



Autoclave Testing in a University Setting

Richard N. Le, Amy L. Hicks, and Janice Dodge

Florida State University, Tallahassee, Florida

Abstract

Potentially infectious wastes, if not properly treated, could expose both humans and the environment to untreated microbes and toxins, and create a potential for illness. Therefore, all pathogenic materials used in research must be destroyed by heat or chemical treatment prior to disposal as biomedical waste. Additionally, bacterial waste from nonpathogenic bacteria carrying antibiotic resistance must be treated before placement in the waste stream to avoid transmission of the antibiotic-resistant trait. In biomedical laboratories, glassware, beakers, test tubes, and other contaminated research material are usually treated by autoclaving before placing them into the waste stream or recycling for continued use in the laboratory.

Introduction

Florida Administrative Code (F.A.C.) Chapter 64E-16, under the Biomedical Waste Code, requires an infectious waste treatment facility to maintain records of temperature and dwell time when wastes are rendered noninfectious by gas or steam sterilization. The F.A.C. further requires biological culture testing to assure proper sterilization of all autoclaved materials. Since this University is not a treatment facility but a generator facility, the University is not required to follow these records and testing requirements. However, in the best interest of the University's environmental and research goals, the Department of Environmental Health & Safety (EH&S) is voluntarily complying with the sterilization aspect of the F.A.C.

As a service, EH&S conducts annual sterilization testing for all departmental autoclave steam cycles

used for biological waste and materials decontamination. This testing is to ensure that the autoclave cycles used are sterilizing all microbial waste.

Background on Steam Sterilization Testing

Researchers who work with potentially infectious materials are at a higher risk of exposure, especially when exposed to untreated infectious waste. Infections may be transmitted through several different routes, including direct contact with untreated infectious waste, indirect contact with contaminated instruments or environmental surfaces, or inhalation of airborne contaminants. Infection via any of these routes requires that all five of the following conditions be present to form "the chain of infection":

1. A pathogen with sufficient infectivity and numbers to cause infection
2. A reservoir or source that allows the pathogen to survive and multiply
3. A mode of transmission from the source to the host
4. A portal through which the pathogen may enter the host
5. A susceptible host (CDC, 2003)

Effective control measures are intended to break one or more of these "links" in the chain, thereby reducing the risk of, or completely preventing, exposure to potentially infectious materials. A key component in this link-breaking process is the proper use of steam sterilizers (autoclaves), which serve as an essential step toward eliminating the "pathogen links" in the chain of infection (DePaola, 2003; Quality America, Inc., 2001).

The Occupational Safety and Health Association

(OSHA) relies on guidelines published by the Centers for Disease Control and Prevention (CDC) as a widely recognized and accepted standard to be followed by employers in carrying out their responsibilities under the Occupational Safety and Health Act. The CDC and OSHA recommend the use of Biological Indicators (BI) for monitoring steam sterilization cycles in autoclaves. The CDC states [for medical autoclaves]...“proper functioning of sterilization cycles should be verified by the periodic use (at least weekly) of biological indicators (i.e., spore tests). Heat-sensitive chemical indicators (e.g., those that change color after exposure to heat) alone do not ensure the adequacy of sterilization cycle...” (CDC, 2004).

In addition, F.A.C. 64E-16.007 states that a treatment facility that disposes of biomedical waste must utilize steam sterilization, incineration, or an alternative process approved by the Department of Health prior to disposal. Treatment shall occur within 30 days of collection from the generator. Additionally, steam treatment facility units should be evaluated for effectiveness with spores of *Geobacillus stearothermophilus* at least once every 7 days for permitted treatment facilities, or once every 40 hours of operation for generators that treat their own biomedical waste.

Sterilizer manufacturers also recognize the importance of routine testing of sterilizers and autoclaves. They recommend that a biological spore test indicator be used weekly in a representative load for sterilization assurance (Ritter; Tilton et al., 2004).

Background on PROSPORE2 Biological Indicators

PROSPORE2 (HealthLink) is a self-contained biological indicator for validating and monitoring steam sterilization of solids. It consists of a paper disc carrier containing *Geobacillus stearothermophilus* (ATCC 7953) spores. The disc is enclosed in a plastic tube along with a glass vial containing growth media for the bacterial spores. Bromocresol purple has been added as a pH indicator to detect spore growth. The outgrowth of spores decreases pH, causing a color change from purple to yellow following a 24-hour incubation period.

A PROSPORE2 biological indicator is placed inside the autoclave and a specific cycle is selected. When the cycle is complete, the PROSPORE2 indicator is sealed by firmly depressing the cap. The glass ampoule of media is crushed inoculating the *Geobacillus stearothermophilus* disc. Then the indicator is incubated at 55°-60°C for a 24-hour time period along with an untreated PROSPORE2 indicator that serves as a control to ensure spore viability.

Once the minimum incubation time of 24 hours has been achieved, both the control and autoclaved PROSPORE2 indicator are examined. If the autoclaved PROSPORE2 indicator retains its purple color, then the sterilization cycle is adequate and the result is recorded as passing. A failed sterilization cycle is indicated by turbidity or a change in color toward yellow, indicating spore growth due to a change in pH. The control indicator should exhibit turbidity and/or a color change to yellow after incubation to ensure the viability of the biological indicators. If the control indicator does not show signs of growth, the test is considered invalid and all the results are unacceptable and not considered.

Evaluation of Autoclaves

PROSPORE2 biological indicators were used to test the steam sterilization cycles of several departmental autoclaves. A total of three runs were conducted for each autoclave. For each autoclave, a test trial run using a PROSPORE2 biological indicator was run to see if the autoclaves were capable of sterilization without any load. The biological indicator in the test trial was placed in a horizontal position, as recommended by the manufacturer, in various locations within the autoclave chamber. This was done to verify the effectiveness of steam sterilization in different areas within the autoclave.

Following the test trial run, two additional runs were performed in which PROSPORE2 biological indicators were placed in a representative load or challenge load to be autoclaved. The challenge load test consisted of placing a PROSPORE2 biological indicator in the center of an autoclave bag containing empty plastic pipette tip boxes. This bag was left partially open to allow for steam penetration and sterilization.

For the results, a “PASS” indicated that the PROSPORE2 biological indicator retained its purple color signifying an adequate sterilization cycle. A “FAIL” resulted in the PROSPORE2 biological indicator having turbidity or a color change toward yellow for that sterilization cycle. Untreated control PROSPORE2 biological indicators were incubated with each autoclaved ran PROSPORE2 biological indicator.

The following results were compiled over a 3-month testing period for several departmental autoclaves. These results are for the challenge load testing only. Challenges were performed using the temperatures preset by the research groups using the autoclaves.

Test Findings

Each test trial run resulted in a passing test for every autoclave. Based on this result, it was concluded that the autoclave was functioning properly.

An adequate amount of steam and heat were produced to sterilize any unobstructed contents such as glassware. However, problems occurred with the challenge load testing. Almost every challenge load test failed to sterilize the biological indicator. Therefore, the testing procedure was modified in an effort to sterilize the enclosed biological indicator. The challenge load volume was reduced by approximately half its original volume and the bag’s opening was increased. In addition, approximately 250 mL of water was added directly to the autoclave bag to generate additional steam within the bag to achieve sterilization.

Biological indicators were also given to two separate departments to autoclave with their normal biohazardous waste load. One was autoclaved for 15 minutes at 121°C gravity cycle and the other load was autoclaved at 121°C for 35 minutes on a liquid setting. Since the biological indicators were placed in actual bags of biohazardous waste, these tests were true representatives of what laboratories were gener-

Table 1

Autoclave	Cycle	Pass	Fail
Amsco 3021-S-A (Amsco)	G15/D30		✓
	L60	✓	
Amsco 3041 (Amsco)	All settings (Liquid and Gravity)		✓
	Addition of water to bag	✓	
Consolidated (Consolidated)	G60/D15	✓	
	G30/D15		✓
Primus Autoclaves-A (Primus)	G20/D15 (Room 511)		✓
	G15/D1 (Room 312)		✓
Amsco 3021-S-B	All liquid cycles		✓
Amsco Scientific (Amsco)	G30/D30 (132°C)	✓	
	L30	✓	
	L15		✓
Amsco 3021-S-C (Amsco)	G15/D30 (132°C)		✓
	L30	✓	
	L15		✓
Primus Autoclave(B)	G15	✓	
Steris, Stage 1 (Amsco)	G30/D15		✓
	G30/D15 (addition of water)		✓

(“L”=liquid cycle, “G”=gravity cycle; and “D”=Dry time. Unless otherwise indicated, the standard 121°C and 15 psi were used at each autoclave)

ating. Unfortunately, both of these tests failed.

Based on the information in the preceding table, it was evident that the results varied greatly with each autoclave due to the type of autoclave and differences in cycle times and temperatures. However, it can be shown that the shorter-timed cycles failed consistently for each challenge load tested. The exceptions to this were the results generated from the Amsco 3041 autoclave and the Amsco Scientific autoclave. For the Amsco 3041 autoclave, the 250 mL of water added to the autoclave bag helped with the sterilization of the materials, resulting in a passing test for the challenge load. The Amsco Scientific autoclave was able to sterilize the PROSPORE2 biological indicator using a 30-minute cycle because of the higher temperature setting. However, the bags used for this autoclave were not compatible with the higher temperature, resulting in complete deterioration of the bags after autoclaving.

Additional Findings

It was observed that not all autoclaves had clear instructions available detailing proper usage and that no user log was present to record the users, cycle times, settings, and autoclave contents. In addition, it was unknown if the temperature sensors on each autoclave were properly calibrated due to inadequate record keeping concerning general maintenance on several autoclaves. However, each autoclave chamber temperature was verified during the runs using a high-low thermometer that was placed in the autoclave with each challenge load run. There was good consistency between the high-low thermometer temperature readings and the temperature indicated on the autoclaves.

In addition, no records were kept to indicate when general or safety maintenance was last conducted on several autoclaves. Maintenance should be done yearly or as recommended by the autoclave manufacturer.

Recommendations

In keeping with the CDC and OSHA recommendations and using F.A.C. 64E-16.007 as a guide-

line, EH&S will use biological indicators for validating the steam sterilization cycles on an annual basis. EH&S recommends that the following adjustments to policies and practices be implemented in order to better achieve sterilization and ensure proper maintenance for each autoclave:

1. Readjustment of Cycle Times

The standard 121°C, 15-psi, and 15-minute dwell time are adequate for sterilization of clean items or smaller loads, but were found to be inadequate for large bulky loads of biohazardous waste. For this type of waste, it is recommended that the sterilization time be increased to a **minimum** of 60 minutes, while still maintaining the standard temperature and pressure. This setting has proven effective for sterilizing larger loads. The addition of water is not recommended because results were not consistent enough for this to be considered an effective option. It is also recommended not to leave the autoclave bag open for actual autoclaving of materials even though this procedure was used in the test procedure. The bag should be secured loosely with a rubberband or closed loosely with a small opening (at least 1 inch in diameter). This will minimize an individual's risk of becoming exposed to hot materials within the bag. It is suggested that a "kill cycle" be set on each autoclave intended specifically for sterilization of contaminated dry waste loads.

The Amsco Scientific autoclave did yield a passing result due to a higher temperature setting. However, the autoclave bags used could not withstand the higher temperature and the bags deteriorated during the procedure. This is a safety concern because the contents within the bag could spill onto the user exposing him or her to the extremely hot contents. To use this autoclave at the higher temperature settings, it is important to use an autoclave bag that is compatible with the 132°C temperature. In addition, the autoclaves should be retested using the new autoclave bags.

2. Proper Autoclave Use

The autoclave bag should never be over-packed or sealed too tightly. It is also important to make sure that the contents of the autoclave bag are kept at a minimum. An over-packed bag will insulate mi-

crobes at the center of the bag compromising effective sterilization for the core contents (Churchill, 2003).

It is the responsibility of each department to ensure that all users understand and know how to use the autoclave correctly. EH&S recommends that proper personal protective equipment be worn when unloading materials from the autoclave. Any exposed skin should be covered when reaching into the autoclave after operation to prevent burns. Heat-resistant, elbow-length gloves and other personal protective equipment are necessary to prevent burns from occurring when removing hot items. In addition, a shallow pan or container should be used when autoclaving bags of biohazardous waste to prevent potential spills of this material onto the user.

3. Record Keeping

The use of an autoclave logbook is recommended for each autoclave. Prior to autoclaving any items, users must fill in all information requested in the autoclave logbook. The logbook should be located adjacent to the autoclave and be maintained by the department. Information that should be part of the logbook includes: user's name, cycle time, cycle setting, materials being autoclaved, contact number, time in, time out, and verification results.

4. Safety Maintenance

General maintenance should be conducted on an annual basis or as recommended by the manufacturer. Specifically, the safety valve should be checked and replaced as required. This information should be posted on the autoclave or maintained in a maintenance logbook for easy reference. In addition, a quick check of the autoclave should be conducted prior to each use to ensure that all parts are properly functioning (e.g., door closes and is sealed properly, the rack is in place, correct settings are being used, the interior is clean, etc.). Autoclaves should be cleaned after every use and the work area should be disinfected as needed. Appropriate cleaning protocols should be obtained from each manufacturer.

5. Training

Each autoclave user should be trained on the proper use of the autoclave. The cycle settings should be posted next to the autoclave to inform each user of the types of settings available, such as gravity and liquid cycles, the temperatures for each cycle, and run times. A written sterilization procedure should be kept near each autoclave and a standard operating procedure should be developed. It should include the appropriate sterilization times for liquid and dry waste goods, identification of standard treatment containers and proper load placement procedures, personal protection equipment required for removing materials from the autoclave, instructions for loading and unloading the autoclave, and instructions on cleaning and maintaining the autoclave.

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