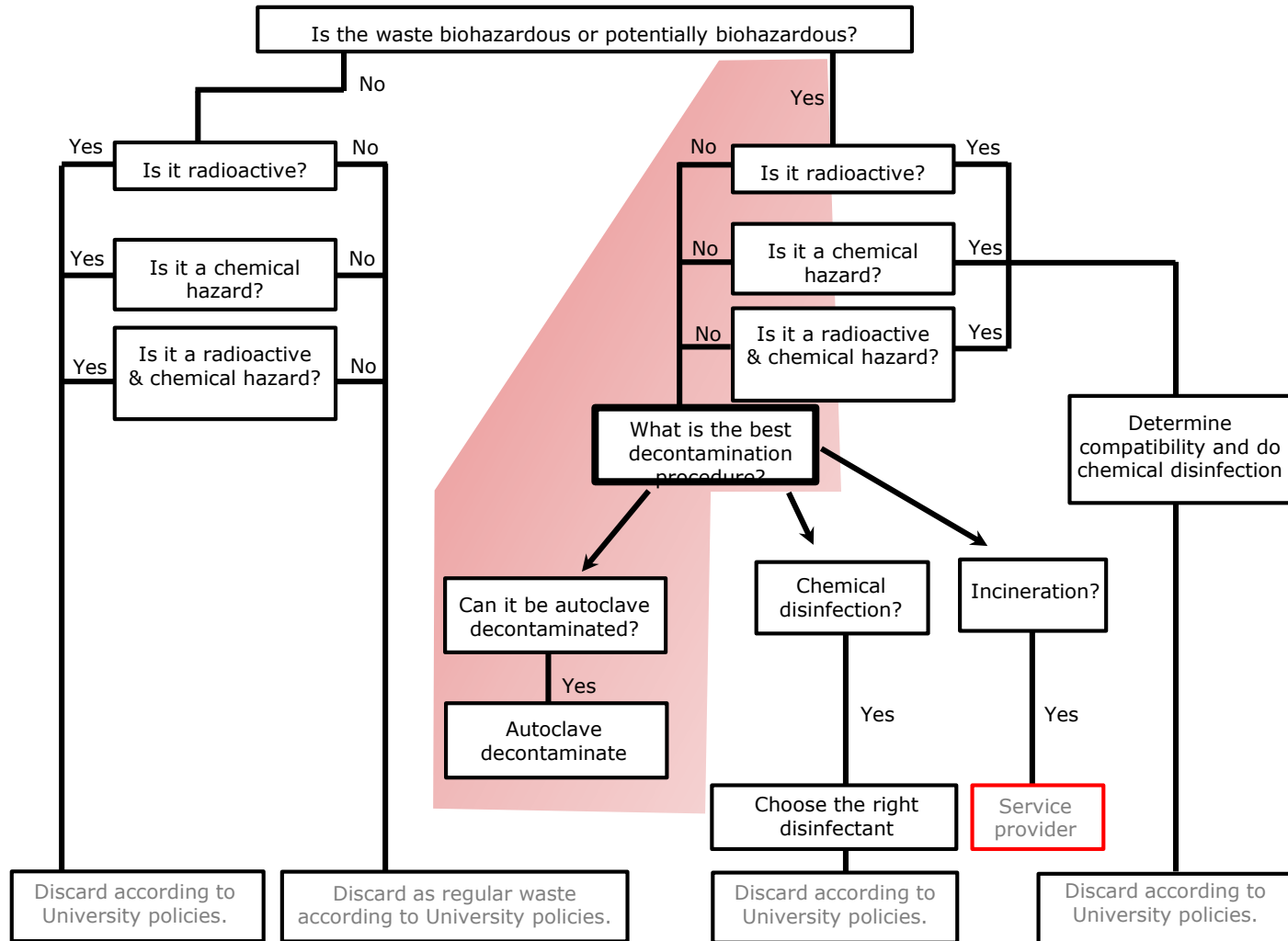


Items That CANNOT be Autoclaved

- **RADIOACTIVE MATERIAL**
- **MATERIAL CONTAMINATED WITH CHEMOTHERAPEUTIC AGENTS**
- Mutagenic chemicals (e.g., Ethidium bromide)
- Sharps



Flowchart



Primary and Secondary Containers

- **Primary containers** are in direct contact with the materials to be autoclaved. They can be borosilicate glassware, plastic containers or plastic bags.
- **Secondary containers** must be used to contain any potential spills that could occur during transfer and/or processing.



Primary and Secondary Containers

- Containers must be:
 - Autoclavable
 - Heat resistant
 - Puncture-proof
 - Water-proof (leak-proof)
 - Very thermally conductive



All Materials Must Be Autoclavable

Autoclavable:

- **Plastics** (Polypropylene (PP) and polycarbonate (PC))
- **Glassware** (Borosilicate type I glass)
- **Stainless steel**



Not autoclavable:

These materials will melt or shatter

- Polystyrene (PS), polyethylene (PE)
- High-density polyethylene (HDPE) plastic
- Household glassware



Personal Protective Equipment

- Lab coat
- Gloves (nitrile, heat-resistant)
 - *Wet gloves offer **zero** insulation/protection.*
- Safety glasses, face shield
- Apron
- Closed-toe shoes



Decontamination of Biohazardous Waste by Autoclaving

Primary Container

Autoclave bag

- Use only approved autoclave bags (for use at 134°C, not 121°C).
- If the outside of a bag is contaminated, double bag or decontaminate its surface.
- Each bag **must bear a fully completed uOttawa hazardous waste label.**



ACCEL SURFACE CLEANER-DISINFECTANT

- > **Formats**
 - > Concentrate
- > **Active Chemistry**
 - > 7% AHP (Concentrate)
 - > 0.5% AHP In-Use
- > **Product Specifications**
 - > 24 month shelf life
 - > 30 days once diluted into an enclosed contain



DÉCHET DANGEREUX / HAZARDOUS WASTE	
Université d'Ottawa University of Ottawa	
PROFESSEUR/PROFESSOR	
ÉDIFICE/BUILDING	TEL/TEL
PIÈCE # / ROOM#	CONTACT PERSON/PERSONNE RESSOURCE
CONTENU/CONTENTS	
INFORMATION SPÉCIALE / SPECIAL INFORMATION	
EN CAS D'URGENCE, COMPOSEZ IN CASE OF EMERGENCY CALL	5411
DÉCHET DANGEREUX / HAZARDOUS WASTE	

Primary Container (cont.)

- **AUTOCLAVE DECONTAMINATION IS ONLY FOR SOLID BIOHAZARDOUS WASTE, not for any type or amount of chemical hazards.**
- Orange biohazardous bags should **NEVER** be used as convenient bins for general lab trash!

Secondary Container

- A secondary container must be used to contain any potential spill that may occur when transferring and/or processing waste.
- It must have same characteristics as the primary containers (e.g., must be autoclavable).



Labs Must Provide Their Own:

- Orange biohazard bags (e.g., Fisherbrand™ Orange Autoclave Bags, 1815C (35 x 24 in). Case of 100 is ~\$110).
- uOttawa hazardous waste labels. Free, but must be requested using the [Hazardous Materials Technical Services Regular Collection Request form](#).
- Autoclavable sterilizing pans (e.g., Thermo Scientific™ Nalgene™ Large Polypropylene Sterilizing Pans, 13-359-20B; case of 6 is ~\$400).



Packaging

For orange biohazardous waste bags:

- Low-density waste: **Do not fill beyond 75% of the holding capacity.**
- Higher density waste: Limit to smaller volume of waste per bag (e.g. 25 to 60% full)
- Do not overfill bags.
- **Do not seal bags tightly.**



Transport to Facility

- Use a cart with guardrails or containment to prevent accidental spillage.
- Autoclave bags, flasks and any other items must be in secondary containers at all times.
- Cap flasks of liquid, whether contaminated or not.



Transport to Facility (cont.)

- Use lids on primary containers of solid waste.
- Never carry biohazardous waste by hand, and never take the stairs with biohazardous waste.



Disposal of Autoclaved Waste

After autoclaving, waste is no longer considered biohazardous. It can be discarded as regular waste, following a defined labelling policy and in accordance with faculty procedures.

General disposal procedure:

- Biohazard symbols must be defaced.
- A University of Ottawa “Autoclaved Biomedical Waste – Non-Hazardous” label must be affixed to each bag and dated.
- Waste bags must then be placed in regular trash bags for disposal.

Autoclave Hazards

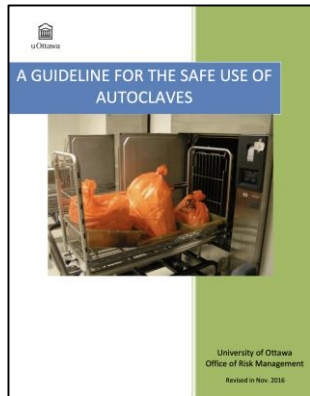
Autoclave related:

- **Heat** (high temperature of parts)
- **Steam** from chamber
- **Pressurized contents and system**

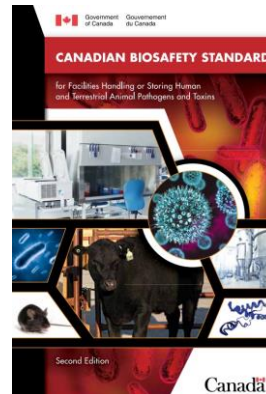
Goods or material related:

- Potential biohazardous material
- Potential prohibited substances (e.g., chemicals, sharps)
- Glassware (shattering)
- Plastics (melting)
- Liquids (hot liquid spillover)

References



[A Guideline for the Safe Use of Autoclaves](#)



[Canadian Biosafety Standards and Guidelines](#)



[Canadian Biosafety Handbook](#)

uOttawa Services

- **For additional assistance:**
 - Supervisor
 - Faculty [health, safety and risk manager](#)
 - Office of Risk Management
 - Ext. 5892 | safety@uOttawa.ca

Quiz

- To successfully complete this awareness workshop, you must [log in and complete the knowledge assessment](#). Only then will your grade be entered in the system.
- This workshop is the first step in the training cycle. Follow your faculty requirements for autoclaving, including site-specific equipment training.