**General Autoclave Safety**

Print a copy and keep one by the autoclave, and store a copy with your
*Integrated Laboratory Management Plan* and/or *Lab Safety Binder*

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| --- | --- |
| **Autoclave Make / Model:** | Enter Autoclave Make / Model |
| **Department:** | Enter Department |
| **Date SOP was approved by PI:** | Enter date |
| **Principal Investigator (PI):** | Enter PI |
| **Office Phone:** | Enter Phone # |
| **Emergency Contact:** | Rich Adickes or Amanda Fletcher-Dembicki |
| R:518-569-5369 or A:281-380-9667 |
| **Location(s) covered by this SOP:** | Enter Building  |
| Enter Room # |

1. **Introduction and Purpose**

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. The autoclaving process is used to destroy microorganisms and disinfect labware or equipment used in Biosafety Level 1 and 2 laboratories at Texas Christian University. The purpose of this document is to provide standard operating procedures for the use of the autoclave.

1. **Risk Management**
	1. Risks and Hazards

Since autoclaves use heat, steam and high pressure for sterilization, the potential hazards and safety risks (the risk is highest when unloading the autoclave) for operators include:

* + - * Broken glassware if the autoclave door is opened too quickly, and sufficient time is not provided for glassware to approach room temperature.
			* Super-heated liquids pose a risk if shaken or moved during the cooling process.
			* Vapors and gases from the accidental autoclaving of volatile chemicals.
			* Heat burns from autoclave chamber walls and doors and/or hot materials.
* Steam burns from steam coming out of the autoclave or materials following completion of cycle.
* Scalds from hot fluids due to boiling liquids and/or spillage in autoclave.
* Explosions from autoclaving certain chemicals, when the seal of the autoclave door malfunctions, or when autoclave is improperly loaded.
* *Sealed containers may explode during the autoclave process.*
* In accordance with local and state regulations, all biohazardous waste must be biologically-inactivated before it is disposed of as regular trash. It is against OSHA regulation to dispose of non-decontaminated waste in the common waste stream.
	1. Health and Safety Expectations

In order to ensure the health and safety of personnel using the autoclave, it is imperative that each department maintain the autoclave equipment per manufacturer and safety guidelines, and ensure personnel are trained in the proper use.

* PI is responsible for ensuring that employees are properly trained before operating autoclave.
* Name of the individual responsible for the autoclave (PI) is posted at the entrance of WIN 409 and technician information on the front of autoclave. Autoclave SOP is present near the autoclave unit (located in Usage Log Binder) for reference.
* Documentation of training, which includes the signature of both the PI and the individual trained, must be kept by the PI.
* Personal protective clothing and equipment must be worn and made available by the Department for autoclave operation.
* Procedural and instructional documents provided by the autoclave manufacturer must be kept, read, and adhered to when working with autoclave unit.
* Autoclave is inspected bi-annually by a certified autoclave technician.
* A basic, visual inspection should be made by the user PRIOR to each use of the autoclave. Potential issues (ex. excess water condensation, spills, abnormal touch screen displays, etc.) should be immediately reported to PI.
	1. Personal Protective Equipment (PPE)

Autoclaves utilize steam, heat, and pressure to complete the sterilization cycle. Therefore, the appropriate Personal Protective Equipment (PPE) must be worn.

* **Eye and face protection – safety goggles / face shield**; safety goggles worn always; face shield worn if there is a possibility of splash back
* **Heat-insulating / autoclave gloves** that provide complete coverage of hands and forearm
* **Lab coats** – long sleeved and knee length
* **Closed-toe shoes** – ensure the foot is entirely covered
* **Long pants or long skirt** – legs must be covered
	1. Training

PI must ensure that all personnel, whether they have taken the online autoclave training or not, have successfully completed an in-person training session on the safe operating procedures prior to using the autoclave. This requirement is applicable to both new and experienced personnel. All in-person training must be documented, dated and signed by both the trainee and trainer, and available to view upon request. Documentation of this and any other in-house training should be kept by the PI for a minimum of 3 years after the person has left the lab.

1. **Autoclave Operation**
	1. Uses and Limitations

Autoclaving is an economical and environmentally friendly way of sterilizing and decontaminating items, but not all materials can be autoclaved. Some materials present specific hazards if autoclaved.

Items that **CAN** be autoclaved include, but are not limited to:

* 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 towels, lab coats, solid and liquid waste
* Items for sterilization such as: glassware, media, liquid solutions
* Some equipment - ask PI for confirmation

Items that should **NOT** be autoclaved include:

* Chemicals (includes most disinfectants; DO NOT AUTOCLAVE BLEACH)
* Samples containing solvents or substances that may emit toxic fumes
* Certain plastics that may melt upon high heat/pressure
	1. Material Preparation

Ensure that all material is prepared properly prior to autoclaving:

* Check to confirm the materials can be autoclaved.
* Inspect glassware for cracks prior to autoclaving. Cracked glassware should **NOT** be autoclaved.
* Plastics must be heat resistant (i.e. polycarbonate (PC), PTFE (Teflon), and most polypropylene (PP)).
* Containers of liquid must NOT exceed 50% full, and always have lids loosened.
* Liquids, bags of agar plates, or any materials that may boil over or leak MUST be placed in secondary container in the autoclave.
* Loose/dry materials must be wrapped/bagged in steam-penetrating paper or loosely covered with aluminum foil.
* Autoclave tape is recommended for each separate container in each load.

*Primary Container*: The container that comes into direct contact with contaminated material or fluid to be autoclaved. Primary container must allow steam penetration while avoiding pressure build-up. Examples include flasks or vials containing liquids, autoclave bags etc. **The container *CANNOT* be sealed – sealed containers may explode in an autoclave!** Loose seals can be achieved by:

* Loosening screw caps (fingertip tight).
* Capping open containers for sterilization with aluminum foil.
* Opening autoclave bags slightly prior to loading them into the autoclave. Autoclave bags:
	+ Are typically made of polypropylene (PP), which is strong and puncture resistant.
	+ Come in many sizes and may or may not have the biohazard symbol printed on it.
	+ Note: Polypropylene does not have good steam permeability. To ensure that steam reaches the contents – autoclave bags MUST be opened prior to autoclaving.

*Secondary Container*: The container used to hold the primary container to secure and contain spills.

* Primary containers must be placed into trays/tubs/pans large enough to contain a total spill of contents.
* Secondary containers must sit flat in autoclave (i.e. no leaning or tilting).
* Secondary containers must be made of a material that can withstand repeated autoclaving.
* No potentially hazardous materials should be on countertops or floors without secondary containment.
* Materials being stored and/or transported to the autoclave must be kept in secondary containers at all times!

*Decontaminated Biohazardous Waste* that has been processed following recommended lab guidelines:

* All decontaminated, solid biohazard waste must be placed in an opaque garbage bag and placed outside of the lab, behind the door to be picked up for disposal by TCUs Custodial Staff.
	+ ***Custodial Staff does NOT handle biohazard bags*** unless it is concealed in an opaque garbage bag.
* Liquefied agar waste must be cooled completely before bagging.
* Hot agar liquid must be drained off into a separate pan to cool completely and disposed of later.
* DO NOT POUR LIQUID AGAR DOWN THE DRAIN!
	1. Loading Procedures

Prior to using the autoclave, verify that it has been functioning correctly by reviewing the previous cycle log recordings (time, temperature, pressure, and notes).

* Wear the appropriate PPE required to safely handle the material being loaded into the autoclave (lab coat, eye/face protection, autoclave gloves, closed-toe shoes, long pants/skirt).
* Place material in autoclave. Do not mix solid and liquid, or incompatible materials.
* DO NOT overload the chamber or compress the contents: this will impede steam penetration.
* Containers holding liquids should not be more than 50% full. This allows for liquid expansion, thereby preventing overflow.
* Ensure secondary containers are sitting flat, not angled or leaning inside the autoclave.
* Place empty flasks or tubes horizontally to prevent trapping air pockets.
* Ensure containers do not touch each other so all surfaces are sterilized.
* Ensure all containers allow steam penetration (slightly open autoclave bags).
* Close and latch autoclave door firmly.
* Door must be snug in order to compress and seal the gasket around the door.
	1. Operating Procedures – Ensure operating procedures match autoclave components (i.e. touchscreen)

Before autoclave operation, all users must have documented in-person training, at minimum.

* Verify autoclave door is closed and latched firmly.
* Press “Cycle Select” and choose the appropriate cycle (i.e. gravity, liquid, or vacuum) for the material. Consult the PI or autoclave manual for assistance in choosing a cycle.
* Choose length of time (15 min, 30 min) and press “YES” to acknowledge the door is closed.
* If you pressed an incorrect button and need to start over, select “Main Menu”
* Start your cycle and fill out the Autoclave User Log with operator and load information.
* A completed cycle usually takes between 45 min - 1.5 hours, depending on type of cycle.
* Do not attempt to open the door while autoclave is operating.
* If problems arise, abort the cycle and report to your PI immediately.
	+ Touch the “Abort Cycle” button or
	+ Switch power to off, if necessary
	1. Unloading Procedure

***The greatest risk of personal injury occurs during the process of unloading the autoclave.*** Refer to the Risks and Hazards (Section II A) for a list of potential hazards and/or risks operator may encounter; exercise caution to eliminate the possibility of such occurrences when operating the autoclave.

* Required PPE: autoclave gloves, eye protection, lab coat, closed-toe shoes, and clothing that covers legs.
* Ensure that the cycle is complete and both the temperature and pressure have returned to a safe range ***The touch screen will tell you when the door is safe to open***. (adjust if no touchscreen)
* Stand to the side of the autoclave (NOT directly in front!) and carefully unlatch the door, taking care to avoid escaping steam.
* Once door is completely unlatched, open door NO MORE than 1 – 2 inches. This allows the remaining steam to escape while simultaneously allowing the pressure within liquids and containers to stabilize.
* Let the autoclaved load to stand for a few minutes in the chamber. This allows steam to clear and trapped air to escape from hot liquids, reducing operator risk.
* Do not disturb containers of super-heated liquids (they can boil over when agitated) or remove caps prior to unloading these materials.
* Wearing autoclave gloves, remove items and place on sable surface or structure to cool.
	+ Stable structure may be a water bath for liquids which need to remain molten.
	+ Stable surface includes carts or designated laboratory benchtops.
* Do not agitate or remove caps until the liquids have cooled to a safe level.
* Check autoclave tape for color change and cycle log recorder for time and temperature attained.
* Shut the autoclave door and partially latch, if necessary, to keep closed.
1. **Autoclave Waste Treatment Record**

Entries must be placed in the Autoclave Waste Treatment Record (i.e. Autoclave Log) each time the autoclave is used. These records are used for maintenance/service schedules and reporting of incidents, accidents, and/or faults.

* Entries should include: operator's name or initials, lab which the individual works, date, time, cycle duration, chamber pressure, and condition of the autoclave.
* The log book must be kept adjacent to the autoclave.
* An Autoclave Waste Treatment Record example is provided in this document.
1. **Maintenance and Repair**
* Autoclave must not be used by anyone unless it is good repair.
* **DO NOT ATTEMPT TO MAKE REPAIRS** - only qualified professionals are permitted to make repairs to the autoclave.
* Report possible malfunctions to the PI, as soon as possible, so that repairs can be scheduled with our contracted autoclave servicer.
* Monthly maintenance should be performed on the autoclave using the following guidelines:

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| **Inspect door gasket for damage and cleanliness**  | With the autoclave switched OFF and the door OPEN, visually inspect the gasket and its mating surfaces for signs of debris, damage and corrosion. Clean by wiping with a damp cloth. |
| **Check the autoclave temperature probes for signs of damage** | With the autoclave switched OFF and the door OPEN, visually inspect the probe at the rear of the inside of the vessel for signs of debris or damage. |
| **Door operation** | Close the door to check all functions are correct in conjunction with the operating manual. |
| **Water condition**  | Check the condition of water in the water tank or trough.  |
| **Make sure that the chamber drain strainer (which can be found in the front drain hole of the sterilizer) is free of debris.**  | This should ideally be done once per day because a clogged chamber drain strainer will prevent your autoclave from sensing temperature. |
| **Wipe up any spills in the chamber with a chamber cleaning solution or water.** | Do not use hypochlorites that contain bleach, corrosive chemicals, or seawater, as these can damage the stainless-steel chamber. |
| **Visually check for water and/or steam leaks** |  If you see a leak, identify the root cause and call in a professional for repairs as soon as possible. |

1. **Equipment Malfunction**

If the autoclave malfunctions, do not attempt to fix the problem. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected.

* Record the problem in the Autoclave User Log.
* Contact the autoclave PI to report the problem.
1. **Incident Response**

All incidents, including a spill or release of biohazardous materials, MUST be reported to the autoclave PI and the Hazardous Materials Safety Manager (Rich Adickes – Safety@tcu.edu).

* If any injury occurs, seek first aid and/or medical assistance as necessary.
* If clothing absorbs hot water/steam, remove clothing, and apply cool water to the injury.
* A notice must be placed on the autoclave to indicate that the unit is out of service until the cause of the incident is identified, pro-active measures are taken to prevent such incidents in the future, and the autoclave is deemed safe for operation.
* A near-miss report must be filled out if an injury/accident nearly occurs.
1. **Spill Clean-Up**

Spills may occur due to a boil-over or breakage of containers during the autoclave procedure.

* No operation of the autoclave is allowed until the spill is cleaned up.
* Secondary containers will make spill cleanup MUCH easier.
* The operator is responsible for the clean-up of the spill.
* Wait until the autoclave and materials have cooled down to room temperature before attempting to clean-up the spill.
* Contain spilled materials using paper towels or the laboratory spill kit, if necessary.
* Dispose of cracked or broken glassware in the broken glass box.
* All spills should be reported to the autoclave PI, and record of spills should be kept.
1. **Autoclave Validation**

Regular testing must be conducted on the autoclave to validate it is working properly. Using biological indicators (BI) that contain heat-tolerant spores is an effective way to test the autoclave cycle is reaching microbial “kill.”

* Autoclaves shall be validated to ensure sterilization utilizing biological indicator assays
	+ Time tape or steam indicators DO NOT validate sterilization
	+ Typical biological indicator assays involve sterilization or inactivation of spores which demonstrate sterilization rather than just heat.
* Validation utilizing a biological indicator (BI) assay should be conducted by inserting the

BI into a run to be validated.

* + It is critical that the validation run is similar to a regular run, both in terms of sterilization time, but also load conditions.
	+ Example: If an autoclave is regularly utilized for a liquid run of 10 L of liquid, 10 L of liquid need to be validated with the BI placed in one of the liquid containers
	+ Individual labs are encouraged to conduct their own validation on their lab-specific loads
* A log of autoclave validation should be kept on or near the autoclave.
	+ Autoclaves should be validated every six (6) months, at minimum.
	+ The CDC, American National Standards Institute (ANSI), and Association for the Advancement of Medical Instrumentation (AAMI) recommend monitoring steam sterilizers weekly.
* An Autoclave Validation Record example is provided in this document.
1. **References**
* CDC Biological and Infectious Waste <http://www.cdc.gov/nceh/ehs/etp/biological.htm>
* Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition, 2020. <https://www.cdc.gov/labs/BMBL.html>
* Oregon State University, Standard Operating Procedures; Safe Autoclave Operations.

<https://ehs.oregonstate.edu/sites/ehs.oregonstate.edu/files/pdf/osu_autoclave_sop_template.pdf>

* University of Wyoming, Standard Operating Procedures; Safe Autoclave Operations. [www.uwyo.edu/safety/\_files/Docs/Procedures/SOPs/Biosafety/Autoclave\_SOP.pdf](http://www.uwyo.edu/safety/_files/Docs/Procedures/SOPs/Biosafety/Autoclave_SOP.pdf)
* Richard Adickes, Hazardous Materials Safety Manager, Texas Christian University
* Julie Nunnally, Laboratory Coordinator, Biology Department, Texas Christian University

**Principal Investigator SOP Approval**

By signing and dating here the designee certifies that the Standard Operating Procedure (SOP) for General Autoclave Safety is accurate and effectively provides standard operating procedures for laboratory personnel.

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Signature Printed Name/Title Date

**Documentation of Training** (Signature of All Users Is Required)

* Prior to conducting any work with the autoclave, designated personnel must provide training to laboratory personnel specific to the hazards involved in working with this equipment, work area decontamination, and emergency procedures.
* The Principal Investigator must provide this SOP to all laboratory personnel.
* The Principal Investigator must ensure that laboratory personnel have attended appropriate laboratory safety or refresher training.

By signing this document, I acknowledge that I have read and understand the content of this SOP and have had in-person autoclave specific training:

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| **Name** | **Signature** | **Date** |
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| **Autoclave Waste Treatment Record** |
| **Building & Room:**  |  |  |  |  |
| **Primary Contact for the Autoclave:**  |  |  |
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| **DATE OF TREATMENT**  | **START TIME** | **RUN TIME** | **CHAMBER PRESSURE** | **CONDITION OF AUTOCLAVE** | **USER NAME** |
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| **Autoclave Validation Record** |
| **Building & Room:**  |  |  |  |  |  |  |
| **Primary Contact for the Autoclave:**  |  |  |  |  |
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| **DATE OF TEST**  | **BIOLOGICAL INDICATOR USED** | **START TIME** | **RUN TIME** **(30 min. minimum)** | **CHAMBER PRESSURE****(15 PSIG minimum)** | **TEMP****(121°C minimum)** | **RESULT (PASS / FAIL)**  | **PERSON PERFORMING VALIDATION** |
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