# Appendix J: Autoclave Operational and Safety Guide



## PRECAUTIONS

Autoclaves can be complex to operate. They must also be safely operated to prevent burns, other injuries, and property damage. If you have never used an autoclave or are unsure about using a particular autoclave, please have a qualified individual, such as one of the Microbiology Department Laboratory Managers (Prairie Springs Science Center, room 4017) instruct you on its use. The following safety precautions are mandatory when using autoclaves.

1. Always wear safety glasses or goggles. Unexpected steam or hot liquid from the autoclave can seriously damage the eyes.
2. Always wear thermally protective hand protection and a lab coat when removing materials from the autoclave. Autoclave gloves are available. Use autoclave gloves that provide protection from heat and moisture.
3. Take the following actions when autoclaving liquids.
   - Use shock resistant glassware or tubes. Glassware that is shock resistant has a fused label on the glassware or tube. Two of the more common labels are Pyrex and Kimax. If the glassware does not have a label, it most likely is flint glass. Flint glass will often break or crack with the high temperatures associated with autoclaving. If you have to use flint glass, please use caution.

   - Inspect all glassware for any visible flaws, stars or cracks. Do not used damaged glassware.

   - Any glassware or tube with a cap should have a vented cap or have a loosened cap. Never tighten caps prior to autoclaving. Only tighten caps on glass containers after the temperature has fallen below 100 °C. The container could potentially implode.

   - Never tighten plastic containers before or after autoclaving until they have reached room temperature. They will collapse as they cool if the caps are on tight.

4. Beware of superheating. The majority of burns are the result of superheating. Superheating can occur in autoclaved or microwaved liquids. Superheated liquids are liquids that are heated beyond the boiling point without actually boiling. Thus, the liquid is so hot that it is unstable. This condition can last for several minutes. When jarred or agitated, the liquid rapidly boils out of its container. In some cases, it almost explodes out of the container. It often takes very little agitation to have it boil over. The following precautions can be used to reduce the risk of superheating.

- When an autoclave has finished a sterilization cycle, open the door slowly and just a few inches to allow steam still trapped in the chamber to escape. Stand away from the door. Steam can burn. Opening the door slowly will also allow you to check for a rare occurrence. The exhaust trap can become clogged allowing a lot of water to build up in the chamber. In this instance, opening the door rapidly could result in burned feet. Allow liquid containers to remain in the autoclave with the door open for approximately 10 minutes. This normally will allow the liquid to cool to a point where the potential for boiling over is significantly reduced. **CAUTION:** While the chance of superheating has been reduced, the liquid will still be hot enough to cause serious burns.

- Gently remove liquid containers. Do not shake, swirl, or agitate them at this time. Do not hold them close to your body in case they boil over or the container breaks. Set them gently on a cart, on a counter top, or in a water bath.

5. When possible, autoclave liquid containers in shallow pans. If containers do boil over or break, it is safer in the pan than in your hand. Try to use stainless steel pans with short sides. The stainless steel will transfer the heat better than plastic pans and the short sides will allow the steam to flow easily around the containers. Try to avoid using deep plastic pans. If possible, fill containers to a maximum of 2/3 full. They are safer to handle and this reduces the possibility of boil over in the autoclave. Full containers or tubes will often boil over when autoclaved. Media that boils over in the autoclave and is not caught in pans could plug the steam traps and cause the unit to be shut down for repairs.

| Autoclave repairs are expensive. Please use pans! |
|---|

6. Make visual checks of the autoclave control panel prior to and after each run cycle. A lot of information is available if you take the time to look. Verify that the cycle was not interrupted or aborted. The control panel will also indicate when a cycle is complete. If a print out is available, make sure that you check it. Check it for the correct time, temperature, and pressure. It only takes seconds to look at the print out. You may wish to keep the print out for your records. If an autoclave does not successfully complete a cycle, you should assume that the materials are not sterile. As soon as possible, inform a laboratory manager or your instructor. Include the print out if possible. If you cannot open the autoclave door, it may mean that there is still some steam pressure in the chamber. Check the pressure gauge, the control panel and the print out for more information. The autoclaves are equipped with door interlocks to prevent anyone from opening the door when there is pressure in the autoclave chamber.

   If there is any pressure in the chamber, it may be an indication that it has not finished the sterilization cycle or the exhaust cycle. It may also indicate that a trap has failed which will not allow the autoclave to go into an exhaust cycle. **Never attempt to force a door open**. **If you think the trap may have failed contact your instructor or laboratory manager.**

7. Do not autoclave items containing solvents, volatile, or corrosive chemicals (phenol,

trichloroacetic acid, ether, chloroform, etc.), hazardous waste or radioactive waste. Autoclaving these materials could create a dangerous situation. Contact Environmental Health and Safety with questions regarding waste disposal.

8. Autoclave bags used for disposing petri dishes should normally only contain petri dishes, but some laboratories allow small numbers of contaminated gloves or small amounts of contaminated paper products. Do not discard sharps, any glass containers or glass products, pipette tips, Eppendorf tubes, pipets, Pasteur pipets, or anything that could potentially puncture the autoclave bag. **Never discard any chemicals or hazardous waste in a petri dish discard container.** Petri dishes should be double bagged and placed in a stainless steel tray. It is usually a good idea to add approximately 1 Liter of water and approximately 50 mL of a odor neutralizer to the bag to help generate additional steam and to control unpleasant odors. **Do not seal the bags**. The bags should be placed in the autoclave with the top opened so that the steam can enter the bag. Check the recommended sterilization times on page 5ApxJ. Most autoclave bags have an indicator on the bag. If the bag has an indicator, make sure it has changed color before disposal. Put the autoclaved bag on a cart. Place it close to a fume hood to control any unpleasant odors and allow to cool until the media has solidified. Transfer the bag to a large garbage bag and dispose in the solid waste dumpster at the southeast corner of Prairie Springs Science Center.

9. There are specific guidelines for autoclaving and disposal of sharps containers. **Anyone needing to autoclave or dispose of sharps must obtain a copy of these guidelines**. There is some basic information about sharps containers that everyone should know.

    - All sharps containers should be handled with caution.
    - All sharps should be segregated. For example bacterial or blood contaminated sharps should not be mixed with sharps contaminated with chemicals or toxic materials.
    - Bacterial or blood contaminated sharps need to be autoclaved prior to disposal.
    - **Sharps contaminated with chemicals or toxic materials should not be autoclaved!**
    - **All sharps containers must be collected for disposal.** Contact Environmental Health and Safety to arrange for pick up and disposal.

## GENERAL STERILIZATION PROCEDURES

Please be aware that autoclaving procedures slightly vary from lab to lab based on the type of operations completed at the site or within individual labs. For example, the procedures in a research lab may vary from a state certified lab doing water analysis. The following recommendations should cover most autoclaving situations at UWL.

1. There are two cycles on most autoclaves. A few also have an isothermal cycle which offers an option of processing heat coagulable and heat sensitive materials at the lower

temperature range of 70 - 100 °C. The two cycles are LIQUIDS (SLOW EXHAUST) and GRAVITY (FAST EXHAUST). **All liquids MUST be autoclaved on the liquids cycle.** A gravity cycle is generally used for empty glassware, pipets, or any materials that do not contain liquid. A drying time is also available on the gravity cycle. During the drying cycle a vacuum of 0-2" Hg will be maintained and hot air will be drawn into the chamber through a bacteria retentive filter. While it is possible to sterilize glassware and other items on the liquids cycle, **liquids cannot be done on a gravity cycle without total loss or a significant loss of volume.** You most likely will end up with a big mess in the autoclave using a gravity cycle to sterilize liquids.

2. The following are some recommended sterilization times and some general guidelines about sterilization. Read directions on media bottles and any reference literature to see if there are any special autoclaving instructions. There are some media that are only autoclaved for 10 minutes, but the majority of media or glassware need a **minimum of 15 minutes at 15 pounds/square inch of pressure and a temperature of 121 °C.** Please remember that this is the minimum. It assumes that the media was heated to a boil or melted prior to autoclaving and that the volume is within acceptable limits for that time. **As a rule the larger the volume the more the autoclaving time needs to be increased.** The following table of volumes and times provides a baseline recommendation for cycle times based on treatment volume.

| Liquid Volume (mL) | Number of Containers (maximum) | Length of Time (minimum) |
|---|---|---|
| 500 | 30 | 29 |
| 1000 | 20 | 44 |
| 2000 | 10 | 47 |
| 3000 | 8 | 53 |
| 4000 | 5 | 62 |

Note that the time must be increased significantly with large volumes. To better understand this information look at the 500 mL volume. The majority of volumes used in the Preparation Room and in most research laboratories are between 100 and 1000 mL. We know from experience that one or two containers with a 500 mL volume would need to be autoclaved for 15-20 minutes. However, notice the minimum time necessary for 30 containers. One must then assume that 15 containers would need to be autoclaved somewhere between 15 and 29 minutes. As needed, discuss cycle times with one of the Microbiology Lab Managers.

**Other Factors that Affect Autoclave Times**

- The total volume being autoclaved affects run time. An autoclave that has well spaced containers can be autoclaved for less time than one with the contents packed closely together.

- Containers autoclaved in pans need to be autoclaved longer than containers that are not in pans. Pans that are packed need to be autoclaved longer than pans that have few containers.

The provided table identifies historical times that the Microbiology Laboratory Managers have used for sterilization. Please remember that very large numbers of containers or a packed autoclave will alter these times.

| | |
|---|---|
| **Tubed Media (Standard 16 X 150 mm tube)** | 15 - 17 minutes |
| **Flasks or bottles of Broth Media**<br>10 - 350 mL<br>350 - 750 mL<br>750 - 1200 mL | <br>15 - 17 minutes<br>20 - 21 minutes<br>22 - 30 minutes |

Flask or bottles of media with agar not melted prior to sterilization. (For media melted prior to sterilization subtract 2 minutes)

| | |
|---|---|
| 10-350 mL | 17-20 minutes |
| 350-750 mL | 20-23 minutes |
| 750-1200 mL | 24-30 minutes |

**Other Autoclave Times**

| | |
|---|---|
| 99 mL dilution blanks | 30 minutes |
| Pans of 99 ml dilution blanks | 45 minutes |
| Boxes of micro-pipette tips (1 – 6 boxes) | 20 minutes + 15 minutes dry |
| Boxes of micro-pipette tips (6+ boxes) | 30 minutes + 15 minutes dry |
| Pipet cans (1 – 6 cans) | 30 minutes+ 15 minutes dry |
| Pipet cans (6 or more) | 45 minutes + 15 minutes dry |
| Small discard bags (NO petri dishes) | 30 minutes |
| Large discard bags with petri dishes | 80 – 90 minutes |
| Large discard pan of tubes | 80 – 90 minutes |
| To obtain RNAase free tubes or glassware | 120 minutes |

Glass pipets, glassware, or wrapped dry supplies can also be sterilized in a dry air oven for 2 hours at 170 °C. Note: most plastic will melt in a hot air oven. Plastic inserts or plastic rings in caps will often melt. Make sure that whatever you put in a hot air oven will withstand the temperature and will not ignite.

## ADDITIONAL AUTOCLAVING TIPS

Last Updated March, 2019

- Many ingredients added to media are heat labile and must be added after sterilization when the media has cooled to 50-55°C in a water bath. If you are making additions to media prior to autoclaving make sure that the additions can withstand high temperatures associated with autoclaving.

- Prepared media containing carbohydrates or amino acids needs to be sterilized for the shortest possible time. They should also be removed from the autoclave as soon as it is safe to remove them. Do not leave them in the autoclave. Amino acids can be damaged by excessive heat and carbohydrates can be broken down or will caramelize. Caramelization is a term used to describe what happens when sugar is burned or overheated. The same thing can happen to carbohydrates in laboratory media when they are left at high temperatures or autoclaved too long.

- Some media may require the aseptic additions of filter sterilized solutions of amino acids or carbohydrates after sterilization and cooling to 55 °C. Some examples of ingredients that are normally added after sterilization are vitamins, blood, most antibiotics, and X-Gal. If you are not sure about an ingredient check with your instructor or with a laboratory manager. Some ingredients such as X-Gal are very expensive.

- It is usually good laboratory practice to put a few small pieces of time tape on several bottles, containers, or pipet cans. The tape will turn color upon reaching the correct sterilization temperature. Note: This does not guarantee sterility. It only verifies that the autoclave reached the normal operating temperature.

- Any individual needing the use of an isothermal cycle or sterilization at temperatures below 121°C should check with a Microbiology Laboratory Manager for procedures.

- Check with a laboratory manager or your instructor if you have any questions or are unsure about any autoclaving procedure.

## AUTOCLAVE ASSESSMENT

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Name: _____

Instructor or Supervisor Name: _____

1. What three types of personal protective equipment should be worn for removing materials from an autoclave?

_____

_____

_____

2. What type of glass is generally safe to use when autoclaving?

_____

3. Inspect all glassware for the following issue(s) prior to autoclaving.

_____

4. Standard test tube caps and the caps that are used on some culture flasks are vented to allow pressure changes in them during autoclaving. Screw cap tubes or glassware with screw caps do not have any vents. What should you do to these tubes or glassware prior to autoclaving?

_____

5. What is the greatest hazard from a liquid being superheated?

_____

6. List two action to minimize the hazards associated with superheating.

_____

_____

7. Name one item to check if you think an autoclave did not complete a sterilization cycle.

_____

8. Name three (3) types of potentially hazardous materials that should not be autoclaved.

_____

_____

_____

9. Normally, what is the only thing that should be placed in a petri dish discard can?

_____

10. How are bags of petri dishes disposed after autoclaving?

_____

11. Who do you need to contact to dispose of sharps?

_____

12. What two cycles are available on most autoclaves? Also, list an example of what could be autoclaved in each of these cycles.

_____

_____

13. What is the minimum time, temperature, and pressure required to obtain sterilization?

_____

_____

_____

14. Time tape does not always indicate sterility. What does it indicate?

_____

15. What are two factors that can affect the amount of autoclave time?

_____

16. List two heat labile ingredients that would normally be added after sterilization.

_____

_____

17. What should you do if the door on an autoclave does not open?

a. Force it open.
b. Beat on the door with a blunt object.
c. Check the print out and the control panel for possible reasons why it will not open. If the control panel or the print out does not provide a possible reason contact your instructor or a laboratory manager.
d. Go home and worry about it later.

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# AUTOCLAVE ASSESSMENT KEY

1. Safety glasses or goggles, lab coat or apron, and autoclave gloves.

2. Shock resistant glass such as Kimax or Pyrex.

3. Look for any visible flaws, stars, or cracks (damaged glassware).

4. Loosen the cap.

5. Burn hazard from boiling over.

6. Allow container to remain in autoclave for approximately 10 minutes and autoclave in a pan. Do not shake, swirl, jar, or agitate superheated containers.

7. The print out or the control panel. The print out is best.

8. Organic solvents, volatiles, corrosives, hazardous waste, or radioactive waste.

9. Petri dishes.

10. Placed in a garbage bag and disposed in the solid waste dumpster.

11. UWL Environmental Health and Safety.

12. Liquids (slow exhaust) - tube media, agar media, dilution blanks.
Gravity (fast exhaust) - glassware, pipets, wrapped materials.

13. Fifteen minutes, 15.7 pounds of pressure, and a temperature of 121$^{o}$C.

14. That the autoclave reached the normal operating temperature to turn the color of the tape.

15. Volume or number of containers (how full the autoclave is). If material is autoclaved in a pan.

16. Vitamins, blood, antibiotics, and X-Gal.

17. c. Check the print out and the control panel for possible reasons why it will not open. If the control panel or the print out does not provide a possible reason contact your instructor or a laboratory manager.