

STANDARD OPERATING PROCEDURE

Performance Verification of Steam Autoclave Kill Cycle

AUTOCLAVE ACTION

Sterilization is defined as the complete destruction of all forms of microbial life, including bacterial spores. The meaning of this word is absolute; there is no such thing as "partial sterilization". Something is either sterile or non-sterile. Sterilization can be accomplished by either physical or chemical means. The principal physical means is autoclaving, the most effective and most reliable means of sterilization.

An autoclave is a common piece of lab equipment which 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. Moist heat kills microorganisms by causing coagulation of essential proteins. 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). Increasing the temperature decreases TDT, and lowering the temperature increases TDT. Environmental conditions also influence TDT. TDT decreases with pronounced acidic or basic pH. Fats and oils slow heat penetration and increase TDT. Processes conducted at high temperatures for short periods of time are preferred over lower temperatures for longer times.

Autoclave temperature, pressure, and time settings are very important to ensure adequate decontamination of biohazardous waste and thus render infectious material safe. Higher temperatures ensure more rapid killing. The most standard temperature/pressure combination employed is 121°C (250°F)/15 pounds-force per square inch (lbf/in² or psi). Longer times are needed for larger loads, large volumes of liquid, and denser materials. When proper conditions and time are employed, no living organisms will survive the autoclave kill cycle. Autoclaves may have settings for "LIQUIDS" to be used for liquid materials. "LIQUID" settings run for longer periods at lower temperatures to minimize liquid evaporation and spills. For solid materials, the "DRY GOODS WITH VACUUM" should be used for infectious waste as it is the most effective at moving steam and heat into the deepest parts of large bags producing the best conditions for killing persistent organisms. Exhaust settings should also be appropriate for the type of waste being autoclaved. FAST exhaust should be used for solid items and SLOW exhaust should be used for liquids.

IS YOUR AUTOCLAVE WORKING PROPERLY?

Biological waste is an important occupational hazard for people who work with the waste products of research and teaching laboratories. Biological (or special) waste has been defined in Title 30 Texas Administrative Code (30 TAC), Chapter 330, Section (§) [330.3\(148\)](#) as waste which requires special handling to protect human health or the environment. Biological waste is regulated by [the Texas Commission on Environmental Quality \(TCEQ\)](#) and the [Texas Department of State Health Services \(TDSHS\)](#). It is very important to be able to assure that viable biohazardous organisms are not sent to the landfill. If adequate steam does not have adequate contact with biohazardous materials, microorganisms can survive a trip through the autoclave. Autoclave tape is not a reliable means to determine if the time, temperature, and pressure combination of the process was adequate to penetrate and kill microorganisms contained within the load.

UT Arlington autoclaves used for kill loads are tested semiannually by the Environmental Health and Safety Office (EH&S) for killing effectiveness using **biological indicators**. Spore ampoules are self-contained biological indicators and they are intended for use in the monitoring of saturated steam sterilization cycles at 121°C (250°F). Commercially available *Geobacillus stearothermophilus* spore ampoules have average spore populations of 10⁴ to 10⁶ organisms. Spores are suspended in growth medium containing bromocresol purple to function as a pH indicator. The acid production associated with growth causes a change in color from purple to or towards yellow.

Spore ampoules:

- should be stored in a refrigerator at 2-8°C
- should not be frozen
- should not be used if damaged
- should not be used after expiration date
- should be handled with care since they contain live cultures
- are not intended for flash sterilization processes

Each biohazardous waste load is also monitored and released based on the result of a [Steam Chemical Integrator](#).

In addition to the above-mentioned autoclave testing, autoclaves go through maintenance according to the service contracts, to verify that they are functioning correctly. With the increasing concern for safe handling and disposal of infectious wastes, time must be taken, and every effort made to ensure that UT Arlington autoclaves are working properly. Please contact the Biological Safety Specialist at 817-272-2185 with any questions or comments concerning autoclave testing, and the Life Sciences Core Facility Director at 817-272-9636 with questions concerning performance and maintenance of Life Science Building and Engineering Research Building autoclaves. Contact person for the Chemistry & Physics Building autoclave is Department of Chemistry & Biochemistry Research Engineer (817-272-3823) and for the Science & Engineering Innovation & Research Building (SEIR) autoclaves SEIR Building Facilities Manager (817-272-9046).

AUTOClave PERFORMANCE CHECKS

The time and temperature used for each type of waste in the laboratory should be validated using biological indicators to ensure effective sterilization. Autoclaves used for kill loads are tested semiannually by EH&S. If test results indicate that the autoclave is not sterilizing properly, the autoclave should not be used for waste until it has been repaired. A notice shall be placed on the autoclave indicating that it is not to be used until the problem is diagnosed and corrected. The first load run in the autoclave after repair should be the test run with biological indicators to ensure proper functioning of the autoclave.

AUTOClave WASTE DECONTAMINATION CYCLE TESTING & VERIFICATION

Exposure: Place one or more biological indicators in the most difficult location to sterilize, usually in the middle of the waste bag or material to be autoclaved or suspended in a volume of liquid. Autoclave tape can also be used to secure the test ampoule(s) to the inside of an autoclave bag.

Solid waste. Do not overfill waste bags or the autoclave. This will interfere with steam penetration. Add about 50-100 ml (~1/4 to 1/2 cup) of water to each bag of solid waste. Adding water creates additional steam that drives residual air from the bag. If there is naturally-occurring water in the load, adding additional water is not necessary. Add a Steam Chemical Integrator inside each waste bag. Leave the waste bags slightly open to allow for steam penetration. Bags are placed into stainless steel or polypropylene trays prior to / during autoclaving.

Liquid waste. Liquids should be placed in borosilicate (Kimax or Pyrex) or polypropylene containers for autoclaving. The containers should not be filled to more than 75% capacity. The caps or stoppers on the containers should be loosened. **Never autoclave sealed containers of liquid. This could result in an explosion of superheated liquid.** Liquid containers should be placed in a stainless steel or polypropylene tray with ¼ to ½ inch of water in the bottom of the tray so that the bottles will heat more evenly. The tray should be placed on a shelf in the autoclave and not on the bottom of the autoclave chamber.

Autoclave the waste following normal procedures using the appropriate cycle/settings. For biohazardous solid waste appropriate sterilization time is 50–60 minutes at 121°C (250°F). Allow time for the autoclave to cool down and for pressure to return to atmospheric after the cycle is finished. Wear heat-resistant gloves when first opening the door after a run.

Once the cycle is complete, remove autoclave tray with bag(s) from autoclave using insulated gloves or heat resistant mitts, a rubber apron in addition to rubber sleeve protectors, and a face shield. Let contents cool. Wait no less than ten minutes when you are autoclaving liquids before removing the items. Inspect the [Steam Chemical Integrators](#) and interpret the results.

Caution: After sterilization, handle ampoules with care. Contents of the ampoules are hot and under pressure. Failure to allow sufficient cooling time (10-15 minutes) may result in bursting of the ampoule! Remove the indicator(s) from the waste bag(s) wearing appropriate protective equipment.

Incubation: Place the processed biological indicator ampoule(s) in a vertical position in an incubator at 55-60°C. Mark a control indicator ampoule as such and incubate along with processed ampoules to ensure spore viability. Incubate for 48 hours.

Monitoring: Examine the biological indicator ampoules daily during incubation. Check for signs of growth at regular intervals during the incubation period (24 and 48 hours). Record observations. All positive ampoules should be recorded and then disposed of immediately (see info of ampoule disposal below).

Interpretation:

Control: The control ampoule should exhibit a color change to or toward yellow and/or turbidity. If the control ampoule shows no signs of growth, consider the test invalid.

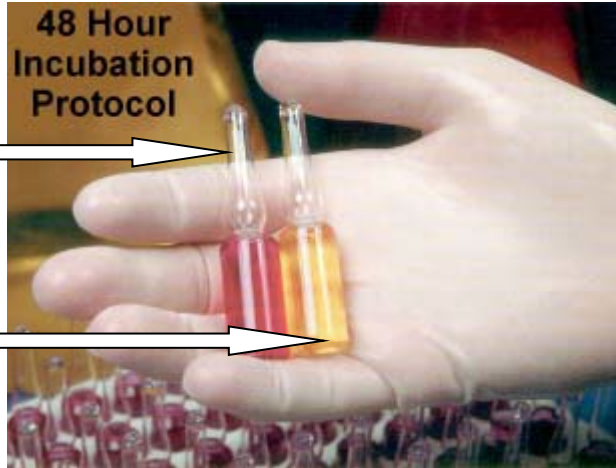
Test: A failed sterilization cycle is indicated by turbidity and/or a change in color to or toward yellow. A test ampoule that retains its purple color indicates an adequate sterilization cycle. If there are signs of growth in the biological indicator ampoule processed with the waste, the waste was not sterilized properly. The time, temperature, and autoclave procedures should be re-evaluated. If an autoclave problem is suspected, contact page 2 listed individuals immediately.

A log of each test should be maintained, which includes the type of indicator used, test date, and result of the test. The [Biological Indicator Test Results Log](#) (CO-EHS-F502) is available for download.

Disposal: All positive and expired units should be incinerated or sterilized prior to disposal.



A test ampoule retaining its purple color indicates an adequate sterilization cycle



A failed sterilization cycle is indicated by turbidity and/or a change in color to or toward yellow

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