

OPERATION OF THE AUTOCLAVES

Part 1. General autoclave wisdom from Howard

Part 2. Specific instructions for the "new" and "old" autoclaves

An introduction to happy autoclaving

The sterilization of materials using steam and pressure is a dependable procedure for the destruction of all forms of microbial life. However, the autoclave must be properly used and understood to be effective. Do not assume that merely pushing the button on an autoclave will result in the proper sterilization of your materials! This document establishes guidelines for the effective use of steam sterilizers (autoclaves) for the decontamination of cultures and other materials, for preparing sterile supplies, and for the safe operation of the autoclave. But let's start off with some history!

History of autoclaving

Ancient history. 1450 B.C.E. Moses (or whomever) wrote in the books of Leviticus, Numbers and Deuteronomy the first sanitary code, involving the purification of materials by fire and boiling water. Later, around, 460-377 B.C.E., Hippocrates poured boiling water onto surgical instruments to clean them.

The modern era of microbiology. An early pioneer in sterilization was Lazzaro Spallanzani (1729-1799) an Italian biologist. He discovered that it took 30 minutes to kill bacteria by heating them in sealed glass flasks.

Later, Pasteur showed that once a medium is sterilized, it will remain sterile until contaminated by microorganisms (i.e., they do not appear by spontaneous generation.). He believed that heating is one of the most convenient methods for sterilization. Simple boiling at 100C, however, is not completely effective for sterilization because many spores can survive this temperature. However, it was recognized that the boiling point of water goes up when exposed to increased pressures. In a pressure cooker where the pressure is 15 pounds/sq inch (1 atmosphere) above standard pressure (760 mm Hg), water boils at 121C. This temperature kills all life forms in 15 minutes or less (remember that what is important is the time that the entire liquid is at these conditions—not the "average" for the autoclave chamber). Such conditions have become the standard for sterilizing apparatus or small volumes of liquid: i.e., treat over boiling water at 15 pounds pressure for 15 minutes (commonly called "15 pounds for 15 minutes").

The current design of the autoclave was largely finalized in the 1880s by Charles Chamberland, a colleague of Pasteur, building up on Denis Papin's marmite. You can read part of



Chamberland (1851-1908),
inventor of the autoclave



*La Marmite de Papin exposée au
Conservatoire des Arts et Métiers à Paris*

Papin's steamcooker

the work in the paper: Chamberland, C. 1884. Sur un filtre donnant de l'eau physiologiquement pure. Compt. Rend. Acad. d. sc. Paris. xcix: 247. This grew out of a Ph.D. thesis written by Chamberland in 1879: *Recherches sur l'origine et le développement des organismes microscopiques*.

Interestingly, the steam pressure cooker, an early version of the autoclave, had been invented in 1681 by Denis Papin (1647-1712), a physicist and one of the inventors of the steam engine. However, this was used solely for culinary purposes until Chamberland's work. Papin called his product the "the marmite de Papin, or the "Papin Digester" (see his publication "La Maniere damollir les os et de faire cuire toutes sor/es de viandes en fort peu de tems et a peu defrais, avec une description de la marmite, ses pro prietes et ses usages." The first commercial steam sterilization system intended for use on medical products was developed in 1889.

The French like to take credit for the invention of the steamcooker. It is true that Denis Papin was born in Blois, France, in 1647 (to a Protestant family). However, when Louis XIV began to persecute the Protestants in 1685, he left France and began a wandering life across Europe. When in Germany, he invented the pressure cooker and his famous steam engine. He constructed a steam boat, but the boatmen, fearing unemployment, broke his machine. He died in utter poverty in London.



French stamp honoring Papin

Why is it called an autoclave? Because it describes a device that automatically locks shut when the pressure rises (to avoid steam spraying out if you open it by accident). The word is French, and comes from the Greek "auto" for automatic and the Latin "clvis," for key (as in lock and key).

Theory of operation

The basics. Why is an autoclave such an effective sterilizer? An autoclave is a large pressure cooker; it operates by using steam under pressure as the sterilizing agent. High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization. This is the amount of heat required to convert boiling water to steam. This amount of heat is large compared to that required to make water hot. For example, it takes 80 calories to make 1 liter of water boil, but 540 calories to convert that boiling water to steam. Therefore, steam at 100° C has almost seven times more heat than boiling water. Steam is able to penetrate objects with cooler temperatures because once the steam contacts a cooler surface, it immediately condenses to water, producing a concomitant 1,870 fold decrease in steam volume. This creates negative pressure at the point of condensation and draws more steam to the area. Condensations continues so long as the temperature of the condensing surface is less than that of steam; once temperatures equilibrate, a saturated steam environment is formed.

Achieving high and even moisture content in the steam-air environment is important for effective autoclaving. The ability of air to carry heat is directly related to the amount of moisture present in the air. The more moisture present, the more heat can be carried, so steam is one of the most effective carriers of heat. Steam therefore also results in the efficient killing of cells, and the coagulation of proteins. When you cook beef at home, for example, it can become tough when roasted in a covered pan in the oven. But just add a little water in the bottom of the pan, and you

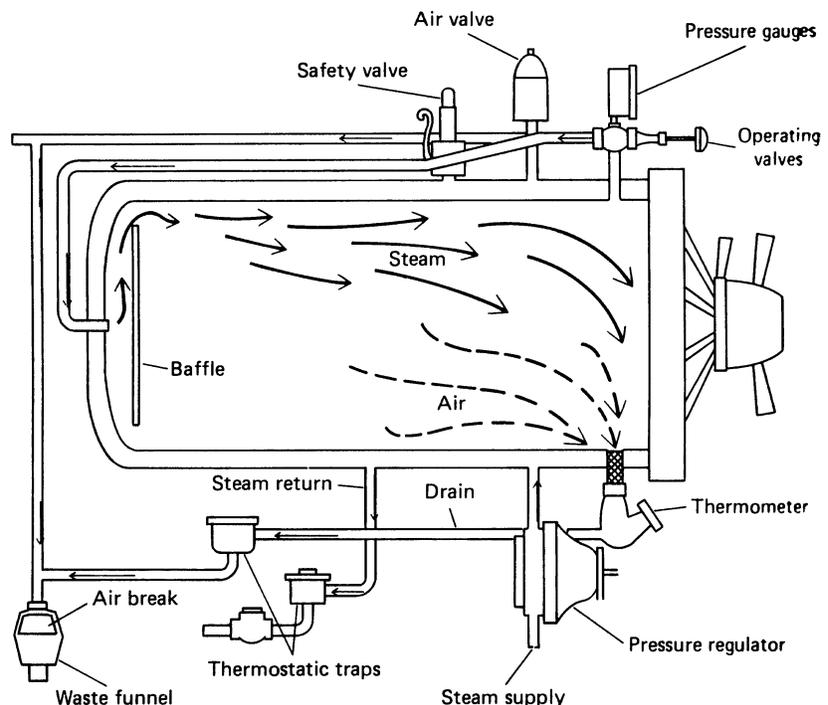
will find that the meat will be tender! The temperature is the same and the time of roasting is the same, but the result is different. Now (as in an autoclave) add another parameter, pressure. By putting this same roast in a pressure cooker you can reduce the time it takes to cook this roast by at least three quarters, and you still get just as tender a finished product.

How does killing occur? Moist heat is thought to kill microorganisms by causing coagulation of essential proteins. Another way to explain this is that when heat is used as a sterilizing agent, the vibratory motion of every molecule of a microorganism is increased to levels that induce the cleavage of intramolecular hydrogen bonds between proteins. Death is therefore caused by an accumulation of irreversible damage to all metabolic functions of the organism.

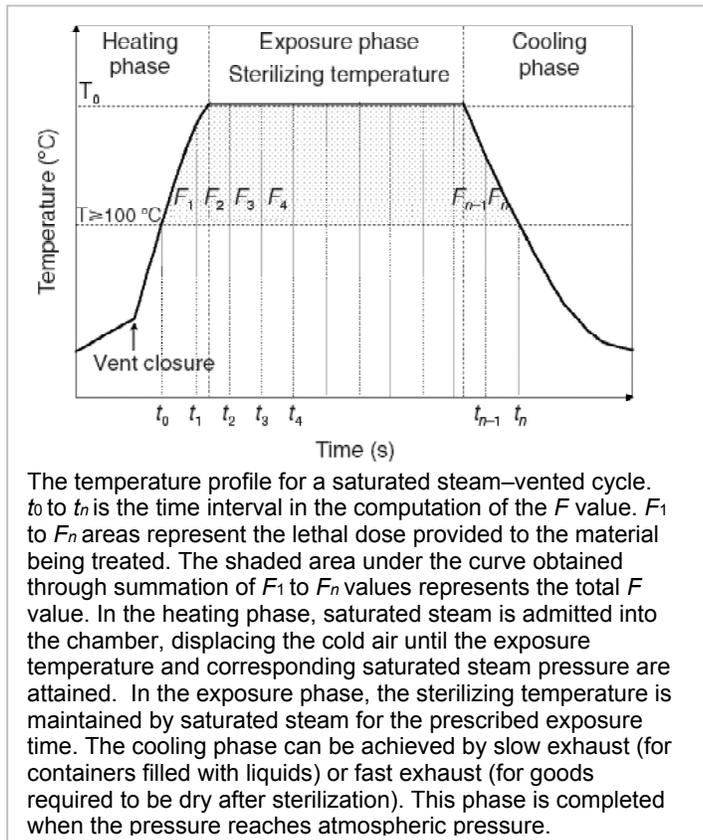
Death rate is directly proportional to the concentration of microorganisms at any given time. The time required to kill a known population of microorganisms in a specific suspension at a particular temperature is referred to as *thermal death time (TDT)*. All autoclaves operate on a time/temperature relationship; increasing the temperature decreases TDT, and lowering the temperature increases TDT.

What is the standard temperature and pressure of an autoclave? Processes conducted at high temperatures for short time periods are preferred over lower temperatures for longer times. Some standard temperatures/pressures employed are 115 °C/10 p.s.i., 121°C/ 15 p.s.i., and 132 °C/27 p.s.i. (psi=pounds per square inch). In our university autoclave, autoclaving generally involves heating in saturated steam under a pressure of approximately 15 psi, to achieve a chamber temperature of a least 121°C (250°F)—but in other applications in industry, for example, other combinations of time and temperature are sometimes used. Please note that after loading and starting the autoclave, the processing time is measured after the autoclave reaches normal operating conditions of 121°C (250°F) and 15 psi pressure, NOT simply from the time you push the "on" button.

How does the autoclave itself work? The diagram of an autoclave depicts the simplicity of its operation. Basically, steam enters the chamber jacket, passes through an *operating valve* and enters the rear of the chamber behind a *baffle* plate. It flows forward and down through the chamber and the load, exiting at the front bottom. A *pressure regulator* maintains jacket and chamber pressure at a minimum of 15 psi, the pressure required for steam to reach 121°C (250°F). Overpressure protection is provided by a *safety valve*. The conditions inside are thermostatically controlled so that heat (more steam) is applied until 121C is achieved, at which time the timer starts, and the temperature is maintained for the selected time.



Are all autoclaves the same? No! There are two general types of steam sterilizers: gravity displacement, in which the displaced air flows out the drain through a steam-activated exhaust



valve; and pre-vacuum, in which a vacuum is pulled to remove the air before steam is introduced into the chamber. With both types, as the air is replaced with pressurized steam, the temperature in the chamber increases. However, the latter is more efficient. Both approaches should result in temperature increases within the load that, under most conditions, are sufficient to treat the materials to be sterilized.

How to achieve perfect sterilization every time.

This is obviously an important issue! Improperly autoclaved materials results in contamination, lost time, and wastes money. Hopefully, by understanding the operation of the autoclave and other issues associated with your sample, you will be successful every time!

Time/Volume/Mass/Insulation/Microbe concentration/Etc. You must think about all of these variables! There is no simple formula for how long a certain item needs to be autoclaved to achieve sterility. Consideration must be given to the type of primary container (the beaker or flask or packet containing the item to be sterilized), the volume of liquid, amount of solid material, and the secondary container (such as a tray containing the primary container). Some examples of the considerations needed are described in the following sections.

Time is critical. As the cycle time will vary with the composition of the load, it is important to determine the appropriate time requirement. Some (ignorant) people assuming that a time of 30 minutes is sufficient, however this often proves to be a very costly mistake.

Secondary Containers. Plastic or steel containers (trays) are commonly used to contain material during autoclaving, since it is important to contain spills. But don't forget that such containers alter the nature of the autoclave run! Polypropylene plastic pans with 6 inch sides are favored over polyethylene and polystyrene because it can withstand autoclaving without melting. (Don't ever autoclave a plastic item -- like a tray-- if you are not sure it can handle the heat. It is no fun to pry out a melted tray!). However, the use of a plastic container increases the time needed for autoclaving, since plastic is a good insulator. Always add 5 minutes when using a plastic tray. On the other hand, stainless steel containers are not only durable, they are a better conductor of heat so the run time will be a little faster.

Do not use overly deep containers (greater than 6 inches), which may prevent displacement of air from the bottom. Also, ensure that steam can flow around the secondary container.

Volume. Obviously, the higher the volume, the more time is needed for sterilization (see general guidelines below). Generally, the volume of liquid per container is a more important consideration than the total volume. A 2-liter flask containing 1-liter of liquid takes longer to sterilize than four 500 mL flasks each containing 250-mL of liquid!

Indicators: These are tools used to validate the autoclaving process. However, use this with caution. Stick-on tape indicators can only be used to verify that the autoclave has reached normal operating temperatures for decontamination—not that the run was long enough. Biological indicators can be used in the efficacy testing of the autoclave process to effectively sterilize the contents being treated, however this is tedious and we don't routinely do this.

JUST TO BE SAFE, when running the autoclave make sure that it reaches the desired pressure and temperature. Also, before using the autoclave, check the drain screen at the bottom of the chamber and clean if blocked. If the sieve is blocked with debris, a layer of air may form at the bottom of the autoclave, preventing efficient operation.

Microbial load. Contaminated items take longer to sterilize than clean items. Consequently, water sterilizes faster than yeast-extract containing media (which contains lots of microbes), or media left at room temperature for a while before autoclaving (which allows the concentration of microbes to increase). Also, some types of microbes are more resistant to autoclaving than others.

Here is some theory related to the kill rate of microbes: When exposing homogenous populations of microorganisms to a sterilizing agent, the microbial inactivation follows an exponential decrease curve. Mathematically, the inactivation of microorganisms can be expressed as a first-order kinetic process. A finite probability of surviving organisms, independent from the magnitude of the delivered sterilizing agent, can be expressed as:

$$N_t = N_0 e^{-kt}$$

in which N_t is the number of surviving organisms after time t , N_0 is the number of microorganisms at time zero (i.e., the bioburden), t is the exposure time, and k is the microbial inactivation rate constant. K will vary with the organisms being killed.

Be cautious of packages wrapped too tightly. Air and steam do not mix readily. Air, being heavier than steam, normally is displaced to the bottom of the sterilizer and is then forced out through the drain. If your dry items are wrapped too tightly, however, air is trapped and cannot escape. It forms cool air pockets at the center of the packages, preventing the items from reaching temperatures sufficient to kill all microorganisms.

If you have a large bag of dry items to sterilize, consider adding some water to the bag. This will create additional steam which will displace the dry air from the bag, increasing the rate of heat penetration.

Can things be autoclaved too long? Yes. Nutrients in media, for example, often break down in the presence of heat. Consequently, a load of media filled containers should be of similar size, shape, content and volume, because exposure time is based on these characteristics. Otherwise some items might cook too long, and others too short.

Packing the autoclave. As much attention must be applied to loading the autoclave as was given to packaging. Again, the determining factor is ensuring heat/steam penetration. Therefore care must be given to avoid overloading the chamber or placing bags in the chamber which are too large. You should also leave room between bags, bottles, etc. for steam circulation. If you must

cram the autoclave very full (or fill a tray full with bottles, you probably have to increase the treatment time by 5-10 minutes.

Some general guidelines. Here are some recommended times for autoclaving liquids of the following volume per container:

75–200 ml	20 minutes
200–500 ml	25 minutes
500–1000 ml	30 minutes
1000–1500 ml	35 minutes
1500–2000 ml	40 minutes

Remember to modify these times as needed!!!! For example, more time is required if the flasks in a plastic tray (which is the standard method); if many bottles are close to each other in the tray; if the chamber is full of several trays; if the liquid contains microbes; if you are using plastic instead of glass containers; etc. Therefore, you probably need to go longer than the times indicated above.

Let's talk about safety!

Due to the fact that autoclaves utilize steam, heat and pressure the risk of personal exposure and potential harm is great. Personnel should wear proper personal protective equipment, i.e. heat resistant gloves, eye protection and a lab coat, particularly when unloading the autoclave.

Regularly inspect the autoclave for proper operation. Do not assume that the temperature and pressure is down before opening the chamber—look at the gauges! Even if the pressure gauge shows "0", open the chamber carefully; crack the door to allow steam to dissipate (don't fling the door open, as steam might come out and burn you). After opening the door, let items sit for five minutes before handling. This will reduce the chance of boil-overs and burns.

Never place sealed containers in an autoclave—they might explode! This allows for expansion during the cycle. Caps must be slightly loose so that pressure created during the cycle does not cause the vessel to break. For screw-cap containers, you can make the lid hand tight and then loosen the lid by one-half turn.

Always leave a few inches of "head room" in your containers. That way, if the item boils, it won't spray out into your face. Liquids to be autoclaved must be in an autoclavable vessel that is at least twice as large as the volume to be autoclaved (i.e. If you are autoclaving 1 liter of media, you need to put it in a flask that hold at least 2 liters)

Always autoclave media in a pan, to contain spills. Agar will clog the drain in the autoclave and break it.

Do not autoclave items containing solvents, volatile or corrosive chemicals (phenol, trichloroacetic acid, ether, chloroform, etc.) or any radioactive materials.

Use of the "old" autoclave in Webber Hall

1. This autoclave is used only on manual. The only control that you should touch is the main control knob—don't touch anything else!
 2. Place your items in the chamber and close it securely.
 3. Turn the knob to "steam." wait a few seconds to ensure that steam is entering the chamber (the temperature and pressure should rise).
 4. Start timing your run (it usually takes about 2 minutes to reach operating pressure—so you can add 2 minutes onto your desired run time and leave).
 5. When the time is up, manually turn the knob to either "fast" or "slow" exhaust. "Dry" never is too effective; don't waste your time with it.
 6. Wait until the pressure comes down—maybe 3 min on "fast" or 15 min on "slow"—you can open the chamber. Look at the gauges to ensure that the pressure has come down to atmosphere. The temperature should also be about 212F or lower.
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The "New" Amsco Autoclave (including programming instructions)

A. To run:

1. Look at the screen. It will show you which programs are for a liquid cycle (slow exhaust), and which are gravity (solids, fast exhaust).
2. Press the number of the program that you desire (#1-4). The screen will then show additional parameters of the program (times, etc.). You may have to browse through several until you find one that you like. Don't assume that a program that you used last time is still the same!! If you need to make a new program, make one. Don't compromise! See Section B.
3. To start, press the number of the program a second time. if you wait too long, the display will reset, and you will have to go back to step 2.
4. The screen will indicate when the run is over. Just to be safe, ensure that the temperature has come down before opening the chamber.

B. To change program parameters:

1. Lift up the sliding panel.
2. Press "change values". Wait until the date and time screen appear before doing anything else.
3. Press "change values" again. This will cause the screen for program #1 to appear. If you want to change program #1, skip to step 4. If you want to change another program, keep pressing "change values" until that program appears.
4. Press the horizontal arrows to scroll to the parameter that you want to change.
5. Press the vertical arrows to change the values (such as time).
6. When done making the changes, press "save values."
7. Once the printer has completed printing the new program parameter list (you have to wait), then start the program as described in Section A2-3 (in other words, just press the number of the program twice).