Fact Sheet: Autoclaving Biological Waste

# What must be Autoclaved In-House (clear bag):

Any autoclave compatible items potentially contaminated with biohazardous materials including, but not limited to: blood, body fluids, human cell culture media, bacteria culture media, viruses, recombinant or synthetic nucleic acid molecules, research animal waste, and plant pathogens, proteinaceous biological toxins, etc. The following are examples of what can be autoclaved in a clear bag:

* Gloves or other potentially contaminated personal protective equipment
* Pipette tips
* Paper towels and absorbent padding
* Glass lab ware
* Culture plates
* Culture media
* Animal cages and bedding from infected animals

# Alternatives to Autoclaving:

* As an alternative to autoclaving, contaminated non-liquid materials can be placed in a red Biohazard bag and be collected as Biohazard waste for off-site autoclaving. Check with your Department Safety Officer (DSO) or Research Building Management if you have questions about red bagging your waste.
* Liquid wastes can be decontaminated with 1:9 v/v bleach (1-part undiluted household bleach to nine parts liquid) for 30 minutes and sewered. (Do not add bleach to materials that may contain ammonia or strong acids as dangerous chlorine gas may be produced.)
* See the [Biological Waste Disposal Template](https://bohd.umn.edu/sites/bohd.umn.edu/files/wastetemplate_1.doc) for more information on biohazardous waste disposal.

# Do Not Autoclave:

* Needles and syringes, broken glass, slides and cover slips, generated in the BSL1 & 2 labs should be placed in sharps containers for pick up. Do not autoclave.
* Liquid wastes decontaminated with bleach should not be autoclaved.
* Low molecular weight (LMW) biological toxins or items contaminated with LMW biological toxins should not be autoclaved since autoclaving with 17 lbs. pressure (121–132°C) for 30-min. is not able to inactivate LMW biological toxins. They must be disposed of in the yellow waste bag.
* Do not autoclave sealed containers or full bottles with narrow necks as they may explode.
* Do not autoclave materials containing solvents, volatile or corrosive chemicals (such as phenol, chloroform, bleach, etc.)

# Background:

Studies have shown that the processing time necessary to achieve decontamination of biological material depends on several loading factors. Load size, type of container, and moisture content all impact decontamination time. A **60 minute** autoclave cycle (excluding exhaust time) is required to allow enough time for the center of the load to come up to temperature and have sufficient steam penetration. This will ensure that the temperature in ALL parts of the load have reached 121°C for at least 20 minutes. If you wish to autoclave waste for less than 60 minutes you must validate effectiveness. Please contact BOHD at 612-626-2008 or UHS@umn.edu for a consultation.

In order to standardize the autoclaving of wastes and to assure that all loads, regardless of size or content, are properly decontaminated, the following procedure must be followed.

# Procedure:

* Place materials in a clear autoclave bag (available from [U Market](https://umarket.umn.edu/)).
	+ No Biohazard symbol should be visible. Do not use red or orange biohazard bags to autoclave biohazardous waste since these bags cannot be disposed of as regular trash. The University needs to pay a premium to dispose of biohazard waste in red bags.
	+ Bag should be loosely packed and not more than 3/4 full.
	+ Do not seal bag shut
		- if using the supplied rubber bands place on loosely, do not twist.
		- bag opening should be at least one inch in diameter.
* Use autoclave indicator tape on outside of bag to show that waste has been processed. Tape does not prove decontamination effectiveness, see **Autoclave Testing** below.
* Place waste material on a large, metal or autoclave safe plastic (Nalgene), leak-proof tray.
	+ Metal containers transfer heat more efficiently than plastic containers.
	+ Container should be large enough and shallow enough to allow for ample steam circulation.
	+ If autoclaving more than one bag at a time, be sure there is ample room between the bags so steam circulation is not impaired.
* Autoclave at 121°C for **60 minutes**.
* After autoclaving is complete, tape bag shut or tighten rubber band and place with regular trash.
* To autoclave liquid waste, place liquid in beaker or flask, not in autoclave bag. Do not seal container with liquid waste to prevent explosion.
* Autoclaved liquid culture waste can be sewered unless hazardous chemical waste is present.
* **Do not sewer** melted agar as it will congeal and clog the plumbing.
* Autoclave tape manufactured before 2008 may contain levels of lead that exceed the hazardous waste limit for trash disposal. Most lead-containing autoclave tape has stripes running across at an angle (///) which appear light beige under normal conditions and darken in the autoclave when exposed to sufficiently high heat and pressure. In some brands of tape, the color changing compound in the angled stripes may contain lead. Check the safety date sheet to determine if your autoclave tape contains lead. Contact the [Regulated Waste Program](https://dehs.umn.edu/environmental-health-safety-dehs/regulated-waste) (612-626-1604 or [hazwaste@umn.edu](file:///%5C%5Cfiles.umn.edu%5Cus%5Cuhs%5CBioSafety%5C15%20-%20Website%20-%20Biosafety%5C2021%5C3.%20For%20BK%20Review%5Chazwaste%40umn.edu)) for the correct disposal method if the autoclave tape contains lead. The use of lead free autoclave tape is strongly encouraged to help the University get the lead out. The following are the vendors and catalog numbers of the lead free autoclave tapes:

o Fisher Scientific – Cat. No. 15-901-110, 15-901-111, 15-901-112

o SPS Medical – Cat. No. LF2-036, LF2-048

o VWR – Cat. No. 10127-460, 10127-458, 10127- 462

Note: Please consult the safety data sheet to determine if the autoclave tape contains barium. Dispose of autoclave tape as hazardous chemical waste if it contains barium that exceeds the maximum contaminant level goals (MCLG) of 2 mg/L or 2 ppm.

# Autoclave Testing:

* All autoclaves used for waste decontamination must be tested monthly for effectiveness.
* Autoclave indicator tape **does not** prove decontamination effectiveness. Tape only indicates that the outside of the container came to temperature, it does not reflect time or conditions inside the load.
* For highest confidence in decontamination effectiveness, each load should be tested. If autoclaving for less than 60 minutes, each load must be tested. 3M Comply (Thermalog) chemical integrator strips provide immediate test results.
	+ Can be purchased from U Market - McKesson item 365492.
	+ Attach the indicator strip to a stick or string and put in the **center** of the load.
	+ Retrieve after autoclaving for confirmation that the entire load has been exposed to the conditions necessary for decontamination.
	+ If the indicator reads unsafe, autoclave again. If load fails again check with designated person in charge of the autoclave for further troubleshooting.
* Monthly autoclave effectiveness test can also be performed by using a biological indicator.
* Autoclave testing data must be kept for two years. An Autoclave Log template is available here: <https://bohd.umn.edu/sites/bohd.umn.edu/files/autoclavelog-9.doc>

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# Personal Safety Precautions:

* When unloading an autoclave, wear heat resistant gloves, eye protection, and lab coat, long pants or skirt and closed toe shoes
* To prevent steam burns, make sure that the autoclave pressure is near zero before opening the door.
* Allow steam to escape gradually by slowly cracking open the autoclave door. Allow load to cool for 10 minutes before removing.
* If autoclaving radioactive materials, please use a liquid cycle to ensure adequate temperature controls and venting take place during the process. A smear survey of the autoclave must be

conducted after each use to ensure that there is no contamination.

# Reference:

Biological Safety Principles and Practices edited by Diane Fleming & Debra Hunt, ASM Press, Washington D.C., page 393-395, 2000