

<b>TITLE:</b>	<b>IBC01-Autoclave Operation and Safety</b>
<b>OVERVIEW:</b>	Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. This policy gives general information on the autoclave process and standards for safe operation.
<b>APPLIES TO:</b>	All individuals using autoclaves.
<b>DEFINITION(S):</b>	<p><i>Autoclave</i> - an instrument for sterilization by means of moist heat under pressure.</p> <p><i>Sterilization</i> - the killing or removal of all microorganisms in a material or on an object.</p> <p><i>Sterility</i> - the state in which there are no living organisms in or on a material.</p>
<b>PROCEDURES:</b>	<p><b>Container selection</b></p> <p><i>Polypropylene containers and pans</i> -polypropylene is a plastic capable of withstanding autoclaving, but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan.</p> <p><i>Polypropylene bags</i> - (commonly called biohazard or autoclave bags) bags which are tear resistant, but can be punctured or burst in the autoclave. These bags are to be placed in a rigid container during autoclaving. Since they are impermeable to steam, the bags should not be twisted and taped shut, but gathered loosely at the top and secured with a large rubber band or autoclave tape to create an opening through which steam can penetrate.</p> <p><i>Stainless steel containers and pans</i> - stainless steel is a good conductor of heat and less likely to increase sterilizing time, though these containers are more expensive than polypropylene containers.</p> <p><b>Preparation and loading of materials</b></p> <p>The following steps should be considered at the time of loading:</p> <ol style="list-style-type: none"> <li>1) fill liquid containers only half full,</li> <li>2) loosen caps or use vented closures,</li> <li>3) put bags of biological waste into pans to catch spills,</li> <li>4) position biohazard bags on the sides with the bag neck taped loosely,</li> <li>5) place empty glassware on the side to allow for steam penetration, and</li> </ol>

	<p>6) leave space between items to allow for steam circulation.</p> <p><b>Cycle selection</b> Use <u>slow exhaust</u> cycle (liquid cycle) when autoclaving liquids to prevent contents from boiling over. Select <u>fast exhaust</u> cycle for glassware and <u>fast exhaust and dry</u> cycle for wrapped items.</p> <p><b>Time/Temperature selection</b> Take into account the size of the articles to be autoclaved. A 2 liter flask containing 1 liter of liquid takes longer to sterilize than four 500 ml flasks each containing 250 ml of liquid. Material with a high insulating capacity (animal bedding, high-sided polyethylene contains) increases the time needed for the load to reach sterilizing temperatures. The time/temperature also varies depending on the type of autoclave used. General parameters include: 1 hour @ 121° C (15 lbs) to decontaminate biohazardous waste; 15 to 20 minutes @ 121° C (15 lbs) for liquid sterilization; and 7 minutes @ 132° C (18 lbs) for unwrapped items [Flash method].</p> <p><b>Removing the load after sterilization</b> Check that the chamber pressure is zero. Wear a lab coat, eye protection, heat insulating gloves, and closed-toed shoes. Stand behind the door when opening and beware of a rush of steam from the door. After slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing.</p> <p><b>Quality assurance monitoring</b> Autoclaves must be tested periodically to assure effectiveness. Two types of test are used, 1] a chemical indicator that fuses when the temperature reaches 121°C (e.g. 3M Comply SteriGage Steam Chemical Indicator #1243) and 2] a biological monitor containing heat resistant spores that are killed by exposure to 121 C for approximately 15 minutes (e.g. Prospore Biological Indicator, MesaLab-Raven). The chemical monitor is generally run with each load and the biological monitor <b>is run at least weekly (or per load if decontamination loads are processed more than a week apart)</b>. Both types of tests should be placed in an area of the autoclave at a point slowest to heating to assure sterilization. A log book of the results of testing must be kept for quality assurance monitoring.</p>
<b>RECORD KEEPING:</b>	The autoclave monitor results are kept on file for at least 3 years.
<b>OTHER</b>	Autoclave Core Facilities will undergo periodic inspection to ensure

<b>INFORMATION:</b>	<p>compliance with University policy (see <b>IBC01-Form 1</b> for inspection checklist)</p> <p>All individuals who handle biohazardous materials must successfully complete the web-based General Biosafety Training program. (Contact the Biosafety Officer for information on access to this training.)</p> <p>Never exceed the manufacturer's recommended pressures and temperatures during the autoclave process.</p> <p>Report all autoclave malfunctions to your supervisor and conspicuously tag the autoclave as "Out-of-Service".</p> <p>Autoclaves are not used unless specific operation instructions are received by the user or the user is working under the direct supervision of an experienced person.</p> <p>Do not stack containers, overload the autoclave, overpak autoclave bags, or autoclave volatile chemicals or radioactive materials.</p> <p>Avoid contact of hands, arms, and face with the walls of the autoclave or the emerging steam.</p> <p>Check autoclave operation periodically during the duration of a run.</p> <p>Do not use the pressure relief override valve to quickly achieve standard pressure so that the autoclave can be opened sooner.</p> <p>Wear insulated gloves or mitts when unloading materials.</p>
<b>REFERENCES:</b>	<p>Widmer, AF and R. Frei. 2007. Decontamination, disinfection, and sterilization. P 65. In P. R. Murray et al (eds.), <i>Manual of Clinical Microbiology</i>. 9th Edition, ASM Press, Washington, DC.</p>
<b>STATUS:</b>	<p>Updated: March 24, 2015</p>