



Ask the Experts

John H. Keene

Biohaztec Associates, Midlothian, Virginia

Do you have a biosafety question and you're not sure who to ask? Send your questions to the "Ask the Experts" column and I'll get them answered for you. Drawing from my own experience or that of other experts in the field we'll try to compile a thorough and comprehensive answer to your question. Please e-mail your questions to jkeene@biohaztec.com or to Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Should the condensate drain from an autoclave be inside or outside the containment area in the design of biosafety level 3 suites?

This question is plaguing many who are currently involved with the design, construction, and operation of BSL-3 biocontainment laboratories. With the assistance of Karen Byers and Lynn Harding, two articles have been located that, on the surface, seem to indicate that there would be a potential release of viable organisms during the initial purging of a steam-jacketed autoclave.

Barbeito and Brookey (1976) modified an autoclave so that they could introduce an aerosol of microbial agents into the chamber prior to initiating the sterilization cycle and then sampled the air and surrounding surfaces for the test agents. They also modified the steam baffles in the autoclave to allow for "unobstructed dispersal of the test organisms." In addition, the authors initiated experiments in a small, unmodified autoclave in which they spiked the loads with large numbers of dry spores or bacteria to obtain a theoretical concentration in the range

of 10^7 to 10^{12} organisms per cubic foot within the chamber.

In these experiments, although a small percentage of spores may have been released when the sterilizer was loaded with spores as a dry powder mixed with animal bedding or on other materials (an unrealistic occurrence when following proper procedures), the authors state: "...no bacteria were released into the atmosphere when spores in a liquid suspension or vegetative cells in a liquid suspension or dry powder were tested. The results of these tests indicated that: (i) bacterial aerosols are not created by the pre-vacuum when bacteria are in suspensions or present as moistened suspensions on discard material..." In addition, a review of the results of the experiments demonstrates that the recoverable organisms, given the extremely large challenge, were in the order of $1 \times 10^{-7}\%$ to $1 \times 10^{-5}\%$. Given the large challenges and the minimal recovery, it is apparent from this work that although release of agents may be possible, it is highly unlikely that under normal operation any organisms would escape from the autoclave during the initial vacuum cycle.

Studies by Marshall et al. (1999) have also demonstrated some release of agents to the environment when the initial vacuum cycle is drawn. The challenge doses were in the range of 10^{10} to 10^{11} cells and the recovery of culturable cells was $\leq 10^2$. The challenge dose in the autoclave chamber exceeded that which would be expected to be present under normal autoclaving procedures since the test plates and slides were not contained in autoclave bags or any other way. In addition, the tests using contaminated materials such as Kimwipes[®] were done with the contaminated materials suspended in close proximity to the exhaust port of the chamber. The au-

thors point out that “under advised sterilization procedures, such exposure would not be expected as it would not represent standard operating conditions.”

The published studies do demonstrate that under extreme conditions, there could be some release of small numbers of infectious agents during the initial vacuum cycle in an autoclave. However, the practical application of this research is not to contain the exhaust but to insure the appropriate compliance with standard operating procedures for loading and operating the autoclave (Byers, 2002).

Now that the published research on this topic has been reviewed and, recognizing that the data generated by the research may be somewhat skewed by the initial concentrations of organisms in the autoclave chamber and the placement of the contaminated materials, let’s look at practical factors to consider regarding autoclave placement in containment facilities. These factors include the heat generated, potential steam release, condensate plumbing, day-to-day operation of the autoclave, the risk of release of agents from the BSL-3 facility to the municipal sewer system, and autoclave location/ease of maintenance.

Heat Generation

The comfort of personnel in the containment facility should be one of the most important concerns of the facility management. If the laboratory space is excessively hot, the laboratory personnel will be uncomfortable and irritated, which can lead to potential failures of compliance with the strict operational procedures required of those working in the containment laboratory. Excessive heat in the laboratory can be generated when the autoclave is fully within the containment space. Therefore, keeping the working parts of the autoclave outside of the containment area can significantly lower the heat load of the facility and more easily facilitate maintenance and repair.

Potential Steam Release

If the autoclave is outside the containment area, and it can be programmed so that the outside door must be opened to remove the autoclaved load following sterilization prior to allowing the inside door to open, then the residual steam released by the

door opening will be vented outside the facility and will not affect the containment area, thus reducing the heat load to the laboratory space. This also minimizes the potential for steam condensate release, which might result in damage to wall and ceiling finishes within the containment area.

Condensate Plumbing

A particular concern that appears to be the major “bone of contention” with regard to the placement of the autoclave is the potential for release of viable infectious organisms to the condensate plumbing with the initial vacuum cycles of the autoclave. Unlike potentially volatile chemicals, microorganisms are not likely to be aerosolized within the autoclave chamber if reasonable care is taken in the placement of the load. These materials should not be aerosolized as a result of pulling a vacuum on the inner chamber of the autoclave; therefore, there should be no contamination of the autoclave exhaust or the condensate.

In addition, there seems to be a misconception, particularly by nonlaboratory personnel that the containment laboratory is somehow always contaminated causing the air in the autoclave to be automatically contaminated with the infectious agents used within the laboratory. This is not a realistic assumption. In fact, when all work with the agent is done in containment devices, as proscribed for BSL-3, there is no contamination of the laboratory air and, therefore, no airborne organisms in the autoclave.

Day-to-Day Operation of the Autoclave

Proper operation of an autoclave requires that materials to be sterilized be contained in appropriate containers while processing. Modern standard procedures for autoclaving infectious materials involve placing potentially contaminated materials into autoclave bags (with water added if they are to be tied or sealed) or into liquid containing pipette/instrument trays (with covers) before placing them in the autoclave. Since the contaminated objects are generally enclosed for sterilization, the probability of release of infectious agents into the autoclave chamber prior to operation is so small as to be nonexistent. Again, it is highly unlikely that any vacuum pulled on the chamber to remove cool air will be strong enough to

pull infectious agents through the autoclave bags or to pull them out of suspension in liquid disinfectant.

Risk of Release of Agents from the BSL-3 Facility to the Municipal Sewer System

As discussed, the risk of release from the autoclave should be extremely small, if any at all. However, what would be the risk if some small number of organisms were released into the condensate plumbing and traveled to the municipal sewer/water treatment plant? Generally, the agents used in a BSL-3 laboratory are, in fact, found in the general population. The reason for containment is because large numbers of these organisms are grown in the laboratory, not because they are unique to the general public. Municipal wastewater treatment plants are designed to treat the water to remove infectious agents that could be transmitted by water to the general public. The miniscule potential for release of agents from an autoclave to the sewer system is further diminished by the fragility of most of these pathogens in the natural environment and the mechanism for treatment of these agents at the municipal water treatment plant.

Autoclave Location and Ease of Maintenance

Finally, having stated reasons for not being concerned about the potential release of infectious agents from the autoclave, there remains an important reason to have the autoclave installed with the bioseal on the input side of the device and the mechanical part of the autoclave outside of containment. That reason is the ease of maintenance. With this configuration, when preventive maintenance or repair of the autoclave must be performed, all of the maintenance work can be completed outside of containment. This allows routine maintenance and repair work to be done without interfering with the day-to-day operation of the laboratory.

Summary

In summary, the research reported in the two referenced articles involves extreme contamination of the sterilizers prior to initiating the sterilization cycles. In both instances, while starting at extremely

high concentrations of organisms ($\geq 10^{12}$ organisms), the total recoverable quantity of infectious agents was in the range of 10^3 organisms. Both papers demonstrate the potential for release of agents from the chamber of the autoclave when large numbers of organisms are present in the chamber. However, both also indicate that if proper procedures are used to contain the infectious agents during the autoclaving process, the likelihood of release is significantly decreased.

It appears that the differential concentration of agents between the "contaminated" chamber and those recoverable from the exhaust is in the range of 10^9 . The likelihood of having such large numbers of agents in the chamber is negligible if appropriate procedures for loading and operating the autoclave are followed. Therefore, the potential for release under actual operating conditions should be virtually nonexistent.

Finally, although there is a move afoot to have the working part of the autoclave within the containment space of a BSL-3 laboratory, such placement does not appear to be necessary and could, in fact, cause interference with normal laboratory work if the laboratory must be shut down for either preventive maintenance or repair of the autoclave. Personnel involved with the planning and design of BSL-3 containment facilities should carefully review the potential risks and make their decisions based on a realistic risk assessment of the facility in question.

References

- Barbeito, M. S., & Brookey, Jr., E. A. (1976). Microbiological hazard from the exhaust of a high-vacuum sterilizer. *Applied & Environmental Microbiology*, 34(5), 671-676.
- Byers, K. B. (2002). Biosafety tips—Standard operating procedures for autoclaving biohazard waste bags. *Applied Biosafety: Journal of the American Biological Safety Association*, 7(2), 94-95.
- Marshall, H. M., Shin-Kym, H., Perlov, D. & Levy, S. B. (1999). Release of Bacteria During the Purge Cycles of Steam-jacketed Sterilisers. *British Journal of Biomedical Science*, 56, 247-252.