



Biosafety Tips

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Biosafety Tips brings you practical approaches to biosafety or "news you can use." If you are looking for a useful and sensible solution to a biocontainment problem or perhaps a reference to help convince a skeptical researcher of the need for caution, this is the place to look. In this column I will share some biosafety insights for managing a variety of situations that arise in the workplace. I welcome feedback or suggestions for future topics. Please send them by e-mail to karen_byers@dfci.harvard.edu or to the Editor, Ira F. Salkin, at irasalkin@aol.com.

Standard Operating Procedures for Autoclaving Biohazard Waste Bags

Too often, laboratory personnel have unrealistic expectations about the process of steam sterilization, and the cycle time chosen is too short to allow for air displacement and steam penetration. This type of error may become apparent if, for example, a large container of broth is autoclaved for 20 minutes at 121°C and the unopened media later become cloudy. However, with biohazard waste sterilization, the only way to effectively determine that adequate treatment procedures are in place is to monitor the process with biological spores.

To sterilize a biological indicator in the center of a bag of biomedical waste, the indicator must reach 121°C and be held at that temperature for 15 minutes. While this may sound straightforward, the actual procedures required to achieve this vary from site to site. The following standard operating procedures (SOPs) for autoclaving bags of biohazard waste in a gravity displacement steam autoclave demonstrate this fact.

1. "Half-filled polypropylene biohazard bags are closed with elastomeric bands. The band allows steam penetration and was easier to manage than

adding water to the bags or requiring incisions to be made in the closed biohazard bag. These bags are processed in the autoclave set at 123°C for 70 minutes; the container used to catch spills is low-sided. Once a week, a biological indicator is placed at the lower front end of the autoclave chamber to verify autoclave effectiveness." (Ozanne, 1993)

2. "If the elimination of *B. stearothermophilus* is used as evidence for sterilization, then the autoclaving of 10-lb or 15-lb bags of waste in a stainless steel container would require a minimum sterilizing cycle of 90 minutes." (Rutala, 1982)

Both SOPs were devised following exhaustive research studies involving many variables. It is sufficient to verify that procedures are effective by placing a biological indicator containing *Bacillus stearothermophilus* spores in a typical load, retrieving the indicator, and then incubating the spores at 56°C for the recommended time. If you have not used biological indicators previously, consult your autoclave manufacturer for recommended products and frequency of use.

When setting up procedures to autoclave biohazardous waste, the first thing to consider is whether the facility has gravity displacement autoclaves, prevacuum cycle autoclaves, or a mixture of both. Prevacuum autoclaves pull out the air at the beginning of the "solid" sterilization cycle and thus result in the sterilization of the biological indicator in a much shorter time. Much longer times are generally required for sterilization of solid materials with gravity displacement autoclaves. Since this vacuum evacuation of the chamber air does not occur during "liquid" cycles, the time for liquid waste sterilization is the same with both gravity displacement and prevacuum autoclaves. Labeling particular autoclaves with the appropriate SOP to be used for biohazard waste would prevent confusion if different

times were to be used in gravity displacement and prevacuum autoclaves within the same facility.

Comprehensive references are available that explain the autoclaving process (Joslyn, 1991; Vesley, 2000) and the regulatory requirements for management of medical waste (Keene, 2002). These should be consulted for a complete discussion of the issues involved in the process. However, when faced with autoclaved test indicators that are positive upon incubation, you might try using the checklist below to evaluate autoclaving procedures as well as increasing the length of sterilization cycle time.

References

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Table 1

Factors that INCREASE time required for sterilization	Factors that DECREASE time required for sterilization
<p>Autoclave Factors</p> <ul style="list-style-type: none"> <input type="checkbox"/> Use of gravity displacement type autoclave <input type="checkbox"/> Running autoclave at 121°C <input type="checkbox"/> Poor quality (wet) steam supply <input type="checkbox"/> Autoclave chamber drain sieve full of debris <input type="checkbox"/> Autoclave chamber drain blocked by container <p>Autoclave Bags</p> <ul style="list-style-type: none"> <input type="checkbox"/> Use of double biohazard bags <input type="checkbox"/> Use of single autoclave bag <input type="checkbox"/> Full bags are densely packed <input type="checkbox"/> Loads with no moisture content <input type="checkbox"/> Stacking bags in two layers in the autoclave chamber <input type="checkbox"/> 4-mil polyethylene bags <p>Biohazard Bag Container</p> <ul style="list-style-type: none"> <input type="checkbox"/> High-sided containers for bags in autoclave <input type="checkbox"/> Vertical containers (opening on top) <input type="checkbox"/> Polypropylene waste containers <input type="checkbox"/> Tightly closing biohazard bags 	<p>Autoclave Factors</p> <ul style="list-style-type: none"> <input type="checkbox"/> Use of prevacuum autoclave on "solid" cycle <input type="checkbox"/> Running autoclave at 123°C <input type="checkbox"/> High quality steam supply <input type="checkbox"/> Autoclave chamber drain sieve emptied after each run <input type="checkbox"/> Autoclave chamber drain unblocked, allowing air to be forced out by steam <p>Autoclave Bags</p> <ul style="list-style-type: none"> <input type="checkbox"/> Use of single biohazard bag <input type="checkbox"/> No autoclave bag <input type="checkbox"/> Bags are half-full <input type="checkbox"/> Loads with high moisture content <input type="checkbox"/> Leaving space between bags in autoclave chamber <input type="checkbox"/> 1-mil polyethylene bags <p>Biohazard Bag Container</p> <ul style="list-style-type: none"> <input type="checkbox"/> Low-sided containers for bags in autoclave <input type="checkbox"/> Horizontal containers (opening on side) <input type="checkbox"/> Stainless steel waste containers <input type="checkbox"/> Elastomeric band closures <input type="checkbox"/> Loosely taping bags closed <input type="checkbox"/> Leaving bags open (if feasible)