

Autoclaving Fact Sheet

What is the purpose of an autoclave? An autoclave is used to sterilize liquid media (including agar), glassware, and the surfaces of small objects.

Sterilization is the complete destruction of all bacteria, endospores, viruses, and fungi. It requires that the media or object be subject to a temperature of 121 °C for a minimum of 15 minutes. This is achieved by increasing the pressure in the autoclave to 18-20 psi (e.g. slightly more than one full atmosphere over normal atmospheric pressure).

Small volumes of liquid or empty glassware will reach this temperature very quickly. Thus, a 20 minute cycle allows sufficient time for the temperature of the liquids to reach 121 °C and remain there for at least 15 minutes.

Large volumes of liquid (>500 mL) will NOT reach 121 °C as quickly as smaller volumes. Thus, a longer cycle (35+ minutes) is needed to allow extra time for the liquids to reach 121 °C. Remember, the entire volume of liquid has to be at 121 °C for 15 minutes in order to achieve sterilization.

Why do I need a slow exhaust when I autoclave liquids? The higher boiling point of water (121°C) in an autoclave is achieved by putting the chamber under pressure. When the cycle is finished, the chamber must return to normal atmospheric pressure (where water boils at 100 °C). As the autoclave exhausts, the boiling point of water will decrease. Thus, the exhaust rate must be carefully controlled in order to keep the liquids from boiling over. You spend a lot of time and money making media—don't waste it by trying to bypass the slow exhaust only to have your media boil over into the tray. Attempts to abort the cycle will only mess up the autoclave (and make others upset). If there is a mechanical problem and the exhaust is taking too long, the autoclave will automatically abort and return to normal pressure.

Why do I have to wait 10 minutes before unloading the autoclave and starting another cycle? The purpose of the waiting time is to allow the hot liquids to cool slightly before moving them. The liquids are likely to be superheated, and abrupt jostling can cause vigorous boiling which can result in serious burns. Exercise care when removing hot items from the autoclave, and do not attempt to override the 10 minute wait time. If you put in another load before the 10 minutes are up, the timer will reset, and you will have to wait another 10 minutes (on top of the time you already waited). So be patient. This is a safety feature.

How full can I fill my tubes and bottles? The liquid in your tubes and bottles will boil in the autoclave. If the bottles or tubes are more than 2/3 to ¾ full, the media will boil over. Make sure caps and lids are loosely closed. If they are tight, the pressure inside the container will build up and potentially cause the container to shatter.

What do I do if something breaks or shatters, or if I make a mess in the autoclave? If there is an explosion inside the autoclave, and there is broken glass all over the place, call Environmental Health & Safety at x6771 or x5617. They can come and help clean out the mess so that things don't go down the drain and clog up the autoclave piping, which will render the autoclave inoperable. No one wants the autoclaves to be out of order, so don't ignore the mess if you made it. After you call and report the problem, post a sign on the autoclave telling others not to use it (and why). DO NOT brush broken glass or clumps of agar into the autoclave drain. That is how water from condensed steam exits the autoclave. If you clog it up, it won't drain.

Who can I call if I have other questions? You can call Tracey Martinson, UAF Biosafety Officer, at x6603.

Helpful hints for autoclaving success

- Use a **tray or bin** to put your materials in when autoclaving them. If agar boils over, the liquid will end up in the tray, not on the bottom of the autoclave (where it will clog up the drains). Trays or bins are also good for supporting autoclave bags.
- **Autoclave bags:** prior to autoclaving, add 100-200 mL water to the bag and then loosely close it. This will help generate steam inside the bag, which will greatly aid in destroying microorganisms.
- Don't fill bottles or tubes more than **2/3 full** to prevent boil-overs.
- Keep lids and caps **loosely** closed. Tightly sealed bottles may explode.
- Use only **Type I borosilicate glass (Pyrex) or autoclavable plastic containers**. Do not use old food jars (peanut butter, pickles...) to autoclave materials because they will break under autoclave conditions.
- Autoclave your materials for the **correct time period** (see Autoclaving Fact Sheet for more information).
- Be sure to **close the door tightly**. If the door is not closed tightly, steam will leak from around the door and the autoclave will make screaming noises. If this happens, abort the cycle (reset the autoclave), tighten the door, and try again.
- Do not open the door until the pressure is down to 0 psi. When you open the door, only open it about one inch at first—this helps ensure that you won't get blasted with steam.
- The door of the autoclave was not meant to hang open (it is hard on the hinges). So, when you are finished using the autoclave, please reseal the door.